University of Zagreb

FACULTY OF SCIENCE
DEPARTMENT OF GEOLOGY

Jasmin Pađan

THE DEVELOPMENT AND APPLICATION OF METHODS FOR VOLTAMMETRIC DETERMINATION AND SPECIATION OF TRACE METALS IN AN ESTUARINE ENVIRONMENT

DOCTORAL THESIS

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Sveučilište u Zagrebu

PRIRODOSLOVNO MATEMATIČKI FAKULTET
GEOLOŠKI ODSJEK

Jasmin Pađan

RAZVOJ I PRIMJENA METODA ZA VOLTAMETRIJSKO ODREĐIVANJE I SPECIJACIJU METALA U TRAGOVIMA U ESTUARIJSKOM OKOLIŠU

DOKTORSKI RAD

Mentor:
Dr. sc. Dario Omanović

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BASIC DOCUMENTATION CARD

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Doctoral thesis

THE DEVELOPMENT AND APPLICATION OF METHODS FOR VOLTAMMETRIC DETERMINATION AND SPECIATION OF TRACE METALS IN AN ESTUARINE ENVIRONMENT

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Abstract: This doctoral research advances both methodological and environmental understanding of trace metal behaviour in estuarine systems, focusing on chromium (Cr), platinum (Pt), and copper (Cu). Highly sensitive voltammetric methods were optimized to overcome interferences from surface-active substances (SAS), enabling accurate determination of trace metal concentrations and speciation in complex natural waters. For Cr, highly negative deposition potentials minimized interference from SAS, allowing redox speciation analysis even in organic enriched samples. Seasonal profiles in the Krka River estuary showed Cr(VI) dominance, while Cr(III) formation and subsequent removal occurred in hypoxic bottom waters, highlighting the sensitivity of Cr speciation to redox conditions and freshwater flow. Ultra-traces of Pt ($< 10^{-12}$ mol L^{-1}) were measured using a combination of optimized deposition potentials, a brief desorption step, and derivative processing of voltamograms, achieving detection down to the femtomolar range. Platinum displayed near-conservative behaviour along the Krka River estuary, reflecting minimal anthropogenic input and providing a rare global reference for sub-picomolar Pt levels. Copper dynamics in the organic-rich Arno River estuary was dominated by complexation with dissolved organic matter (DOM). The use of nonionic surfactant, Triton-X-100, together with controlled deposition and desorption potentials, minimised SAS interference and allowed reliable Cu speciation and characterization of two ligand classes, with strong ligands controlling most dissolved Cu. Localized anthropogenic inputs due to seasonal boat traffic caused non-conservative Cu increases, while seasonal variations in discharge and DOM composition modulated Cu speciation. Comparisons between Krka and Arno highlight four key controls on trace metal distribution: metal-specific chemistry, hydrography, organic matter content, and anthropogenic influence. Overall, this work provides a methodological framework for voltammetric analysis in complex estuarine matrices and new insights into trace metal speciation, seasonal variability, and human impact, offering a reference for future studies on estuarine trace metal biogeochemistry.

(**136** pages, **15** figures, **4** tables, **230** references, original in English)

Keywords: Trace metal speciation; Voltammetry; Chromium; Platinum; Copper; Dissolved organic matter; Surface-active substances; Humic substances; Mediterranean estuaries; Biogeochemical cycling
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RAZVOJ I PRIMJENA METODA ZA VOLTAMETRIJSKO ODREĐIVANJE I SPECIJACIJU METALA U TRAGOVIMA U ESTUARIJSKOM OKOLIŠU

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Sažetak: Ovo doktorsko istraživanje unapređuje metodološko i okolišno razumijevanje ponašanja metala u tragovima u estuarijskim sustavima, s naglaskom na krom (Cr), platinu (Pt) i bakar (Cu). Vrlo osjetljive voltametrijske metode optimizirane su kako bi se prevladale smetnje izazvane površinski aktivnim tvarima (PAT), omogućujući točno određivanje koncentracija metala u tragovima i njihove specijacije u složenim estuarijskim vodama. Za Cr, vrlo negativni potencijali depozicije minimizirali su interferenciju PAT, omogućujući analizu redoks specijacije čak i u uzorcima bogatim organskom tvari. Sezonski profili u estuariju rijeke Krke pokazali su dominaciju Cr(VI), dok se nastajanje i uklanjanje Cr(III) odvijalo u hipoksičnim pridnenim vodama, ističući osjetljivost specijacije Cr na redoks uvjete i protok slatke vode. Ultra-tragovi Pt ($< 10^{-12}$ mol L^{-1}) uspješno su mjereni kombinacijom optimiziranog potencijala depozicije, kratkog desorpcijskog koraka i derivativne obrade voltamograma, postigavši detekciju do femtomolarnog raspona. Platina je pokazala gotovo konzervativno ponašanje duž estuarija rijeke Krke, odražavajući minimalan antropogeni doprinos i pružajući rijedak globalni referentni okvir za sub-pikomolarnu razinu Pt. Specijacijom Cu u organski bogatom estuariju rijeke Arno dominiralo je kompleksiranje s otopljenom organskom tvari (OOT). Upotreba neionskog surfaktanta, Triton-X-100, u kombinaciji s kontroliranim potencijalima depozicije i desorpcije, minimizirala je interferenciju PAT i omogućila pouzdano određivanje Cu te karakterizaciju dviju klasa liganada, pri čemu su jaki ligandi kontrolirali većinu otopljenog Cu. Lokalni antropogeni doprinosi uzrokovani sezonskim prometom brodova izazivali su nekonzervativna povećanja Cu, dok su sezonske varijacije u protoku rijeke i sastavu OOT kontrolirale njegovu specijaciju. Usporedbe estuarija rijeke Krke i Arna ističu četiri ključna faktora koja utječu na raspodjelu metala u tragovima: kemija specifična za metal, hidrografija, sadržaj organske tvari i antropogeni utjecaj. Sveukupno, ovo istraživanje pruža metodološki okvir za voltametrijsku analizu u složenim estuarijskim medijima i nove uvide u specijaciju metala u tragovima, sezonsku varijabilnost i ljudski utjecaj, nudeći polazište za buduća istraživanja biogeokemije metala u tragovima u estuarijima.

(**136** stranica, **15** slika, **4** tablica, **230** literaturnih navoda, jezik izvornika engleski)

Ključne riječi: Specijacija metala u tragovima; Voltametrija; Krom; Platina; Bakar; Otopljena organska tvar; Površinski aktivne tvari; Humični spojevi; Mediteranski estuariji; Biogeokemijsko kruženje metala
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Rad prihvaćen:

Information about the supervisor– Dr. sc. Dario Omanović, Senior scientist

Dr. Dario Omanović (b. 1968) is a Senior Scientist and Head of the Laboratory for Physical Chemistry of Traces at the Ruđer Bošković Institute, Zagreb.

He earned his degree in Chemical Engineering from the Faculty of Chemical Engineering and Technology, University of Zagreb (1993), his MSc in Oceanology from the Faculty of Science, University of Zagreb (1996), and his PhD in Chemistry from the Faculty of Chemical Engineering and Technology, University of Zagreb (2001). His doctoral thesis, *"Pseudopolarography of Trace Metals in Aqueous Solutions"*, was supervised by the late Dr. Marko Branica.

Dr. Omanović joined the Ruđer Bošković Institute in 1993 as a research assistant. He was appointed Research Associate in 2004, Senior Research Associate in 2009, and Senior Scientist in 2015. Since May 2021, he has served as Head of the Laboratory for Physical Chemistry of Traces.

His research focuses on trace metal speciation, electrochemical methodologies, aquatic biogeochemistry, and the application of advanced technologies for environmental monitoring. He has led or collaborated on numerous national and international research projects, including a Croatian Science Foundation project (*MEBTRACE*) and, since 2020, an ESIF IRI2 project on the application of artificial intelligence for online water quality monitoring. He has also coordinated bilateral projects with partners in the UK, France, and China, and has engaged in industry collaborations generating over €300,000 for the Institute.

Dr. Omanović is the author of 111 peer-reviewed publications, with over 85% published in high-impact journals (Q1+Q2). His work has been cited approximately 2,700 times (h-index 30). He has successfully supervised five PhD candidates (including two from France) and three MSc theses.

He was the main organizer of three international conferences, most notably the *XVI International Estuarine Biogeochemistry Symposium*, held in Šibenik in May 2023. He has served as guest editor for four special issues of prestigious journals and is a member of the editorial boards of four international journals. He has acted as a reviewer for a large number (50) of respected scientific journals with more than 200 conducted reviews.

At the international level, he is an active member of the GESAMP Working Group 45 *"Climate Change and Greenhouse Gas Related Impacts on Contaminants in the Ocean"* (a UN advisory body), where he leads the subgroup on trace elements. He maintains strong international collaborations with scientists from ten countries (USA, Canada, Switzerland, Germany, UK, France, Spain, Italy, Greece, Brazil), resulting in a substantial body of joint publications and ongoing projects.

Research papers on which the thesis is based on

Research paper 1.

Pađan, J., Marcinek, S., Cindrić, A.M., Layglon, N., Lenoble, V., Salaün, P., Garnier, C.,Omanović, D. (2019) Improved voltammetric methodology for chromium redox speciation in estuarine waters. *Analytica chimica acta*, **1089**, 40-47. doi: 10.1016/j.aca.2019.09.014

Research paper 2.

Pađan, J., Marcinek, S., Cindrić, A.M., Layglon, N., Garnier, C., Salaün, P., Cobelo-García, A., Omanović, D. (2020) Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology. *Environmental chemistry*, **17**, 2; 74-84. doi: 10.1071/EN19157

Research paper 3.

Pađan, J., Marcinek, S., Cindrić, A.M., Santinelli, C., Retelletti Brogi, S., Radakovitch, O., Garnier, C., Omanović, D. (2021) Organic Copper Speciation by Anodic Stripping Voltammetry in Estuarine Waters With High Dissolved Organic Matter. *Frontiers in chemistry*, **8**, 628749, 16. doi: 10.3389/fchem.2020.628749

PROŠIRENI SAŽETAK

U prirodnim estuarijskim sustavima prisutni su različiti metali u tragovima, čija koncentracija i kemijsko ponašanje odražavaju kombinaciju geogenih, biogeokemijskih i antropogenih utjecaja. Među njima, krom (Cr), platina (Pt) i bakar (Cu) posebno su zanimljivi, iako svaki iz različitih razloga. Krom se ističe zbog svoje dvojne oksidacijske prirode u prirodnim vodama, Cr(III) i Cr(VI), što ga čini osjetljivim na promjene u redoks uvjetima i interakcije sa suspendiranim česticama. Platina se smatra iznimno rijetkim elementom u prirodnim vodama, no postaje sve važnija kao nova onečišćujuća tvar (eng. *emerging contaminant*) koja potječe iz antropogenih izvora, poput katalizatora ispušnih plinova, industrijskih otpadnih voda te njene uporabe u medicini. Ponašanje Pt u estuarijima je nedovoljno istraženo. Bakar, s druge strane, iako sveprisutan i esencijalan za biološke funkcije, u obalnim vodama može doseći potencijalno toksične koncentracije, pri čemu prirodni organski ligandi imaju važnu ulogu u kontroli njegove bioraspoloživosti te ublažavanju toksičnog učinka. Zbog toga, usmjereno istraživanje pojedinih metala omogućuje detaljno razumijevanje njihovih specifičnih kemijskih i biogeokemijskih ponašanja, osjetljivosti na okolišne uvjete i reakcija na antropogene utjecaje, čime se stvara temelj za predviđanje njihove raspodjele, bioraspoloživosti i potencijalnog utjecaja na ekosustave.

Razvoj pouzdanih analitičkih metoda za određivanje metala u tragovima u okolišu ključan je korak u razumijevanju njihove biogeokemije. Tradicionalne voltametrijske tehnike često su ograničene interferencijama površinski aktivnih tvari (PAT) i organskih liganada prisutnih u prirodnim uzorcima. Površinski aktivne tvari obuhvaćaju široku skupinu spojeva, uključujući anionske, kationske, neionske i amfoterne tenzide, koji se odlikuju dvojakom hidrofilnom i hidrofobnom građom što im daje sposobnost nakupljanja na granicama faza i smanjenja površinske napetosti. U morskim sustavima uglavnom potječu iz prirodnih izvora, prvenstveno razgradnjom organske tvari i eksudatima fitoplanktona i mikroorganizama, dok u okoliš mogu dospjeti i antropogenim putem, primjerice otpadnim vodama bogatim deterdžentima, sredstvima za čišćenje i industrijskim otpadom. Ove tvari adsorbiraju se na površinu živine elektrode te se tako natječu s metalima u tragovima za aktivna mjesta na površini kapljice žive potrebna za prekoncentriranje metala i prijenos njihova naboja, što dovodi do iskrivljenja signala, smanjenja ili širenja strujnih vrhova i posljedično, nepouzdane procjene koncentracije i kemijske raspodjele metala u tragovima. S obzirom na ove izazove, cilj ovog doktorskog istraživanja bio je optimizirati elektroanalitičke metode koje omogućuju precizno određivanje redoks specijacije Cr, ultra-tragova Pt i organske specijacije Cu u

estuarijskim sustavima, uz istovremeno razumijevanje njihove prostorne i sezonske dinamike duž gradijenata saliniteta.

Specifični ciljevi istraživanja uključivali su: (i) razvoj protokola za učinkovitu pripremu prirodnih uzoraka, uključujući UV tretman i očuvanje izvornosti uzoraka, (ii) optimizaciju potencijala depozicije i ostalih elektroanalitičkih parametara za smanjenje interferencija PAT u voltametrijskim mjerenjima, (iii) prilagodbu dodataka reagensa za optimalno funkcioniranje katalitičkih procesa kod određivanja Cr i Pt, i primjenu neionskog surfaktanta kod određivanja Cu za uklanjanje interferencija PAT, (iv) primjenu optimiziranih metoda u dva oprečna estuarija: estuarij rijeke Krke, krški estuarij s minimalnim antropogenim utjecajem, te estuarij rijeke Arno, karakteriziran visokim unosom organske tvari i pod velikim antropogenih utjecajem.

Krom se u prirodnim vodama pojavljuje u dva oksidacijska stanja: Cr(III), koji je reaktivan prema česticama i organskim ligandima, i Cr(VI), koji je mobilan i toksičan. Optimizacija voltametrijske metode pokazala je da primjena vrlo negativnih potencijala depozicije značajno smanjuje interferencije humične kiseline i sličnih organskih tvari, čime se omogućuje točno određivanje Cr(VI) u estuarijskim uzorcima. Analiza sezonskih promjena u estuariju rijeke Krke pokazala je da Cr(VI) dominira u oksičnim uvjetima, dok se Cr(III) stvara u hipoksičnim dubljim slojevima, gdje se zatim uklanja adsorpcijom na suspendirane čestice. Ovi rezultati potvrđuju osjetljivost raspodjele iona Cr na hidrološke uvjete, posebno sezonske promjene slatkovodnog protoka i izmjenu voda.

Za Pt, iznimno niske koncentracije ($< 10^{-12}$ mol L^{-1}) zahtijevale su podešavanje parametara elektrokemijske metode, pri čemu je kombinacija odabranog potencijala depozicije i kratkog desorpcijskog koraka omogućila smanjenje utjecaja PAT na elektrodu, poboljšavajući oblik i reprodukciju strujnih vrhova. Primjena derivativne obrade voltamograma dodatno je smanjila interferenciju pozadinskih struja i omogućila detekciju Pt u femtomolarnim koncentracijama. Sezonski i prostorni profili Pt u estuariju rijeke Krke pokazali su gotovo konzervativno ponašanje uz minimalni utjecaj promjene saliniteta i prisutnosti kloridnih iona, što odražava nisku antropogenu prisutnost i inertnu prirodu ovog elementa u sustavima s niskim antropogenim unosom. Rezultati pružaju rijedak globalni referentni okvir za sub-pikomolarne koncentracije Pt u estuarijima, neophodan za razlikovanje prirodnih i antropogenih doprinosa u usporedbi s estuarijima s visokim onečišćenjem.

Bakar, kao element snažno reguliran organskim ligandima, proučavan je u estuariju rijeke Arno, bogatom otopljenom organskom tvari (OOT). U ovom sustavu koncentracije otopljenog organskog ugljika su iznad 2 mg L^{-1}, što je značajno utjecalo na voltametrijske signale. Primjena neionskog surfaktanta, Triton-X-100, u kombinaciji s kontrolom potencijala depozicije i kratkim desorpcijskim korakom, omogućila je pouzdano određivanje signala Cu i analizu kompleksiranja s OOT. Dvije klase liganada, jaki L1 i slabi L2, dominirale su specijacijom Cu, s koncentracijama koje premašuju ukupni otopljeni Cu, čime se potvrđuje ključna uloga organske tvari u kontroli biološki dostupnog Cu. Sezonske varijacije u hidrografiji i antropogenim unosima dodatno utječu na specijaciju Cu. Visoke koncentracije Cu unesene u estuarij rijekom Arno su uglavnom antropogenog porijekla, međutim lokalizirani sezonski doprinosi, poput povećane aktivnosti brodskog prometa u ljetnim mjesecima, uzrokuju dodatni porast koncentracija Cu u srednjem dijelu estuarija. Analiza fluorescentnih komponenti OOT pokazala je da proteinske komponente i autohtoni ligandi igraju dominantnu ulogu u sastavu OOT i u specijaciji Cu, dok kopneni unos i humični spojevi dominiraju tijekom visokog protoka rijeke u proljeće.

Kombinirana analiza rezultata iz Krke i Arna ističe četiri ključna principa kontrole raspodjele metala u tragovima u estuarijima. Prvo, specifična kemija pojedinačnih metala diktira dominantne kontrolne mehanizme: Cr je osjetljiv na redoks uvjete i suspendirane čestice, Pt pokazuje gotovo konzervativno ponašanje, dok je Cu snažno kontroliran dinamikom OOT. Drugo, hidrološki uvjeti i uslojenost utječu na raspodjelu metala, pri čemu slatkovodni protok potiče obnovu vode i aeraciju, što je važno za stanje redoks uvjeta i akumulaciju metala, dok koncentracija OOT i suspendiranih čestica utječe na uklanjanje i biološku dostupnost metala. Treće, antropogeni utjecaji, uključujući korištenje protuobraštajnih boja na brodovima, mogu značajno povećati biološki raspoloživu speciju Cu u obliku slobodnih hidratiziranih iona, čak i u relativno čistim sustavima. Četvrto, niske koncentracije metala, kao u rijeci Krki i njezinom estuariju, služe kao globalni referentni okvir za procjenu ljudskog utjecaja u znatnije urbaniziranim estuarijima.

Ovaj rad doprinosi razvoju metoda za određivanje metala u tragovima i razumijevanju njihovih biogeokemijskih ciklusa u estuarijima, kombinirajući visoko osjetljive voltametrijske metode s primjenom u različitim ekološkim i hidrološkim kontekstima. Razvijene metodološke strategije, uključujući optimizaciju potencijala depozicije, desorpcijskog koraka, primjenu neionskog surfaktanta i derivativnu obradu voltamograma, omogućuju pouzdano određivanje koncentracije i specijacije Cr, Pt i Cu, čak i u složenim medijima uzoraka iz estuarija. Primjena

ovih metoda u oprečnim estuarijima pružila je nova saznanja o specifičnim kontrolama ponašanja ovih metala, sezonskim varijacijama i utjecaju antropogenih aktivnosti, čime se poboljšava globalno razumijevanje biogeokemije metala u tragovima u obalnim sustavima.

Disertacija je strukturirana tako da nakon detaljnog literaturnog pregleda slijedi rasprava koja odražava dvije ključne dimenzije istraživanja: metodološki razvoj i optimizaciju, te primjenu u estuarijima s različitim ekološkim i hidrološkim uvjetima, uz integraciju rezultata u širem kontekstu biogeokemijskih procesa i u usporedbi s globalnim podacima. Rezultati ovog istraživanja pružaju praktične analitičke smjernice i znanstvene uvide, čime se doprinosi razumijevanju prirodnog i antropogenog utjecaja na raspodjelu metala u tragovima u estuarijima, a istovremeno se naglašava vrijednost visoko osjetljivih voltametrijskih metoda u istraživanju okoliša.

1. INTRODUCTION

Trace metals play a fundamental role in estuarine and coastal biogeochemistry, serving both as essential micronutrients and, at elevated concentrations, as potentially toxic contaminants. Chromium (Cr), platinum (Pt), and copper (Cu) represent three distinct trace metals with contrasting chemical behaviours and environmental significance. Chromium is a redox-sensitive element whose distribution and speciation strongly influence its mobility, bioavailability, and toxicity. Hexavalent Cr(VI) is highly soluble and toxic, whereas Cr(III) is particle-reactive and generally less bioavailable. Platinum, by contrast, is a noble metal characterized by extremely low natural concentrations and less known behaviour in marine environments. In recent decades, Pt has emerged as a contaminant in coastal waters, largely due to anthropogenic inputs from automotive catalytic converters, medical applications, and other industrial sources. These anthropogenic sources contribute to increasing Pt levels, making it a contaminant of growing concern. Despite its increasing environmental relevance, there is still very limited information on Pt distribution, behaviour and effects in marine systems, as its extremely low concentrations pose significant analytical challenges, emphasizing the need for sensitive and robust measurement methods. Copper represents a third category, being both an essential micronutrient for aquatic organisms and a potentially toxic metal at elevated concentrations. Its environmental behaviour is closely governed by natural dissolved organic matter (DOM) and its heterogeneous ligand mixture. The interplay between organic ligands and Cu is therefore central to understanding its biogeochemical cycling and associated ecological implications in marine systems. Estuaries are dynamic environments where riverine and marine influences converge, creating pronounced physicochemical gradients that exert strong control over trace metal distributions through a combination of physical mixing, chemical complexation, redox transformations, and particle interactions. The interplay between metal-specific chemistry and estuarine processes therefore creates complex spatial and temporal patterns. Understanding of these processes is essential for elucidating both natural biogeochemical processes and the extent of anthropogenic influence on coastal systems, while achieving this requires high-resolution and sensitive analytical methods. Electrochemical methods provide a powerful approach for trace metal analysis, offering high sensitivity and selectivity, thus allowing determination and speciation of trace metals in complex matrices and under environmentally relevant concentrations. However, in estuarine and coastal waters its application can be hindered by the presence of natural surface-active substances (SAS). Surface-active substances encompass a broad group of compounds, including anionic, cationic, nonionic, and amphoteric surfactants, characterized by a dual structure with a hydrophilic head and a hydrophobic tail, giving them the ability to accumulate at phase boundaries and reduce

surface tension. In marine systems, they mainly originate from natural sources, primarily the degradation of organic matter and exudates from phytoplankton and microorganisms, while anthropogenic contributions can enter the environment through wastewater containing detergents, cleaning agents, and industrial effluents. These organic compounds can adsorb onto electrode surfaces, altering peak shapes, elevating background currents, and biasing estimates of metal concentrations and speciation. Previous studies have demonstrated that organic substances can significantly interfere with the voltammetric detection of Cr, Pt, and Cu in complex marine matrices, underscoring the necessity of addressing such interferences while simultaneously accounting for metal-specific chemistry.

The aim of this doctoral research was to develop and refine electroanalytical methods capable of reliable determination of Cr, Pt, and Cu in natural estuarine waters and to apply these methods to investigate the spatial and temporal dynamics of these metals in contrasting estuarine settings. Methodological improvements were achieved through careful optimization of sample preparation and analytical conditions, effectively minimizing interferences from natural organic matter. Collectively, these developments demonstrate that integrating strategies specific to each metal chemistry allows robust, reproducible, and sensitive electrochemical measurements in complex estuarine matrices. Environmental application of refined methods provided new insights into metal cycling, highlighting the distinct controls on Cr, Pt, and Cu. Seasonal field studies were conducted in two Mediterranean estuaries with contrasting environmental settings. The Krka River estuary in Croatia, a pristine karstic system with minimal anthropogenic impact, low suspended particulate matter, and naturally low metal concentrations, was monitored to assess seasonal variations in Cr and Pt behaviour. In contrast, the Arno River estuary in Italy, strongly influenced by industrial, urban, and agricultural activities, with turbid waters, high dissolved organic carbon (DOC), and elevated trace metal inputs, was studied to evaluate seasonal Cu dynamics. Comparative analysis placed these findings within a global context, highlighting how environmental setting and seasonality shape estuarine trace metal behaviour.

By integrating method development with environmental applications, this research advances marine electrochemistry and estuarine trace metal biogeochemistry, providing a framework for reliable metal speciation measurements in estuarine waters, and offering insights into trace metal dynamics with both local and global relevance.

2. LITERATURE OVERVIEW

2.1 Trace metals in seawater

Metals occur naturally in marine waters which contain virtually every chemical element across an exceptionally broad concentration range, from ~0.6 mol L^{-1} down to ~10^{-14} mol L^{-1} (Mason, 2013). Trace metals typically fall below 10^{-8} mol L^{-1} and include biologically important species such as iron (Fe), Cu, or zinc (Zn), which can become toxic at elevated levels, as well as particularly hazardous metals, such mercury as (Hg), or lead (Pb). Trace metals in natural waters appear in multiple species and in a range of physicochemical forms. By elemental "species" we refer to groupings distinguished by isotopic composition, oxidation state, or the molecular/complex structure (Templeton and Fujishiro, 2017). Speciation describes how a given element is apportioned among its distinct chemical species in a particular system. In seawater, those species include simple cations (e.g., Cu^{+}/Cu^{2+} or Fe^{2+}/Fe^{3+}), oxyanions (e.g., chromate, CrO_4^{2-}), and oxycations (e.g., uranyl ion, UO_2^{2+}), which can exist as free hydrated ions or as organic and inorganic complexes (Bruland and Lohan, 2003). For example, in inorganic form, cadmium (Cd), Hg, Pt, palladium (Pd), and silver (Ag) are dominated by chlorides, whereas Cu, yttrium (Y), Pb, and lanthanoids are dominated by carbonates (Bruland and Lohan, 2003). For some metals, organic speciation is more important for assessing their marine behaviour and environmental effects, such as Cu and Fe whose dissolved fraction is dominated by organic ligands (80–99%), governing their bioavailability (Gledhill and Buck, 2012; Hollister et al., 2021; Jacquot et al., 2014), or for Hg whose most toxic form is methylmercury, which, unlike inorganic Hg, can cross biological membranes, including the blood-brain barrier, leading to neurotoxic effects (Jeong et al., 2024; Wu et al., 2024). For others, redox speciation is driving their fate and effects in marine environment, such as Cr whose toxicity to marine biota depends on it redox state, with Cr(VI) being highly toxic and Cr(III) typically regarded as less bioavailable and also less reactive intracellularly (Qambrani et al., 2016; Singh et al., 2023). Fractionation, in contrast, refers to separations based on physical (e.g., particle size, solubility) and chemical (e.g., binding type, reactivity) properties (Templeton and Fujishiro, 2017). In practice, metals in natural waters are divided by size into particulate and dissolved, operationally separated using a 0.2 µm filter defined by GEOTRACES protocols (Cutter et al., 2017), while species smaller than about 1 kDa are defined as truly dissolved (Wells, 2002) (although often cut-off between 3–10 kDa are used; Marcinek et al., 2022) and are separated from colloidal fraction (1 kDa–0.2 µm) most often by tangential or centrifugal ultrafiltration (Marcinek et al., 2022; Schlosser et al., 2013). Metals within the particulate and colloidal pools are adsorbed to or incorporated within organic and

inorganic particles, whereas dissolved forms occur as free hydrated ions or as complexes with organic or inorganic ligands. This partitioning, encompassing the distinction between species and fractions, the operational size-based definitions, and the modes of metal occurrence in particulate, colloidal, and truly dissolved pools, is synthesized and illustrated in **Figure 1**.

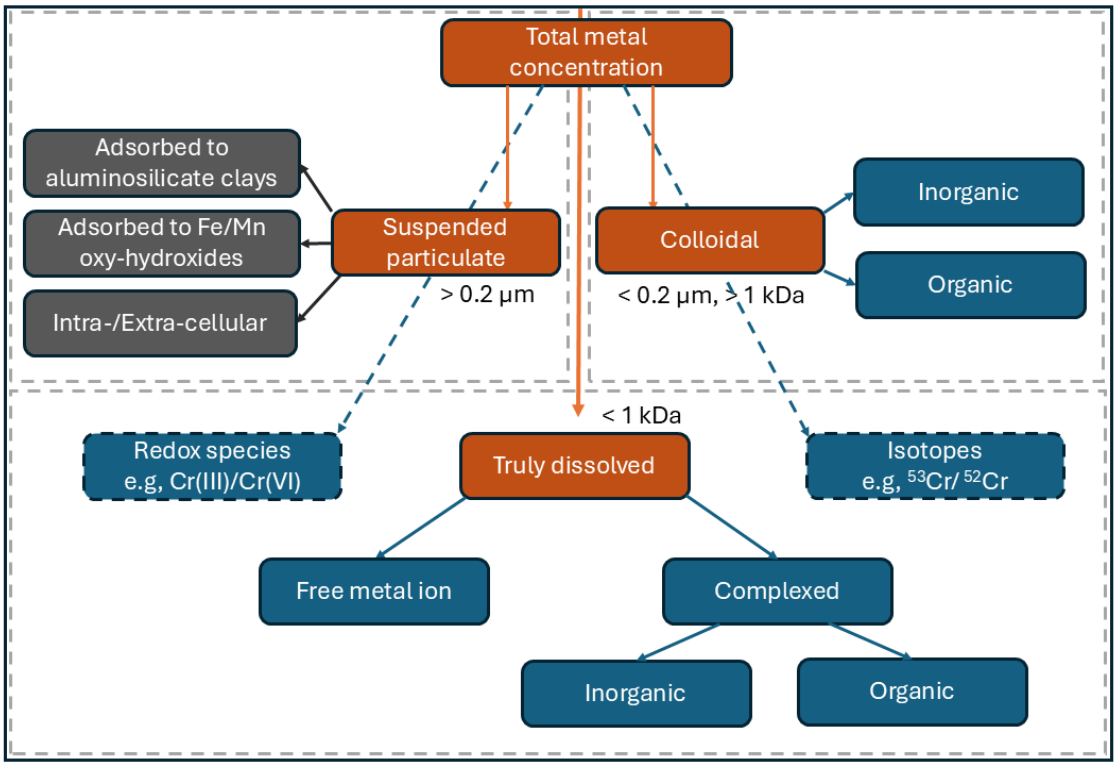

Figure 1. Metal speciation (blue) and fractionation by size (orange) and binding type (grey) in seawater.

Metal ion concentrations exceeding natural background levels in coastal waters arise mainly from human activities, though certain natural processes such as continental runoff, volcanic and hydrothermal activity, atmospheric deposition, and sediment resuspension also contribute. Since the mid-20th century, anthropogenic inputs, including mining, transportation, industrial and municipal wastewaters, agriculture, sewage discharge, landfills, and tourism, have become the dominant sources. Unlike organic pollutants, trace metals are non-degradable; once introduced in marine environment, their cycling is controlled by complex biogeochemical processes and physical mixing. Over time, metals accumulate in sediments and biota, serving as indicators of anthropogenic contamination and posing risks to aquatic ecosystems and, indirectly, to human health. Sediments act as both the main sink and a potential secondary source, depending on changes in redox conditions or ligand breakdown. Removal from the water column occurs through biological uptake, precipitation and scavenging, but resupply can

take place via remineralization or desorption. As outlined earlier, metal toxic effects are governed by their chemical speciation dictating not only their cycling, but also their bioavailability and biological effects. In the following text three specific metals that are focus of this thesis are described in detail, including (i) their sources, (ii) processes driving their behaviour in the marine environment, and (iii) resulting environmental implications.

2.1.1 Chromium in marine environment

2.1.1.1 Sources

Naturally, Cr is present in soils, rocks, and sediments, from which it may be mobilized into surface waters through weathering, erosion, and runoff processes (Ao et al., 2025). Anthropogenic contributions dominate in many coastal settings and arise from industrial applications such as electroplating, ferrous and nonferrous alloys, refractories, pigments for paints and inks, leather tanning, corrosion inhibitors, drilling muds, textile dyes, catalysts, and wood and water treatment chemicals (Singh et al., 2023; Vaiopoulou and Gikas, 2020). Chromium emissions enter the atmosphere in particulate form and can be transported over varying distances before deposition onto aquatic surfaces via dry or wet precipitation, with particle size significantly affecting deposition rates (Bielicka et al., 2005). Local inputs are particularly important in estuaries, harbours, and other nearshore environments affected by anthropogenic effluents. Studies have shown that Cr concentrations in industrially contaminated water can be tens of thousands of times higher than natural background values and are a central driver of water quality degradation and bioaccumulation (Ao et al., 2025; Lazo, 2009; Lin et al., 2013).

2.1.1.2 Biogeochemical cycling

Once in seawater, the fate of Cr is largely dictated by its redox speciation and chemical interactions. In oxic, alkaline waters, Cr(VI) is thermodynamically favoured as the soluble chromate anion (CrO_4^{2-}), with Cr(VI)/Cr(III) ratios predicted to exceed 10^{21} (Elderfield, 1970). However, environmental observations indicate that Cr(III) can constitute a substantial fraction of dissolved Cr in surface waters, with ratios of Cr(VI)/Cr(III) in the range of 1.6–3.1 (Hirata et al., 2000), predominantly as hydrolysed species such as $Cr(OH)^{2+}$ and $Cr(OH)_3^0$ (Semeniuk et al., 2016). The presence of Cr(III) in surface is attributed to reduction of Cr(VI) mediated by multiple interconnected processes, including interactions with Fe(II), Cu(I), hydrogen sulphide, superoxide, and organic ligands such as oxalate, and citrate, as well as hydrogen peroxide, superoxide radicals, and other reactive oxygen species produced by biological

activity or light-driven reactions (Li et al., 2009, and references therein). For example, photochemical reduction of Cr(VI) is enhanced by transition metals complexed with organic ligands, such as Fe(III)-hydroxyl or Fe(III)-multi-hydroxyl-acid complexes and Cu(I), which absorb ultraviolet and visible light and facilitate electron transfer to Cr(VI) (White et al., 2003; Zhang et al., 2002). In addition, the ratio of Cr(III) to Cr(VI) has been observed to increase during periods of high biological productivity, reflecting the role of phytoplankton in facilitating reduction via exudates in the presence of light (Connelly et al., 2006). Conversely, Cr(III) can be oxidized to Cr(VI) in sediments or on particle surfaces, often mediated by manganese (Mn) oxides or Fe(III) (Zhang, 2000).

The behaviour of Cr in seawater is also strongly influenced by particle interactions and planktonic activity. Chromium(III) is highly particle-reactive, forming colloids and adsorbing onto suspended particulate matter, Fe-hydroxide minerals, and extracellular minerals associated with phytoplankton cells (Semeniuk et al., 2016). In contrast, Cr(VI) can weakly adsorb to mineral surfaces and remains largely in dissolved form. In coastal waters, plankton can remove Cr from the water column through uptake, adsorption onto cell surfaces, or incorporation into extruded particulate organic matter and faecal pellets (Connelly et al., 2006; Semeniuk et al., 2016). Flocculation with faecal pellets or detritus further enhances sediment incorporation of Cr, limiting its transport over long distances. Observations in the Sargasso Sea and the English Channel show correlations between particulate Cr and particulate organic carbon, indicating that phytoplankton-mediated export contributes significantly to Cr cycling (Auger et al., 1999; Connelly et al., 2006). In sediments, Cr may be remobilized under oxidizing conditions or through solubilization of Cr(III), contributing to pore water Cr concentrations and ongoing redox cycling. Its release from sediments is controlled by key mediators such as Mn oxides and organic matter, which regulate Cr redox transformations at the sediment-water interface (Liang et al., 2021; Tebo et al., 2004). The slow oxidative release of Cr(III), combined with the higher mobility of the Cr(VI) formed, makes sediments a continuous source of Cr to overlying waters (Ao et al., 2025; Bruggmann et al., 2019). However, systematic understanding of how sediment components influence Cr oxidation and its release remains limited.

2.1.1.3 Ecological implications

The biological effects of Cr in marine environments are strongly redox-dependent. Hexavalent Cr is highly toxic and a known carcinogen, capable of entering organisms via sulphate transporters under high Cr(VI) concentrations, whereas Cr(III) is much less bioavailable due

to its particle-reactivity (Ao et al., 2025; Qambrani et al., 2016). Some studies suggest that Cr(III) may be taken up by phytoplankton through internalization as well as surface adsorption (Semeniuk et al., 2016). In marine organisms, the high toxicity of Cr(VI) arises from its intracellular redox activity. Once Cr(VI) enters cells, it is reduced stepwise to Cr(V), Cr(IV), and finally Cr(III) by cellular reductants such as glutathione, ascorbate, and other thiol-containing molecules (Costa and Klein, 2006). This reduction generates reactive oxygen species, which can damage DNA, proteins, and lipids, leading to oxidative stress, metabolic disruption, and in some cases cell death (DeLoughery et al., 2014; Velma and Tchounwou, 2010). Chromium(VI) intermediates also form covalent adducts with nucleic acids, causing genotoxic effects, while Cr(III), already in a stable trivalent state, lacks such redox reactivity and produces minimal intracellular damage (Qambrani et al., 2016; Singh et al., 2023). Consequently, Cr(VI) poses a far greater threat to marine phytoplankton, zooplankton, and other coastal biota than Cr(III), even at similar cellular concentrations. In conclusion, the combined effects of redox transformations, photochemical reactions, organic complexation, particle adsorption, and biological interactions determine the bioavailability and toxicity of Cr in coastal ecosystems, influencing both the exposure of marine organisms to Cr(VI) and the removal of Cr from the water column via sedimentation.

2.1.2 Platinum in marine environment

2.1.2.1 Sources

Platinum is one of the rarest elements in the Earth's crust, with an average abundance of ~0.5 ng g^{-1} (Rudnick and Gao, 2003). It is introduced into the oceans through a variety of natural processes that can be broadly categorized as endogenous, exogenous, and extraterrestrial (Berezhnaya and Dubinin, 2024). Endogenous contributions are linked to volcanic and hydrothermal activity, while exogenous inputs include riverine and glacial runoff, coastal abrasion, atmospheric deposition, and aeolian dust transfer. Of these, suspended and dissolved material carried by rivers is thought to represent the most significant natural exogenous source of Pt to the ocean, although uncontaminated rivers generally exhibit sub-picomolar concentrations of dissolved Pt (Cobelo-García et al., 2013; Pađan et al., 2020; Soyol-Erdene and Huh, 2012). Extraterrestrial delivery represents another, albeit minor, natural source; estimates based on meteorite fluxes and Pt content in chondritic meteorites suggest a cosmogenic input of ~29 kg yr^{-1} (Soyol-Erdene and Huh, 2012). Aeolian transport of Pt-containing material contributes comparably little, at around 7 kg yr^{-1} (Soyol-Erdene and Huh,

2012). Altogether, these natural pathways establish low background flux of Pt to the oceans, reflected in natural dissolved concentrations in clean coastal environments averaging between 0.1 and 0.6 pmol L^{-1} (Cobelo-García et al., 2013; Pađan et al., 2020; Soyol-Erdene and Huh, 2012). Against this low natural baseline, anthropogenic activities have become the dominant driver of Pt inputs into marine, especially coastal environments. Current estimates suggest that over 80–85% of Pt fluxes in the environment are anthropogenic in origin (Sen and Peucker-Ehrenbrink, 2012). The largest single source is vehicular traffic, via the release of Pt and other platinum group elements from catalytic converters. These devices, while effective in reducing gaseous pollutants, release Pt-bearing particles through mechanical abrasion and thermal stress during vehicle operation (Artelt et al., 1999). The resulting nanoparticles are widely dispersed into the environment by urban runoff and atmospheric deposition, and are eventually transported into rivers, estuaries, and coastal waters (Abdou et al., 2020, 2016). Hospitals and sewage systems represent a second major anthropogenic source, linked to the extensive use of Pt-based anticancer drugs such as cisplatin, carboplatin, and oxaliplatin (Ravindra et al., 2004). These compounds are excreted by patients, entering hospital effluents and municipal sewage, and persist through wastewater treatment plants, thereby discharging directly into rivers and coastal seas. Platinum concentrations in such effluents can be exceptionally high, reaching up to 890 pmol L^{-1} (Abdulbur-Alfakhoury et al., 2021; Vyas et al., 2014), with documented riverine and estuarine concentrations exceeding natural riverine and oceanic levels by one to two orders of magnitude (Abdulbur-Alfakhoury et al., 2021; Monteiro et al., 2021). Other anthropogenic pathways include industrial effluents, urban stormwater runoff, and, indirectly, discharges from desalination facilities and wastewater outfalls, which increase Pt burdens in receiving waters (Panagopoulos and Giannika, 2024; Rowe and Abdel-Magid, 2020). These inputs make urbanized estuaries and coastal systems major global hotspots for Pt contamination.

2.1.2.2 Biogeochemical cycling

Despite the growing recognition of Pt as a contaminant of concern, its geochemical behaviour in seawater remains incompletely understood. Early studies of dissolved Pt in the Atlantic, Pacific, and Indian Oceans revealed inconsistent profiles, conservative, recycled-type, or scavenged-type, raising questions about methodological uncertainties and true basin-to-basin differences (Colodner et al., 1993; Goldberg et al., 1986; Jacinto and van den Berg, 1989; López-Sánchez et al., 2019). Once in the marine environment, Pt from anthropogenic sources can undergo transformation through interactions with natural organic ligands, inorganic

complexes, particle associations or biological activity, or can be influenced by other hydrodynamic and human perturbations (such as dredging) enhancing its mobility and bioavailability (Dahlheimer et al., 2007; Hollister et al., 2024). Estuarine studies have demonstrated that in estuarine mixing zones, Pt is predominantly present in dissolved species, with non-conservative increase along the salinity gradient due to stabilization by chloride complexation (Cobelo-García et al., 2013, 2008; Turner, 1996; Turner et al., 2007), while depth-related increases in coastal Pt concentrations suggest remobilization from sediments as another source to bottom waters (Hollister et al., 2024; Mashio et al., 2017). However, the paucity of estuarine and coastal field studies means that the key processes governing Pt cycling at land-ocean interfaces remain poorly constrained. Evidence for long-range atmospheric transport of Pt to remote environments, such as Greenland and Antarctic ice, underscores its persistence and potential for global redistribution (Frache et al., 2001; Soyol-Erdene et al., 2011). Nonetheless, it is in near-shore, urbanized coastal systems that Pt concentrations reach their highest and most ecologically relevant levels.

2.1.2.3 Ecological implications

The biological implications of Pt enrichment in marine systems are only beginning to be understood. Bioaccumulation of Pt has been demonstrated in variety of organisms in a field and laboratory experiments, including marine macroalgae (Abdou et al., 2023; Hodge et al., 1986), bivalves such as mussels and oysters (Abdou et al., 2023, 2019, 2018, 2016; Neira et al., 2015), gastropods, fish (Ruchter et al., 2015; Zimmermann et al., 2015), and even dolphins (Essumang, 2008). Time-series biomonitoring over two to three decades has revealed up to an eightfold increase in Pt concentrations in mussels, directly corresponding to the expansion of catalytic converter use (Abdou et al., 2019; Neira et al., 2015). Laboratory and field studies have also shown Pt to be toxic and genotoxic to plants and animals (Gagnon et al., 2006), though most experimental concentrations tested (5–50 μmol L^{-1}) were far above those encountered in natural waters. The degree to which environmentally relevant, sub-picomolar to several-picomolar concentrations of Pt affect marine biota remains unclear. At the base of the food web, phytoplankton play a central role in the cycling of trace metals, both by direct uptake and by transporting contaminants to depth via cell sinking and grazing pathways. However, no study to date has quantified Pt concentrations in phytoplankton, leaving a major gap in understanding potential entry points of Pt into marine food webs. Given its bioaccumulation in higher organisms and persistence in coastal systems, concern exists over the possibility of biomagnification and long-term ecological risks (Ruchter et al., 2015;

Zimmermann et al., 2015). Overall, while natural Pt levels in open ocean waters appear to pose limited ecological concern, the combination of anthropogenic enrichment, bioavailability, and evidence of accumulation in coastal biota highlights the urgent need for further investigation into its environmental and biological effects.

2.1.3 Copper in marine environment

2.1.3.1 Sources

Copper is a naturally abundant trace metal, present in the Earth's crust at concentrations ~50 $\mu g\ g^{-1}$. It enters marine systems through multiple pathways and displays a pronounced gradient between coastal environments and open ocean (Roy, 2000). Marine sediments can often accumulate more than 100 $\mu g\ g^{-1}$, and in seawater its concentration ranges from 0.2–4 nmol L^{-1} in the open ocean up to 100 nmol L^{-1} in coastal areas (Blossom, 2007; Dulaquais et al., 2020; Jacquot and Moffett, 2015; Whitby, 2016). Rivers are a dominant natural source of Cu to estuarine and coastal systems, although 20–40% of dissolved Cu may be removed during estuarine flocculation (Karbassi et al., 2013). Other important natural inputs include atmospheric deposition, resuspension from sediments, continental shelf margins, and hydrothermal systems, with the latter potentially contributing up to 14% of deep-ocean Cu (Little et al., 2014; Sander and Koschinsky, 2011). At the same time, a large fraction of Cu is removed during the formation of oxic sediments, which account for 80–90% of its long-term burial (Little et al., 2014). Since the mid-20th century, however, anthropogenic activities such as mining, industrial production, agriculture, and use of Cu-based antifouling paints on vessels and marine infrastructure have markedly increased Cu inputs into coastal waters (Amara et al., 2018; Carić et al., 2021; Lagerström et al., 2018). In semi-enclosed estuaries and harbours, where dilution and circulation are limited, Cu can accumulate to levels that approach or exceed regulatory thresholds (Buck et al., 2007; Moffett et al., 1997; Umbría-Salinas et al., 2021), raising concerns for ecosystem health. Together, these multiple sources establish coastal zones as hotspots for Cu enrichment and variability.

2.1.3.2 Biogeochemical cycling

Once in the marine environment, Cu undergoes complex transformations that determine its mobility, bioavailability, and eventual fate. In oxic seawater, Cu inorganic speciation is dominated by carbonate complexes (~60%), hydroxide (~32%), and chloride and sulphate forms, with only ~4% present as free Cu ions (Van Den Berg, 1984). Although Cu(II) is thermodynamically the most stable state (Turner et al., 1981), significant proportions of Cu(I)

have been measured in estuarine waters, with up to 89% (Buerge-Weirich and Sulzberger, 2004; Crmarić et al., 2024). The vast majority of dissolved Cu (> 99%), however is bound to natural organic ligands, which strongly control its behaviour (Arnone et al., 2024; Buck and Bruland, 2005; Ruacho et al., 2022; Vraspir and Butler, 2009). This strong complexation acts as a buffer that mitigates potential toxicity, although during high-input events the ligand-binding capacity can be saturated, leaving more labile thus bioavailable Cu species in solution (Buck et al., 2007; Moffett et al., 1997). These Cu-binding ligands are broadly categorised into strong (L1) and weak (L2) ligand classes based on the stability constants of resulting Cu-complex, which are operationally defined depending on the used measurement method (e.g., anodic stripping voltammetry, cathodic stripping voltammetry or ion-selective electrodes) (Bruland et al., 2000; Marcinek et al., 2025, 2021; Pađan et al., 2021; Town and Filella, 2000). The stronger, L1 ligands are often produced by biological activity *in situ*, potentially in response to elevated Cu concentrations, and include thiols such as glutathione, cysteine, and related compounds (Croot et al., 2000; Moffett and Brand, 1996; Ruacho et al., 2022; Whitby et al., 2017), as well as other low molecular weight compounds produced by microbes, such as methanobactin (Hakemian et al., 2005; Kim et al., 2004). The weaker, L2 ligands include humic substances (HS) from both, terrestrial and marine sources (Kogut and Voelker, 2001; Marcinek et al., 2025; Ruacho et al., 2022; Whitby and van den Berg, 2015). These ligand pools determine the balance between soluble and particulate Cu, influence redox dynamics, and buffer toxicity by keeping free Cu ions at very low levels (Crmarić et al., 2024; Marcinek et al., 2025; Tercier Waeber et al., 2012).

2.1.3.3 Ecological implications

The organic speciation of Cu directly regulates its dual ecological role, as it is simultaneously an essential micronutrient and a potential toxicant. At low concentrations, Cu is required for key enzymatic processes in photosynthesis, respiration, iron acquisition, and denitrification (Amin et al., 2013; Glass and Orphan, 2012; Peers and Price, 2006). However, when concentration of free Cu ions exceeds the buffering capacity of ligand pools, toxicity can occur. Toxic effects have been observed at free Cu concentrations as low as ~10 pmol L^{-1} for many phytoplankton and zooplankton taxa (Brand et al., 1986; Sunda et al., 1990, 1987), and even 2 pmol L^{-1} for some cyanobacteria (Mann et al., 2002), with sublethal toxicity manifesting in reduced growth, impaired reproduction, altered enzyme activity, and disrupted osmoregulation, and effects cascading through marine food webs. Sensitivity to Cu varies markedly across taxa, with microorganisms and primary producers among the most vulnerable (Jakimska et al., 2011; Kiaune and Singhasemanon, 2011). However, coastal species often display higher tolerance

due to regular exposure to fluctuating metal inputs, whereas open-ocean organisms are more prone to Cu limitation and sensitivity (Guo et al., 2010; Peers et al., 2005). Phytoplankton, cyanobacteria, and other microorganisms actively regulate Cu uptake, and in some cases excrete strong ligands to mitigate toxicity under elevated Cu conditions, observed in both laboratory and field studies (Croot et al., 2000; Moffett and Brand, 1996; Sander et al., 2015; Zitoun et al., 2019). Coastal environments are particularly vulnerable due to their proximity to anthropogenic sources and their dynamic conditions of salinity, organic matter, and light, which modulate Cu speciation and bioavailability (Marcinek et al., 2025; Pađan et al., 2021). Elevated Cu concentrations are frequently reported in harbours, shipyards, and other industrialized coastal settings (Buck et al., 2007; Liang et al., 2024; Lin et al., 2013; Schiff et al., 2007), while laboratory and field studies indicate that high Cu levels can negatively affect marine organisms, including effects of altered phytoplankton assemblages, stress responses in invertebrates, and biodiversity losses (Campbell et al., 2014; Hartland et al., 2019; Pérez et al., 2010; Yu et al., 2023). The strong control of ligands on free Cu levels highlights their central role in mitigating these impacts, yet due to spatial and temporal variability in ligand production and stability, Cu in these environments can switch readily between functioning as an essential micronutrient and exerting toxic effects (Buck et al., 2007; Mellett and Buck, 2020; Sander et al., 2015). This highlights the importance of understanding Cu speciation and bioavailability in coastal waters to better predict and mitigate its potential ecological impacts.

2.2 Voltammetric methodologies

Voltammetry refers to a group of electroanalytical methods in which information about analyte is obtained by measuring the dependence of current signal on applied potential, with peak currents occurring at characteristic oxidation/reduction potentials due to the redox reaction at the surface of the working electrode. The signal reflects the concentration of the analyte and thermodynamic parameters, such as standard potentials, as well as kinetic parameters, including diffusion rates, electron transfer rates, and the reversibility of the electrode process. Altogether this allows sensitive, selective, and quantitative analysis of a wide range of chemical species and reduction processes. Modern voltammetric measurements are usually performed in a three-electrode cell, consisting of a working electrode, a counter electrode to complete the circuit, and a reference electrode that maintains a constant potential and allows precise control of the working electrode (Borrill et al., 2019). Mercury electrodes are often used as working electrodes because of their practicality, particularly the hanging Hg drop electrode (HMDE)

and the Hg thin-film electrode (TFE). Their main advantages are the wide cathodic potential window and high hydrogen overpotential, which enable measurement of many reduction processes (Borrill et al., 2019). The HMDE also provides a renewable surface which is reducing fouling interferences. However, Hg has relatively low oxidation potential (+0.4 V vs. saturated calomel) restricting its use for metals with more positive formal electrode potentials. Other electrode materials, including gold (Au), Pt, and modified carbon electrodes are also employed (Borrill et al., 2019).

To achieve high sensitivity required for trace analysis, pulsed techniques, differential pulse (DP) and square-wave (SW) voltammetry are widely used. By recording current differences in response to potential pulses, these methods suppress background currents, enhance resolution, and lower detection limits to about 10^{-8} mol L^{-1} (Scholz, 2015). For even greater sensitivity, preconcentration-based stripping techniques are applied. In these methods, the analyte is first accumulated on the electrode surface, then removed (stripped) during the measurement step, producing a current signal related to its concentration. Depending on the mechanism of preconcentration and stripping, anodic stripping voltammetry (ASV) and adsorptive cathodic stripping voltammetry (AdCSV) can be distinguished. In ASV, labile metal species are preconcentrated by reduction into a Hg amalgam at potentials more negative than the half-wave potential and subsequently reoxidized during the stripping step. Due to this preconcentration step, the small sample volume is able to produce locally higher metal concentrations, increasing Faradaic currents and lowering detection limits. In AdCSV, a synthetic ligand is added to the sample to form an electroactive metal-ligand complex that adsorbs onto the electrode surface without undergoing redox reactions during preconcentration. The accumulated metal is then reduced by shifting the potential negatively. In ASV, accumulation conditions, such as potential, time, stirring, or electrode rotation, influence the amount of deposited metal and thus the sensitivity of the measurement, while in AdCSV, the sensitivity is primarily determined by the accumulation period and the stability of the metal-ligand complex. These approaches allow detection limits down to 10^{-12} mol L^{-1} (Scholz, 2015) and have been widely used since the 1970s for sensitive determination of trace metals in environmental and other aqueous samples (Plavšić et al., 1982; Šipoš et al., 1977). For achieving even lower detection limits, catalytic processes can be employed, in which the analyte participates in a redox cycle that amplifies the measured current. This approach has been successfully applied to metals, including Cr and Pt, where catalytic reactions on the

electrode surface enhance the stripping signal and allow detection of concentrations down to 10^{-15} mol L^{-1} (Pađan et al., 2020; Scholz, 2015).

2.2.1 Catalytic adsorptive cathodic stripping voltammetry for Cr redox speciation

Chromium speciation in natural waters requires careful consideration of sampling, preservation, preconcentration, and detection steps, as each can alter the original redox distribution of Cr species. Sample storage is particularly critical because Cr(III) and Cr(VI) exhibit very different stabilities under common storage conditions. While Cr(VI) is stable at natural pH of seawater (~8), especially if a CO_2 blanket is applied (Vercoutere et al., 1998), Cr(III) is rapidly removed from solution due to adsorption onto container walls within minutes to hours. Acidification, commonly used for metal preservation, can conversely induce reduction of Cr(VI) to Cr(III) via reactions with organic matter, complicating redox speciation analysis. The relatively low concentrations of Cr in aquatic systems (0.1–16 nmol L^{-1} in seawater, 0.5–100 nmol L^{-1} in freshwater) further challenge analytical determinations, which is reflected in the limited number of studies describing the actual distribution of Cr(III) and Cr(VI) in natural waters (Boussemart et al., 1992; Korolczuk and Grabarczyk, 1999; Li and Xue, 2001; Sander et al., 2003a; Sander and Koschinsky, 2000). Analytical methods most commonly applied for Cr redox speciation include high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC/ICP-MS) (Markiewicz et al., 2015) and catalytic AdCSV in the presence of diethylenetriaminepentaacetic acid (DTPA; H_5Y) (Bobrowski et al., 2009; Boussemart et al., 1992; Cuéllar et al., 2016; Espada-Bellido et al., 2013).

In AdCSV method, Cr redox speciation is based on the selective formation of electro-inactive complex which enables differentiation between Cr(VI) and Cr(III) through controlled complexation. Chromium(III) is initially present in solution as a stable hexaaquo complex which forms complexes with DTPA present as H_3Y^{2-} and H_2Y^{3-}, whereas Cr(VI), present as chromate, does not (Sander et al., 2003b). Upon addition of DTPA, a 1:1 Cr(III)-DTPA complex (**Fig. 2A**) forms within approximately 14 min, which is electrochemically active but shows limited adsorption onto the electrode. This 1:1 complex slowly reacts with a second DTPA ligand (H_3Y^{2-}) to form a 1:2 Cr(III):DTPA complex. In this complex, all six Cr(III) coordination sites are occupied, five by the first DTPA ligand and the sixth by a carboxylate group of the second ligand, effectively shielding Cr(III) and rendering it electrochemically inactive (Cuéllar et al., 2016; Sander et al., 2003b). The slow formation of this electro-inactive complex

underpins the selective determination of Cr(VI). Complexation can be accelerated by increasing the temperature (Grabarczyk and Korolczuk, 2003). Chromium(VI) is reduced irreversibly at about -0.05 V to Cr(III), which immediately forms a complex with the excess DTPA adsorbed on the electrode surface (Cuéllar et al., 2016) (**Fig. 2B**). The resulting Cr(III)-DTPA complex is further reduced to Cr(II) at about -1.2 V in a quasi-reversible reaction. Reoxidation to Cr(III) can be initiated by nitrate, which is reduced to nitrite in the process, resulting in a catalytic enhancement of the analytical signal (Cuéllar et al., 2016; Sander et al., 2003b).

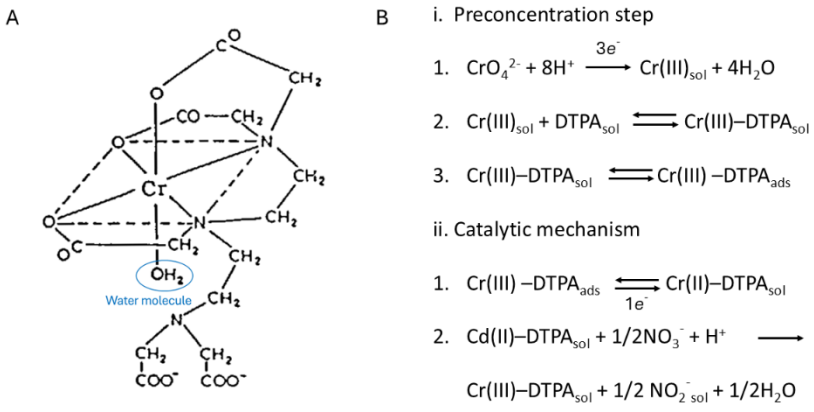

Figure 2. A) Cr-DTPA 1:1 complex (Bucci et al., 1991). B) Electrode mechanism (Cuéllar et al., 2016).

Solutions for this analysis are typically buffered at pH 5–6.5 to maintain complex stability and reproducible electrode response (Boussemart et al., 1992; Korolczuk and Grabarczyk, 1999; Li and Xue, 2001). Deviations from this pH range can reduce sensitivity due to incomplete complexation or altered adsorption kinetics. Total Cr is determined after 24 h of UV irradiation at acidic pH, which converts all Cr(III) to Cr(VI), while Cr(III) is calculated as the difference between total Cr and Cr(VI). Since Cr(VI) measurement has to be performed in non-UV-treated samples, which is common solution for decomposition of unwanted organic material, presence of SAS can interfere in measurements, which is an important challenge in Cr analysis in natural samples. In this thesis, particular attention was given to minimizing this influence (Pađan et al., 2019).

2.2.2 Catalytic adsorptive cathodic stripping voltammetry for Pt measurements

The determination of Pt in natural waters represents a significant analytical challenge due to the extraordinarily low concentrations at which this element typically occurs, necessitating the application of analytical techniques that combine both extremely high sensitivity and selectivity in order to accurately quantify trace levels of Pt in complex matrices. Among the most widely employed approaches, inductively coupled plasma mass spectrometry (ICP-MS) coupled with a pre-concentration step has proven to be effective through the preconcentration of Pt from large sample volumes prior to detection, thereby achieving detection limits in the range of 15 to 20 picomolar (Fischer et al., 2018; Mashio et al., 2017; Turetta et al., 2003), while catalytic-AdCSV has emerged as an equally powerful alternative, capable of reaching even lower detection limits in the femtomolar range, through the exploitation of a highly sensitive Pt catalysed hydrogen reduction process at the Hg electrode surface (Cobelo-García et al., 2014b, 2014a, 2013; Obata et al., 2006; van den Berg and Jacinto, 1988). Although linear scan voltammetry was the first technique applied for this method (van den Berg and Jacinto, 1988), square wave (Locatelli, 2005) and differential pulse voltammetry have also been successfully employed (Cobelo-García et al., 2014a).

The voltammetric approach relies fundamentally on the formation of a stable complex between Pt(II) and formazone, a reagent generated *in situ* through the reaction of formaldehyde with hydrazine (**Fig. 3A**). This reduces present Pt(IV) species to Pt(II), therefore no discrimination between them is possible. The resulting Pt(II) reacts with formazone through the amine group to form a stable complex that adsorbs onto the surface of the Hg electrode, where Pt(II) is reduced to metallic Pt(0). It was suggested that the Pt(II)/Pt(0) reduction potential coincides with the hydrogen reduction current, and that active catalytic sites of formed Pt(0) decrease the hydrogen reduction overpotential (van den Berg and Jacinto, 1988; Zhao and Freiser, 1986), which would explain the drop in hydrogen production, generating the characteristic catalytic voltamogram during a cathodic scan, with well-defined current peak whose height is proportional to the amount of complex accumulated on the electrode (**Fig. 3B**). Sensitivity can be further enhanced by extending the preconcentration time of the complex on the electrode surface, with longer accumulation periods, even up to 20 to 30 minutes (Grabarczyk and Korolczuk, 2003).

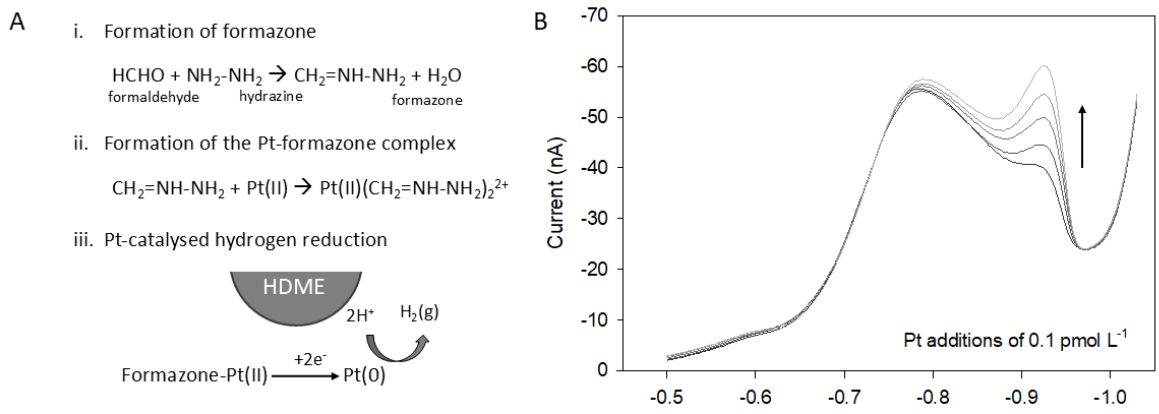

Figure 3. A) Solution and electrode mechanism schematics (Cobelo-García et al., 2014b; van den Berg and Jacinto, 1988). B) Voltamograms of hydrogen reduction in presence of Pt-formazone complex (Pađan et al., 2020).

The sensitivity and reliability of the method, however, critically depend on the careful optimization of chemical and experimental conditions, including reagent concentrations, acidic medium, and sample preparation. The formation of the formazone complex is highly sensitive to the concentrations of formaldehyde and hydrazine, with studies indicating that the peak increases sharply with increasing reagent concentration up to approximately 0.012% formaldehyde and 0.0015% hydrazine, beyond which further addition produces negligible enhancement, although the presence of excess ligand can generate a broad background wave, which may interfere with accurate quantification (van den Berg and Jacinto, 1988). The analytical performance of the voltammetric method is also strongly influenced by the composition and concentration of the acidic medium, with sulfuric acid producing an increase in peak height when present in concentrations up to 0.5 mol L^{-1} before plateauing, while hydrochloric acid shows a more complex behaviour, in which concentrations above 0.3 nmol L^{-1} can lead to decreased response, an effect that does not appear to be directly related to chloride complexation given the high chloride concentration typical of seawater (van den Berg and Jacinto, 1988). Prior to measurement, the removal of organic matter from the sample is crucial, as the presence of even trace amounts of organic compounds can substantially suppress the catalytic hydrogen signal (Cobelo-García et al., 2013; Obata et al., 2006). This is commonly achieved through UV irradiation, which degrades the majority of organic material in the sample; however, the process is not always completely effective, and traces of residual organics can still interfere with the analysis, an effect that was specifically addressed in this thesis (Pađan et al., 2020).

2.2.3 Differential pulse anodic stripping voltammetry for Cu speciation

Voltammetric methods are widely used to investigate Cu organic complexation in natural waters, providing a means to assess ligand capacity to bind free Cu at elevated concentrations (Han and Pan, 2021; Pižeta et al., 2015). Voltammetric methods allow direct measurement of Cu organic complexation by titrating a sample with Cu at natural pH, assuming that different ligands can be represented by average properties, such as conditional stability constants. Complexation capacity of natural ligands in the sample is expressed in Cu equivalents, and it is a function of the total concentration of ligand classes (L1, L2, …) and their conditional stability constants with Cu (logK') at given pH and salinity. Obtained titration curves are mathematically transformed to determine these complexation parameters (Pižeta et al., 2015) (**Fig. 4**), while software, such as ProMCC, facilitates the processing of complexometric data (Omanović et al., 2015).

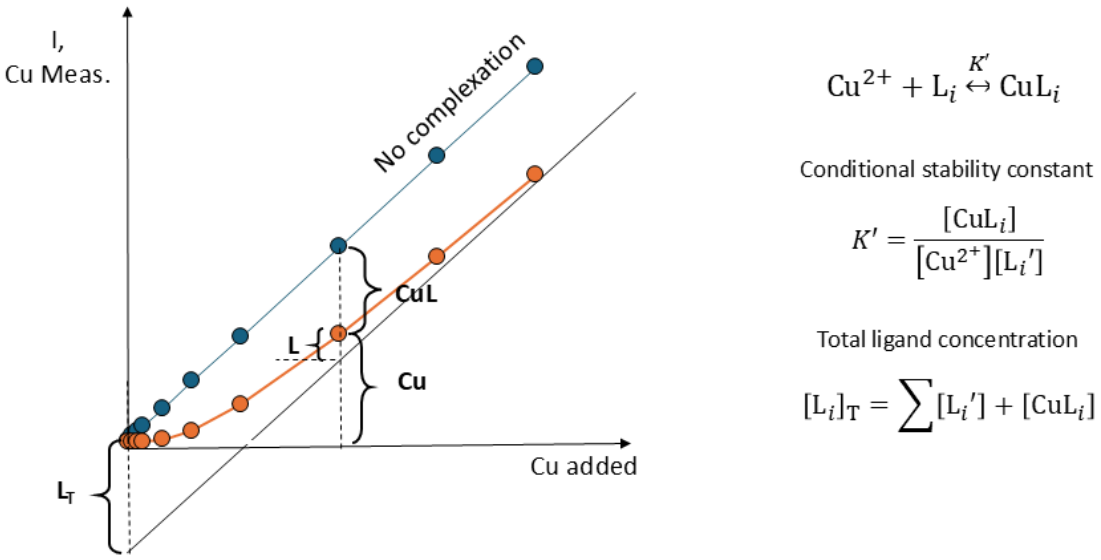

Figure 4. Example of titration curve and simplified mathematical process of complexing parameter calculation.

It is important to note that different voltammetric approaches capture different fractions of ligands, defined by a "detection window" that depends on the thermodynamic and kinetic properties of the complexes (Bruland et al., 2000; Town and Filella, 2000). While AdCSV combined with competitive ligand exchange utilizes synthetic ligand for competition with natural ligands in the sample, making the signal straightforwardly assignable to that complex, in complexometric ASV experiments, the current arises from free Cu ions at the electrode

surface, those that are diffusing directly from solution as well as those released from labile complexes in the diffusion layer, hindering the determination of the Cu species that actually contribute to the current. In seawater, species contributing to reduction include free Cu and labile complexes with small inorganic ligands as well as labile organic ligands such as citrates or fulvic acids (Gibbon-Walsh et al., 2012; Omanović and Branica, 2004; Town and van Leeuwen, 2019). To prevent reduction of organic complexes, the accumulation potential must be set so that all free Cu and inorganic complexes are reduced while all organic complexes remain electro-inactive (Omanović et al., 1996), however, some unwanted reduction is unavoidable, leading to slight underestimation of complexation parameters and consequently overestimation of free Cu (Dulaquais et al., 2020). Despite this, ASV is considered a reliable indicator of bioavailable Cu (Sánchez-Marín, 2020 and references therein), although, recent studies have demonstrated that this is only accurate at elevated Cu concentrations (> 20 nmol L^{-1}) (Barber-Lluch et al., 2023). Alternative techniques, such as diffusive gradients in thin films (DGT) or ion-selective electrodes (ISE), may provide complementary assessments under specific conditions (Cindrić et al., 2020; Marcinek et al., 2021; Strivens et al., 2019; Umbría-Salinas et al., 2021).

Pseudopolarography, or more correct term being, scanned stripping voltammetry /chronopotentiometry, can be used to study electrodeposition processes more directly by generating pseudopolarograms (Branica and Lovrić, 1997; Branica et al., 1977; Komorsky-Lovrić et al., 1986; Lovrić and Branica, 1980; Omanović and Branica, 2004). These are constructed by performing a series of voltamograms at successive accumulation potentials and plotting peak signal versus potential, producing waves analogous to classical polarographic signals (**Fig. 5**). Complexation of metal with a ligand causes a shift to more negative potentials in the polarographic wave relative to the free metal ion. The magnitude of this shift is directly related to the stability of the complex and the concentration of the complexing ligand. Each pseudopolarographic wave corresponds to a Cu-ligand complex (or a group of complexes with similar stability if continuous increase is observed) while electrochemically inert species do not produce waves but strongly decrease the limiting current of the pseudopolarograms. The position and shape of the waves reflect the stability of the complex and the reversibility of chemical and electron-transfer processes.

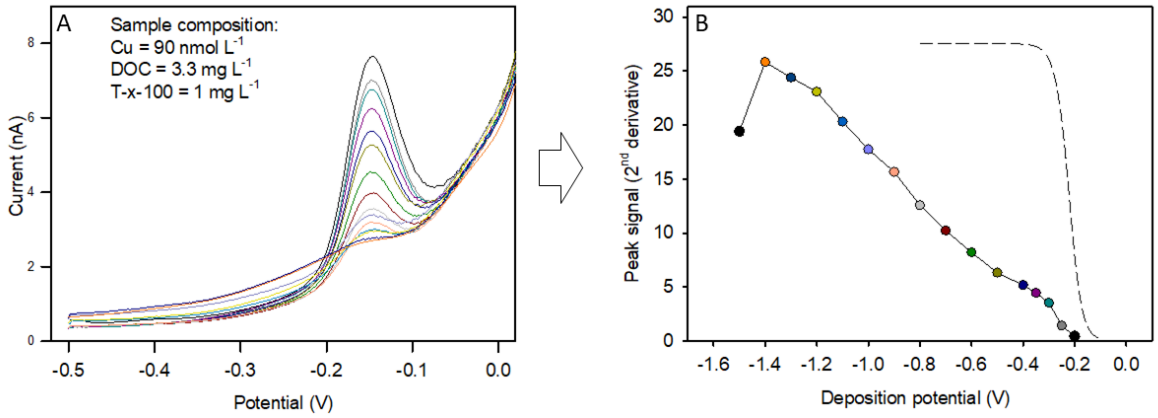

Figure 5. A) Voltamograms of Cu-amalgam oxidation obtained at different deposition potentials represented by different colours. B) Pseudopolarogram constructed from individual voltamograms in A in relation to pseudopolarogram obtained in organic-free sample (dashed line) (Pađan et al., 2021).

Overall, voltammetric techniques provide powerful, but method-dependent insights into Cu organic complexation in seawater, with each approach offering distinct advantages and limitations that must be carefully considered and possibly combined when interpreting bioavailability and stability of Cu-ligand complexes. Additional complementary approaches, such as UV/Vis spectrometry and fluorescence spectroscopy, allow indirect characterization of optically active fractions of DOM, providing insights into average aromaticity, molecular weight, and the presence of humic-, fulvic-, and protein-like substances (Marcinek et al., 2020; Santos et al., 2016; Yamashita et al., 2008). More direct analyses of molecular composition of DOM isolated from estuarine water are possible using techniques such as Fourier-transform infrared spectroscopy (FTIR) or nuclear magnetic resonance spectroscopy (NMR) (Alhassan and Aljahdali, 2021; Zhang et al., 2018), which can reveal the distribution of functional groups and molecular features of Cu-binding ligands, although, these approaches require specialized instrumentation and extensive sample preparation, while former offer rapid, cost-effective, and accessible insight into the DOM quality, making them particularly useful for routine studies. Incorporating such information alongside voltammetric data enhances our understanding of the sources, chemical identity, and binding characteristics of natural organic ligands, thereby providing a more comprehensive picture of Cu complexation in marine environments (Marcinek et al., 2025; Wong et al., 2019).

2.2.4 Advantages and limitations of voltammetric approaches

Voltammetric techniques are distinguished by their exceptional sensitivity and their capacity to resolve complex speciation patterns directly in natural waters. Unlike bulk concentration methods, they provide quantitative insight into the distribution of different redox species, bioavailable metal fractions, ligand interactions, and conditional stability constants, making them invaluable both for studies of biogeochemical processes and for environmental assessments where the chemical form of a metal, rather than its total concentration, determines reactivity and mobility. Nevertheless, the accuracy and reproducibility of voltammetric measurements are influenced by a variety of interfacial and kinetic factors. These limitations are arising primarily from their reliance on processes occurring at the electrode-solution interface. The measured current is highly sensitive to interfacial phenomena, such that subtle changes in the electrode surface or the immediate chemical environment can significantly alter the voltammetric response. Various processes may modify the electrode surface and thereby influence the voltammetric signal. By adsorbing onto the Hg electrode, presence of interfering species can alter capacitive currents and modulate Faradaic processes, with particularly pronounced effects during the stripping step. Adsorption of electroactive species is driven by interactions between the electron cloud of the Hg surface and active molecular moieties and is especially significant in the presence of aromatic compounds due to π-electron interactions, while electro-inactive species may either inhibit electron transfer by forming a blocking layer or facilitate it by mitigating electrostatic repulsion in the double layer. In stripping analysis, these interactions manifest as distortions of the voltammetric signal, including reduced peak currents, broadening or shifting of peaks, or the appearance of humps, complicating both quantification and speciation analysis. Among these interferences, the adsorption of SAS, which are ubiquitous in natural waters, especially in productive coastal areas, represents one of the most pervasive interferences. For example, HS, an important constituent of natural waters, contain abundant redox-active functional groups such as phenols, quinones, carbonyls, and carboxyls, endowing them with significant electron transfer capacity, a recently recognized property that has opened potential applications in environmental remediation, sensing, and energy storage (Ajjan et al., 2019; Nurmi and Tratnyek, 2002; Wang et al., 2023). It is upon adsorption at Hg electrode, HS can undergo partial, irreversible reduction, giving rise to this "pseudocapacitive current" (Wasiński et al., 2014). While this property is promising for direct electrochemical detection of HS (Marcinek et al., 2023), in trace metal AdCSV analysis it can act as an interference, elevating the background current, decreasing linear signal range and

masking and distorting signals of target analytes, as was evident in Cr measurements where increase of the baseline current due to this HS effect gradually masked Cr-DTPA reduction signal (**Fig. 6**) (Pađan et al., 2019).

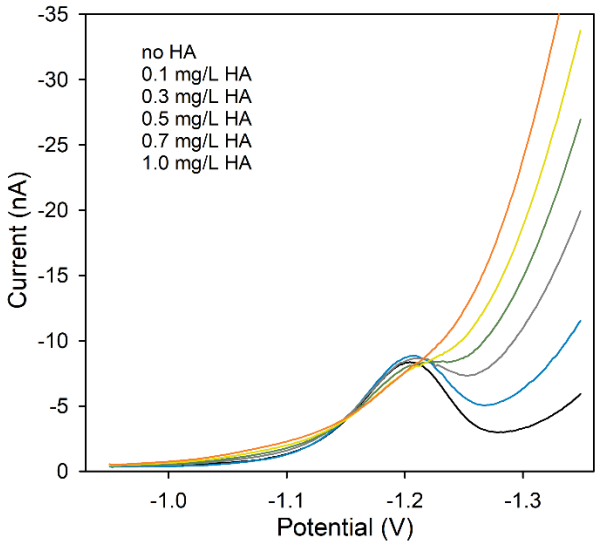

Figure 6. Influence of humic acid (HA) on Cr-DTPA reduction signal in AdCSV.

Other matrix constituents can also interfere with stripping voltammetry. Overlapping signals from multiple redox-active species, such as other metals, organic compounds, iodides, or sulphides, may produce merged or distorted peaks. These effects underscore the need for careful experimental design and data interpretation in complex natural matrices. In ASV, additional kinetic limitations arise from the preconcentration step. The measured signal depends not only on the thermodynamic properties of the analyte and its complexes, but also on the effective residence time of species within the diffusion layer during preconcentration step. This kinetic behaviour can be modulated by adjusting deposition potential, deposition time, and hydrodynamic conditions such as stirring, or electrode rotation, which control the thickness of the diffusion layer. Decreasing the diffusion layer thickness enhances the availability of labile species for electron transfer, improving both sensitivity and reproducibility. However, failure to account for the dynamic nature of ASV may lead to misinterpretation of derived complexation parameters, which reflect the combined influence of thermodynamics and kinetics rather than true equilibrium constants.

In summary, while stripping voltammetry provides exceptional sensitivity and detailed speciation information, its reliability is governed by interfacial and kinetic factors. Adsorption of SAS constitutes the primary limitation, but overlapping peaks, reactive matrix components,

and the kinetic behaviour also play critical roles. Proper understanding and control of these parameters are essential for obtaining accurate and interpretable data in complex matrices.

2.3 Environmental settings

Field studies were conducted in two contrasting Mediterranean estuaries: the Krka River estuary in Croatia, a near-pristine karstic system with naturally low organic matter input, and the Arno River estuary in Italy, a heavily modified system impacted by urban and industrial discharges with high organic matter loading. Trace metal behaviour in estuaries is governed by bio-physico-chemical factors including salinity, pH, water hardness, organic matter, colloids and competition from major cations (Tercier Waeber et al., 2012). Changes in salinity can cause colloid aggregation, promoting associated trace metal removal and sedimentation. Organic matter and Mn/Fe-rich colloids act as major carriers for metals, while biotic processes like metal uptake and microbial activity further affect their distribution. Some metals, however, can be released from colloidal aggregates due to chemical competition (e.g., Zn, Cd, and Pt affected by the interplay of divalent cations and chloride) (Cobelo-García et al., 2008; Turner, 2007) or released back into water due to sediment resuspension (Cindrić et al., 2015; Oursel et al., 2013). In Krka and Arno River estuaries seasonal variations in river discharge, biological activity, and anthropogenic pressures modulate these processes, creating dynamic patterns of trace metal cycling. These contrasting spatial and temporal conditions provide an ideal framework not only to examine how differences in hydrology, organic matter sources, and human activity govern trace metal cycling, but also to test and validate improved analytical approaches for metal detection and speciation across varying environmental settings.

2.3.1 Krka River estuary (Croatia)

The Krka River estuary, located on the eastern Adriatic coast of Croatia, is a 23.5 km long, permanently stratified system that extends from the Skradinski Buk waterfall through Prokljan Lake to the sea (**Fig. 7**). Its hydrology is controlled by karstic geology, with river flow ranging from 5 m^3 s^{-1} in late summer to 400 m^3 s^{-1} in spring, averaging 52.9 m^3 s^{-1} (Bužančić et al., 2016). Limited tidal influence (~30–40 cm) reinforces the persistence of a sharp halocline, typically located at 1.5–5 m depth, which acts as a physical barrier for vertical mixing and particle transfer (Cindrić et al., 2015; Legović et al., 1994, 1991; Marcinek et al., 2020). This interface is enriched with nutrients, trace elements and dissolved and particulate organic matter, including microorganisms of both freshwater and marine origin (Cauwet, 1991; Louis et al.,

2009; Marcinek et al., 2022; Viličić et al., 1989). Seawater renewal is highly dependent on river flow, ranging from 50–100 days during high river flow, and up to 250 days during low river flow, which can lead to occasional hypoxia in most upstream part of the estuary (Legović et al., 1991; Marcinek et al., 2020).

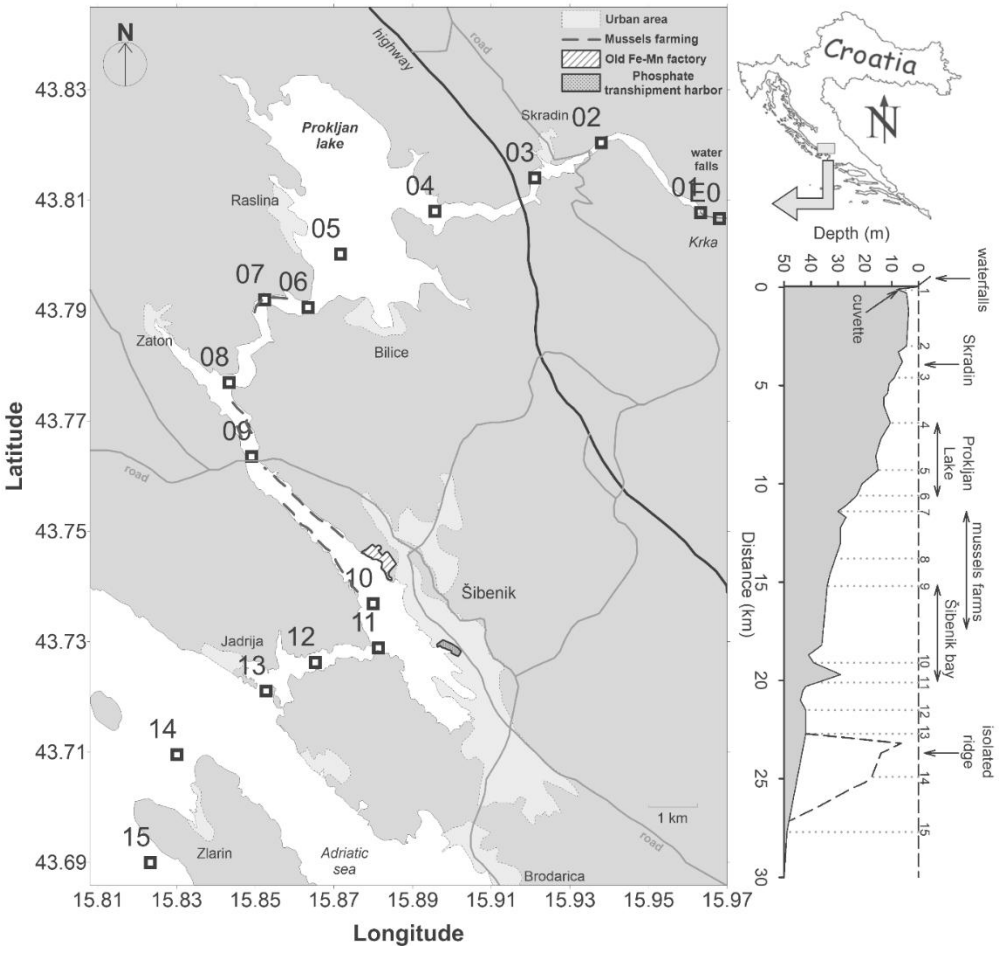

Figure 7. Map of the Krka River estuary with marked sampling stations (from the waterfall, station E0 to island Zlarin, station 15) and specific urban influences. The right panel shows the shape of the bottom depth with the indication of sampling stations and specific regions.

Owing to the river's self-purifying properties, low sediment and organic matter load, and the absence of heavy industry in the catchment, the Krka represents one of the cleanest estuarine environments, with riverine concentrations of DOC and trace metals such as Cd, Cu, Zn, Pb, and Pt generally lower than those in the adjacent coastal sea (Cindrić et al., 2015; Cukrov et al., 2008; Legović et al., 1994; Marcinek et al., 2020; Pađan et al., 2020). Good water quality supports mussel cultivation concentrated in the lower estuary (**Fig. 7**). Anthropogenic pressures are relatively limited, with localized influences from harbour activities in Šibenik and seasonal boating and tourism, which can affect water quality during the summer months

(Carić et al., 2021; Cindrić et al., 2015, 2020; Marcinek et al., 2025). Recent observations indicate seasonal restructuring of the DOM pool with scarce, yet humic-rich DOM dominating in winter and freshly produced DOM prevailing in summer, that modulates trace-metal complexation and potential bioavailability (Marcinek et al., 2020, 2025). Together, the interplay of karstic hydrology, strong stratification, DOM seasonality, and minimal but seasonally variable anthropogenic inputs creates a distinctive setting where trace metal distribution and cycling can be assessed in two contrasting conditions: high-flow winter periods reflecting near-pristine inputs, and biologically active summer months marked by touristic influences.

2.3.2 Arno River Estuary (Italy)

The Arno River, the fifth largest river in Italy, drains a basin of 8,228 km^2 and discharges into the Ligurian Sea approximately 10 km below Pisa (**Fig. 8**), forming a 12 km long, highly stratified estuary. Its hydrology is characterized by pronounced seasonal extremes, with flows ranging from as low as 6 $m^3\ s^{-1}$ in summer to peaks of 2000–3000 $m^3\ s^{-1}$ during winter and autumn flood events, with a mean discharge of about 82–100 $m^3\ s^{-1}$ (Retelletti Brogi et al., 2020). The river basin is dominated by low-permeability lithologies (clays, marls, sandstones, schists) that enhance the sensitivity of discharge to precipitation patterns (Cortecci et al., 2009; Dinelli et al., 2005). Along its course, the Arno is subject to significant anthropogenic pressures, particularly downstream of Florence, where inputs from paper mills, tanneries, textile industries, and electrochemical plants contribute organic matter, nutrients, and trace metals (Cortecci et al., 2009, 2002). Domestic and agricultural wastewaters, as well as effluents from industrial districts, such as the leather-processing area along the Usciana tributary, further elevate contaminant and particulate loads, leading to increased chemical oxygen demand, nutrient enrichment, and high concentrations of dissolved solids (Cortecci et al., 2002). These inputs are reflected in the estuary, which alone contributes about 7% of the total dissolved organic carbon flux to the western Mediterranean (Retelletti Brogi et al., 2020). In addition to riverine contamination, local sources such as anchored recreational and sailing vessels in the lower estuary represent potential contributors of Cu. Together, the combination of highly variable hydrology and intense anthropogenic pressures shapes the estuarine biogeochemistry, providing a complex and dynamic setting for the study of trace metal speciation.

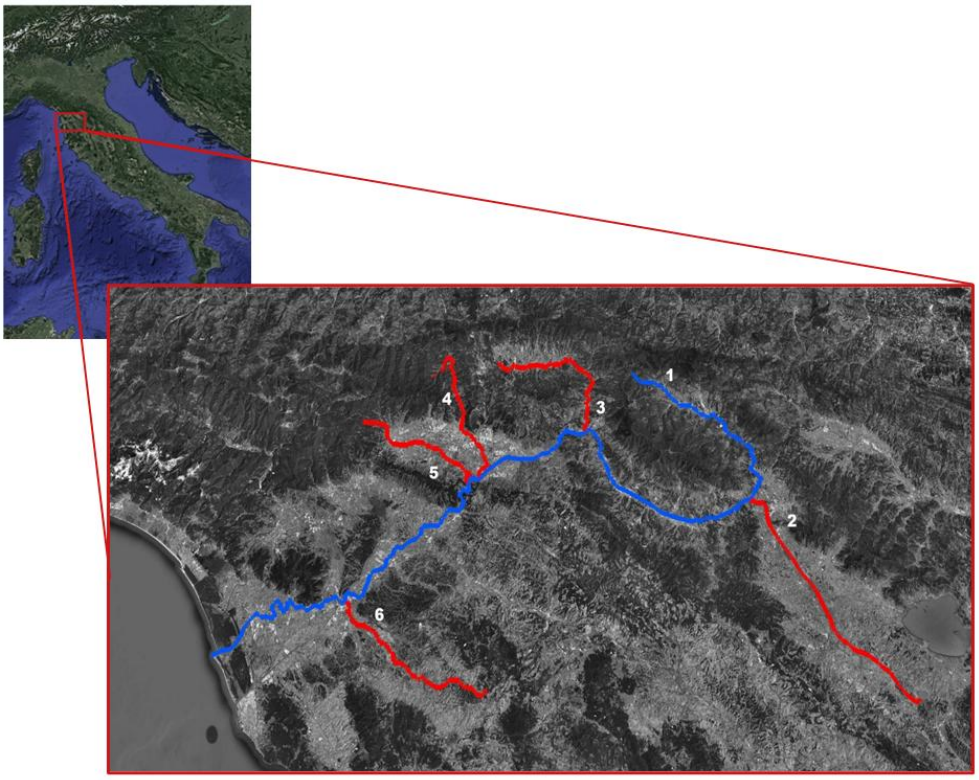

Figure 8. The Arno River basin, showing Arno River (1) and its main tributaries: Canale della Chiana (2), Sieve (3), Bisenzio (4), Ombrone (5) and Era (6). (Image source: Google Earth Pro).

3. OBJECTIVE AND HYPOTHESIS

The aim of this doctoral research is to develop and refine electroanalytical methods that allow reliable measurement of the concentration and speciation of Cr, Pt, and Cu, and to investigate their spatial distribution in estuarine settings across salinity gradients and contrasting seasons. The work focuses both on methodological robustness and on understanding how these metals behave under specific natural environmental conditions.

The research proceeds from several hypotheses. First, the reliability of electrochemical determinations for all three metals is strongly influenced by the concentration of natural organic matter and by how samples are prepared; however, by tuning experimental conditions and carefully preparing natural samples, these interferences can be fully removed or reduced to negligible levels. Second, in the Krka River estuary the ratio of Cr(III) to Cr(VI) should depend on physicochemical parameters, with redox speciation differing between winter and summer seasons, and with oxidized Cr, Cr(VI), prevailing and displaying non-conservative behaviour. Third, because the Krka River is characterized by low suspended matter, low organic matter, and presumably low anthropogenic Pt concentrations, Pt is expected to behave conservatively along the salinity gradient of this estuary. Fourth, anodic stripping voltammetry for Cu speciation is anticipated to be significantly affected by elevated DOC concentrations. Finally, in natural waters Cu is expected to bind predominantly to strong complexes with organic ligands, thereby reducing its toxicity, and optical characterization of organic matter should substantially aid in identifying the types of these ligands.

4. DISCUSSION

Marine electrochemistry has long recognized surface-active substances (SAS) as the dominant source of artefacts in voltammetric measurements of natural waters. These organic films adsorb onto Hg electrodes and compete for the same interfacial sites required for preconcentration and charge transfer, leading to well-known distortions: depressed or broadened peaks, sloping backgrounds that resist subtraction, and biased estimates of metal speciation. The research presented in this thesis addressed these challenges for three trace metals in sequence, with proposed solution to eliminate or strongly reduce the negative influence of SAS on their determination and speciation, each in a dedicated publication: (i) Cr, where the focus was on minimising SAS effects through deposition potential control; (ii) Pt, where method optimisation, by combining adjustment of instrumental parameters based on a short desorption step with a voltamogram processing, provided a desired effect of minimising SAS influence; and (iii) Cu, where nonionic surfactant that does not interfere with Cu chemistry was introduced as a masking agent for SAS adsorption onto the sensor surface. The first part of this discussion revisits the specific methodological improvements achieved in those studies and brings them together into a broader perspective on tackling SAS interference. The second part moves beyond method development to application, presenting new environmental observations from two contrasting estuaries: the Krka River estuary (Cr, Pt), a stratified karstic system with low terrestrial and anthropogenic input, and the Arno River estuary (Cu), a dynamic, turbid system heavily influenced by anthropogenic inputs and higher organic load. Here, the optimised approaches are used to resolve seasonal dynamics of Cr, Pt, and Cu, providing insights that extend the thesis beyond analytical refinement, offering new insights into cycling of these elements in estuarine systems and demonstrating the broader value of improved voltammetric methods in marine biogeochemistry. The final chapter integrates these findings, providing a comparative framework to understand how natural and human factors interact to shape trace metal dynamics in contrasting Mediterranean estuarine environments, alongside relevant global comparisons.

4.1 Advances in voltammetric methods for trace metal analysis

4.1.1 Mitigating SAS interference in Cr voltammetry

The optimization of voltammetric determination of Cr in natural waters revealed that the choice of deposition potential strongly influences both the signal intensity and the extent of interference from natural organic matter present in the sample solution. Initial tests, using a

common deposition potential of −1.0 V for the applied method, showed variable peak shapes and sensitivities across different estuarine samples, indicating that this potential was insufficient to overcome sample-specific effects. In contrast, applying a more negative potential, such as −1.65 V, consistently produced better-defined Cr peaks with improved stability. This observation aligns with previous reports suggesting that more negative deposition potentials enhance peak definition, particularly in the presence of interfering substances or when using vibrating amalgam electrodes (Espada-Bellido et al., 2013; Korolczuk, 2000; Korolczuk and Grabarczyk, 1999).

As mentioned in Section 2.2.4, the presence of humic acid (HA) increased the baseline current and gradually suppressed the Cr peak when common deposition potential of −1.0 V was applied, with complete peak disappearance at 1 mg L^{-1} HA (equivalent to ~0.5 mg L^{-1} DOC) (**Fig. 6**). This observation is consistent with prior reports that SAS can strongly distort voltammetric signals of Cr, as documented by Niewiara et al., 2007, who removed organic interference using a fumed silica column. However, at −1.65 V, the baseline remained largely unaffected, and the Cr peak was preserved, demonstrating that very negative potentials efficiently minimize SAS interference and allow accurate quantification of Cr(VI) even in organically rich samples (**Fig. 9A**). Therefore, determination at −1.0 V becomes unreliable in presence of high concentrations of organic matter, highlighting the importance of deposition potential in controlling matrix effects. However, findings indicate that negative deposition potentials minimize the impact of adsorbed organic substances, similar to what has been observed for Cu (Louis et al., 2008; Pađan et al., 2021) and Pt voltammetry (Pađan et al., 2020).

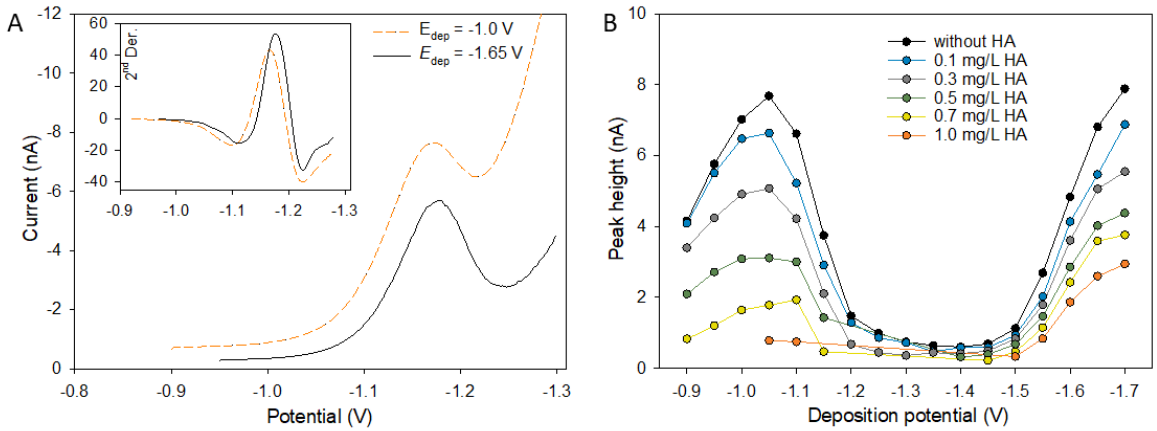

Figure 9. A) Voltamograms obtained at two contrasting deposition potentials with inset showing use of 2nd derivative transformation in peak quantification. B) Influence of deposition potential on Cr-DTPA reduction peak in presence of humic acid (HA).

The representation of the peaks signal intensities in relation to applied deposition potential provided further insight into the interplay between deposition potential and organic interference (**Fig. 9B**). Peak intensities initially increased when less negative deposition potentials were used, reaching a maximum around −1.05 V, then decreased sharply before recovering at the most negative potentials. At potential of −1.05 V, the peak vanished at the highest HA concentration of 1 mg L^{-1}, while the signal at −1.7 V remained. This U-shaped relationship mirrors observations reported in previous studies at vibrating amalgam electrodes (Espada-Bellido et al., 2013) and confirms that organic ligands suppress Cr deposition at intermediate potentials, whereas very negative potentials allow efficient Cr reduction at the Hg electrode surface. Similar patterns were observed in our estuarine samples across different salinities, suggesting that the phenomenon is generalizable across different environmental matrices, although with improved sensitivity and higher negative deposition potential limit in freshwater.

Although some decrease in peak height at deposition potential of −1.7 V was observed at the high HA concentrations (**Fig. 9B**), likely reflecting minor residual surface effects, the Cr peak remained well shaped and this effect did not compromise the overall reliability of the method. Further analysis obtained recoveries of ~100%, confirming that accurate Cr quantification was feasible at more negative deposition potential even in the presence of elevated HA concentrations. Occasional dislodgement of the Hg drop at very negative potentials was observed, but this did not affect the overall analytical performance due to short deposition times and replicate measurements, demonstrating that method robustness can be maintained under field conditions. Second-derivative transformations further enhanced peak clarity, reducing baseline curvature and allowing lower detection limits (**Fig. 9A**) (Cobelo-García et al., 2014b; Pađan et al., 2020).

Overall, these results confirm that deposition potential have important role in mitigating SAS interferences in Cr voltammetry. Very negative potentials improve peak resolution and reproducibility, enabling accurate Cr(VI) determination even in complex matrices, while data processing techniques such as derivative transformations enhance signal reliability. The observations are consistent with previous work on both Cr (Espada-Bellido et al., 2013; Korolczuk, 2000; Korolczuk and Grabarczyk, 1999) and other metals including Cu and Pt (Louis et al., 2008; Pađan et al., 2020), demonstrating that SAS can occasionally dominate voltammetric outcomes and that careful optimization of analytical parameters generates accurate trace metal analysis in natural waters.

4.1.2 Mitigating SAS interference in Pt voltammetry

The voltammetric determination of Pt in natural waters required careful optimization due to the expected ultra-low concentrations (< 1 pmol L^{-1}) and the steep baseline currents at the Pt peak position. Previous studies had established suitable concentrations of reagents, formaldehyde, hydrazine, and sulfuric acid (Cobelo-García et al., 2013; Obata et al., 2006; van den Berg and Jacinto, 1988), allowing the focus to be placed on optimizing stripping parameters and deposition potential. Optimization targeted not only the intensity of the Pt peak but also its resolution relative to the steep baseline, which is critical for accurate quantification at such low analyte levels. Variations in pulse and interval times, as well as modulation amplitude, were adjusted to improve peak definition, and a deposition potential of −0.65 V was selected as optimal from the graphs of peaks signal intensities in relation to deposition potential (**Fig. 10A**), balancing signal intensity and peak shape.

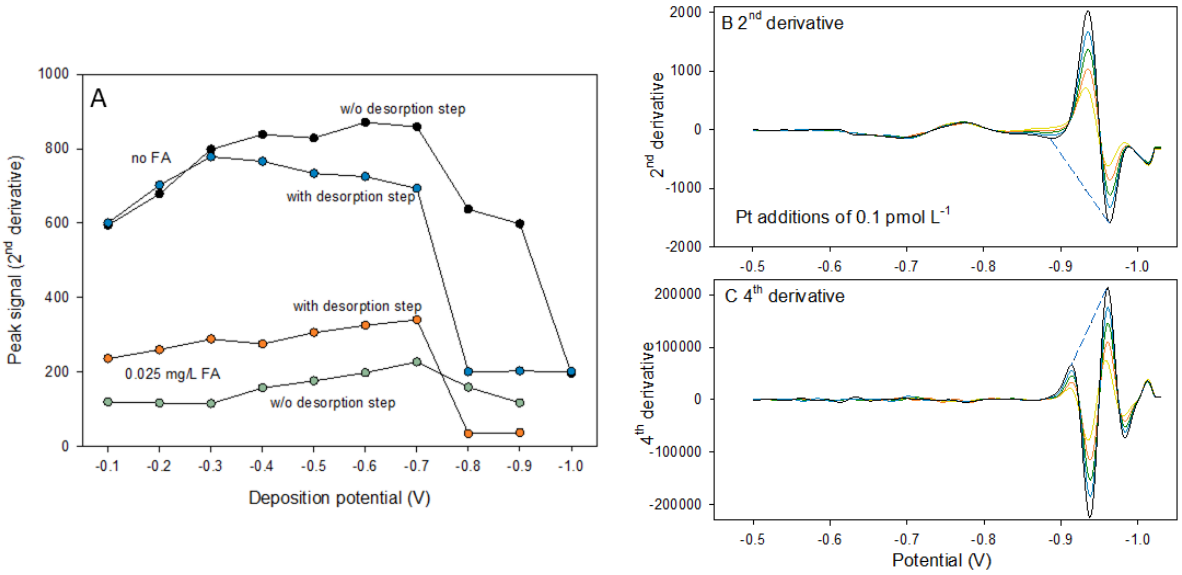

Figure 10. A) Influence of deposition potential and desorption step on Pt signal in presence and absence of fulvic acid (FA). Details of measurement listed within the figure. B/C) Use of derivative transformations in Pt peak quantification.

The presence of organic matter, even at very low concentrations, strongly influenced the voltammetric signal. Fulvic acid (FA) at 0.025 mg L^{-1} significantly suppressed the Pt peak (**Fig. 10A**), reflecting either adsorption of surface activity of FA on the electrode or its potential complexation of Pt(II), as proved possible by previous studies in seawater (Cobelo-García et al., 2013; van den Berg and Jacinto, 1988), although not confirmed especially at low pH conditions at which the method performs. To mitigate this interference, a brief 2-s desorption

step at −1.35 V was applied, following approaches previously used for Cu (Louis et al., 2008). The desorption step substantially improved peak resolution and intensity in the presence of FA, demonstrating that it can efficiently remove relatively low concentrations of SAS from the electrode surface (**Fig. 10A**). Standard addition experiments in MilliQ water confirmed the accuracy of the optimized procedure. Although the sensitivity in these experiments decreased with FA addition, the desorption step consistently improved analytical response compared with the procedure without desorption step, and recoveries for both procedures were around 103%. In natural seawater, the desorption step was essential to recover detectable Pt peaks along with UV irradiation treatment. UV irradiation is a necessary step in sample pretreatment for decomposition of organic matter in Pt voltammetric measurement, but its effect could be variable (sometimes insufficient), depending on the UV lamp type and intensity. Same as for Cr, derivative transformations of voltamograms further enhanced analytical performance. Both 2^{nd} and 4^{th} derivative treatments produced well-defined peaks, even at extremely low Pt concentrations, by flattening baseline curvature and reducing interference from background currents (**Fig. 10B**). The 4^{th} derivative provided a slightly better baseline and detection limits down to 10^{-15} mol L^{-1}, supporting its use in ultra-trace applications.

4.1.3 Mitigating SAS interference in Cu voltammetry

The voltammetric measurements of Cu speciation in samples with high DOM content (DOC > 2 mg L^{-1}) revealed the substantial influence of SAS on electrochemical signals. The absence of a well-defined Cu oxidation peak under these conditions reflected the strong complexation of Cu by organic ligands. However, the unusually high background currents observed suggested that the problem extended beyond simple complexation (**Fig. 11A**), pointing to significant adsorption of SAS at the electrode–solution interface. This interference complicated the direct interpretation of voltamograms and highlighted a key limitation of ASV when applied to organically rich waters, even when Cu was present at elevated concentrations (20–50 nmol L^{-1}). The introduction of a desorption step (at −1.5 V for 1–3 s) partially alleviated this issue and improved signal clarity, but it did not fully restore well-defined Cu peaks (**Fig. 11A**). This outcome emphasized that desorption alone is insufficient when organic matter content is high, as residual adsorbed SAS continued to perturb the stripping process. Even deposition potentials more positive than the Cu redox potential (where SAS is less adsorbed) did not fully restore peak definition. The introduction of the nonionic surfactant Triton-X-100 that does not affect Cu speciation, however, proved to be an effective approach for masking the SAS influence on

voltammetric response. By occupying the electrode surface without interacting with Cu in the bulk solution, Triton-X-100 enabled the recovery of narrow, well-resolved Cu peaks (**Fig. 11A**).

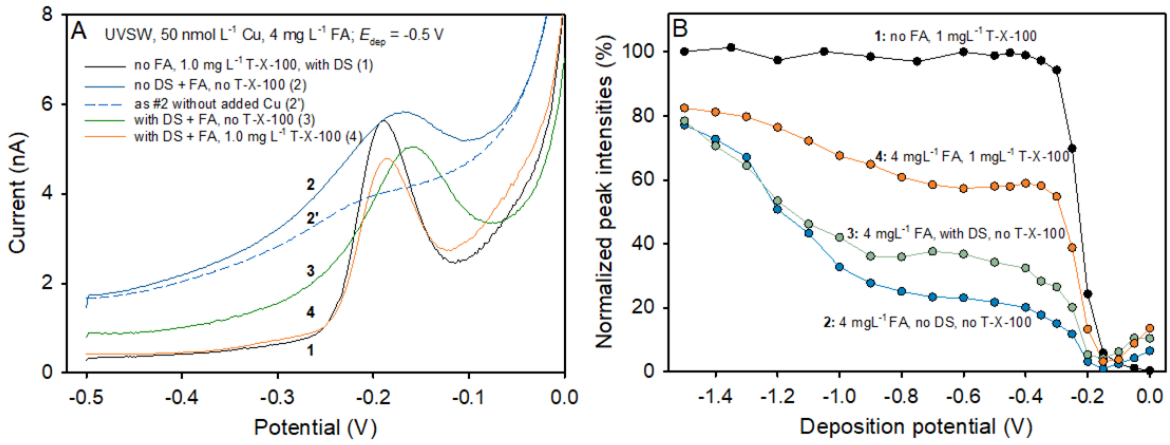

Figure 11. Influence of various scanning conditions (indicated within the figure) on voltamograms (A) and pseudopolarograms (B).

Addition of Triton-X-100 has been previously employed in other voltammetric studies to improve peak separation and signal quality for Cu and iodine (Krznarić et al., 1994; Luther et al., 1988; Omanović et al., 1996; Plavšić et al., 1994; Žic et al., 2012), confirming that surface-active additives can selectively suppress electrode-surface adsorption without altering bulk speciation. The significant improvement of the Cu signal with Triton-X-100 also confirmed that the broad, undefined peaks observed previously were primarily due to surface effects rather than inherent limitations of the electrochemical technique. Pseudopolarographic measurements in sample with high DOC (3.3 mg L^{-1}) revealed the complexity of Cu speciation using ASV with the shift of pseudopolarographic waves toward negative potentials, in respect with the sample free of organic matter (**Fig. 5B**). Namely, the progressive reduction patterns indicated the presence of multiple heterogeneous Cu-binding sites on DOM in the sample (Bi et al., 2013; Lewis et al., 1995; Pinheiro et al., 2020; Serrano et al., 2007), with steep increase of intensities at potentials more negative than −0.9 V suggesting the presence of very strong, irreversibly reduced, organic complexes (Branica and Lovrić, 1997; Gibbon-Walsh et al., 2012; Lewis et al., 1995).

Verification experiments in UV-digested seawater (UVSW) and in UVSW with 4 mg L^{-1} of fulvic acid (FA) (DOC equivalent of 2 mg L^{-1}) added as a model ligand, clarified the

mechanism of the observed effects. Voltamograms and pseudopolarograms obtained in UVSW confirmed that Triton-X-100 does not complex with Cu at the concentrations used (1 mg L^{-1}), confirming its inert behaviour in the bulk solution. In the presence of FA, addition of Triton-X-100 restored well-defined Cu peaks (**Fig. 11A**) and pseudopolarographic waveforms (**Fig. 11B**) much closer to those obtained in ligand-free conditions, effectively overcoming interference from adsorbed organic matter. Diminished effectiveness of Triton-X-100 at very negative deposition potentials suggested that either a portion of FA remained adsorbed during the deposition step or some strong Cu-FA complexes were irreversibly reduced, consistent with literature observations (Gibbon-Walsh et al., 2012; Town and Filella, 2000).

Overall, the differences between measurements conducted with and without Triton-X-100 underscored that electrode surface interactions can obscure the underlying speciation. Importantly, these results suggested that without addressing adsorptive interference, the determination of complexation parameters could be significantly biased, leading to overestimation of the stability of Cu complexes. The findings also implied that methods relying solely on deposition potential or desorption steps may fail in the presence of significant organic matter, and that integrating surface-active agents like Triton-X-100 is good measure to disentangle true Cu signals from surface artifacts. Moreover, in this study we highlighted the importance of pseudopolarography as a diagnostic tool to assess complexation heterogeneity and electrode interferences, providing a more nuanced understanding of metal-ligand interactions in complex environmental matrices. These results have broader implications for trace metal speciation studies, emphasizing that accurate characterization requires careful consideration of surface chemistry effects in addition to conventional complexation analyses.

4.1.4 Consolidating key method developments

The comparative examination of voltammetric determinations of Cr, Pt, and Cu highlights common challenges and methodological considerations inherent to trace metal analysis in natural waters, particularly regarding the influence of SAS and organic matter **(Table 1)**. Across all three metals, the increased presence of adsorbing organic matter significantly affected voltammetric signals, either by modifying the baseline, suppressing peak intensities, or altering peak shapes. These interferences, if not addressed, can compromise the reliability of analytical results and the extraction of metal speciation information. A central insight emerging from these studies is the effectiveness of carefully optimized deposition potentials in mitigating the impact of SAS, with pseudopolarograms proving as an effective guide.

Table 1. Summary of adapted electrochemical methods with associated preparation and operating conditions for measurement of Cr, Pt, and Cu.

Parameters	DP-AdCSV Cr	DP-AdCSV Pt	DP-ASV Cu
UV irradiation	Only for total Cr	Yes	No
pH	5.5 5 mmol L^{-1} 2-(N-morpholino) ethanesulfonic acid (MES)	< 2 600 mmol L^{-1} H_2SO_4	8.2 10 mmol L^{-1} borate/ammonia buffer
Reagents	1.25 mmol L^{-1} diethylenetriamine-pentaacetic acid (DTPA); 500 mmol L^{-1} $NaNO_3$	3.5 mmol L^{-1} formaldehyde; 0.45 mmol L^{-1} hydrazine sulfate	1 mg L^{-1} Triton-X-100
Deposition potential (V)	–1.65	–0.65	–0.55
Duration (s)	60	300–450	120
Desorption potential (V)	-	–1.35	–1.55
Duration (s)	-	2	3
Equilibration time (s)	5	5	5
Modulation time (s)	0.04	0.025	0.05
Interval time (s)	0.1	0.1	0.1
Potential scan (V)	–0.95 to –1.35	–0.5 to –1.03	–0.55 to 0
Step potential (V)	0.002	0.002	0.002
Modulation amplitude (V)	0.01 or 0.04	0.04	0.02

Three implementations reported in this thesis followed the same general pattern but emphasized different levers depending on the analyte's pathway and matrix constraints. First, for Cr, where analytical mechanism was catalytic-adsorptive and SAS competed within the conventional window, the deposition potential was shifted to a negative deposition regime where SAS were intrinsically less competitive, and buffer and deposition time were adjusted to ensure Hg-drop stability and repeatability, producing well-resolved, reproducible peaks even at environmentally relevant concentrations. Similarly, for Pt with the same analytical

mechanism and with detectability at ultra-trace levels as the primary limitation, the combination of an appropriately chosen deposition potential with a short desorption step efficiently removed low concentrations of adsorbing organics from the electrode surface, enhancing peak definition and reproducibility, while derivative processing of voltamograms for both methods improved method sensitivity. For Cu, SAS dominance was observed directly in the native matrix (wide background features; absent Cu peak), and previous steps of deposition potential adjustment and desorption step, on their own proved insufficient in most high-DOC samples. This led to the application of a controlled surfactant that does not interfere with Cu speciation and desorption step retained as a brief, non-disruptive safeguard, allowing accurate extraction of Cu peak intensities and enabling organic speciation analysis. These approaches illustrate that tailoring electrode conditions to the specific chemistry of each metal is a key factor in overcoming organic matter interference. These improvements build on previous studies that investigated SAS behaviour and trace-metal analysis under similar conditions: Case studies identified SAS as a major, strongly adsorbing component of seawater DOM and linked their adsorption to distorted, suppressed stripping responses (Louis et al., 2008). Studies of organic adsorption and catalytic mechanisms underscored that the deposition potential is a control parameter for the interfacial regime, enabling analysts to disfavour SAS while preserving the desired pathway (Gibbon-Walsh et al., 2012; Korolczuk, 2000; Korolczuk and Grabarczyk, 1999). Very short "excursions" to negative potentials were proposed to desorb these films and improve reproducibility without damaging the drop (Gibbon-Walsh et al., 2012; Louis et al., 2009, 2008). Finally, practical experience with nonionic surfactants in saline matrices established competitive adsorption as an operational tool that standardizes the interface without altering trace metal equilibria at analytical time scales (Krznarić et al., 1994; Omanović et al., 1996; Plavšić et al., 1994).

These studies collectively reinforce the importance of understanding the surface chemistry at the electrode interface and the bulk speciation of metals in the presence of organic ligands. Strategies such as desorption steps, competitive adsorption, or the selection of negative deposition potentials do not act uniformly across all metals but can be tailored to the specific electrode behaviour and complexation properties of each target element. When combined with careful calibration, replicate and control measurements, and optional UV treatment to remove residual organics (for total metal measurements), these methods enable robust, accurate, and reproducible voltammetric determinations even in samples with substantial DOC concentrations. Overall, the methodological improvements developed here for Cr, Pt, and Cu

highlight a general framework for enhancing voltammetric analysis in natural waters: (i) mitigation of SAS and organic matter effects through electrode treatment or solution modification, (ii) optimization of deposition and stripping parameters for well-resolved peaks, and (iii) use of derivative transformations of resulting voltamograms to reduce baseline interference and improve detection limits. By applying these strategies **(Table 1)**, reliable trace metal measurements can be achieved in complex environmental samples, supporting accurate assessment of metal concentrations and speciation.

4.2 Environmental applications: Insights into estuarine metals dynamics

The environmental applications focus on two contrasting estuarine systems along the Mediterranean: the Krka River estuary in Croatia, a karstic system with minimal anthropogenic influence and largely unaltered riverine waters, and the Arno River estuary in Italy, a heavily modified system shaped by industrial, agricultural, and urban pressures. Both systems are permanently stratified and exhibit pronounced seasonal variations in river discharge which are driving estuarine biogeochemistry, yet they differ fundamentally in the sources and magnitude of dissolved and particulate inputs. In the Krka, low sediment and organic matter loads, combined with minimal contamination, provide a setting in which natural hydrological and biogeochemical processes dominate trace metal behaviour, with only slight seasonal anthropogenic signal, mostly associated with Cu. In contrast, the Arno receives large inputs of organic matter, particulates, and trace metals from paper, textile, tanning, and electrochemical industries, as well as urban and agricultural runoff, resulting in a highly dynamic environment where anthropogenic pressures strongly modulate metal distributions. In both estuaries seasonal contrasts between high river flow winter/spring and low river flow summer periods are evident in biogeochemical parameters, reflecting variations in riverine inputs, water residence time, and biological activity.

4.2.1 Seasonally influenced Cr and Pt dynamics in the pristine Krka River estuary

The estuarine behaviour of trace metals in the Krka River estuary, represented here by Cr and Pt, was strongly shaped by seasonal hydrography and physicochemical conditions. Seasonal changes in salinity, temperature and oxygen are presented in **Fig. 12**.

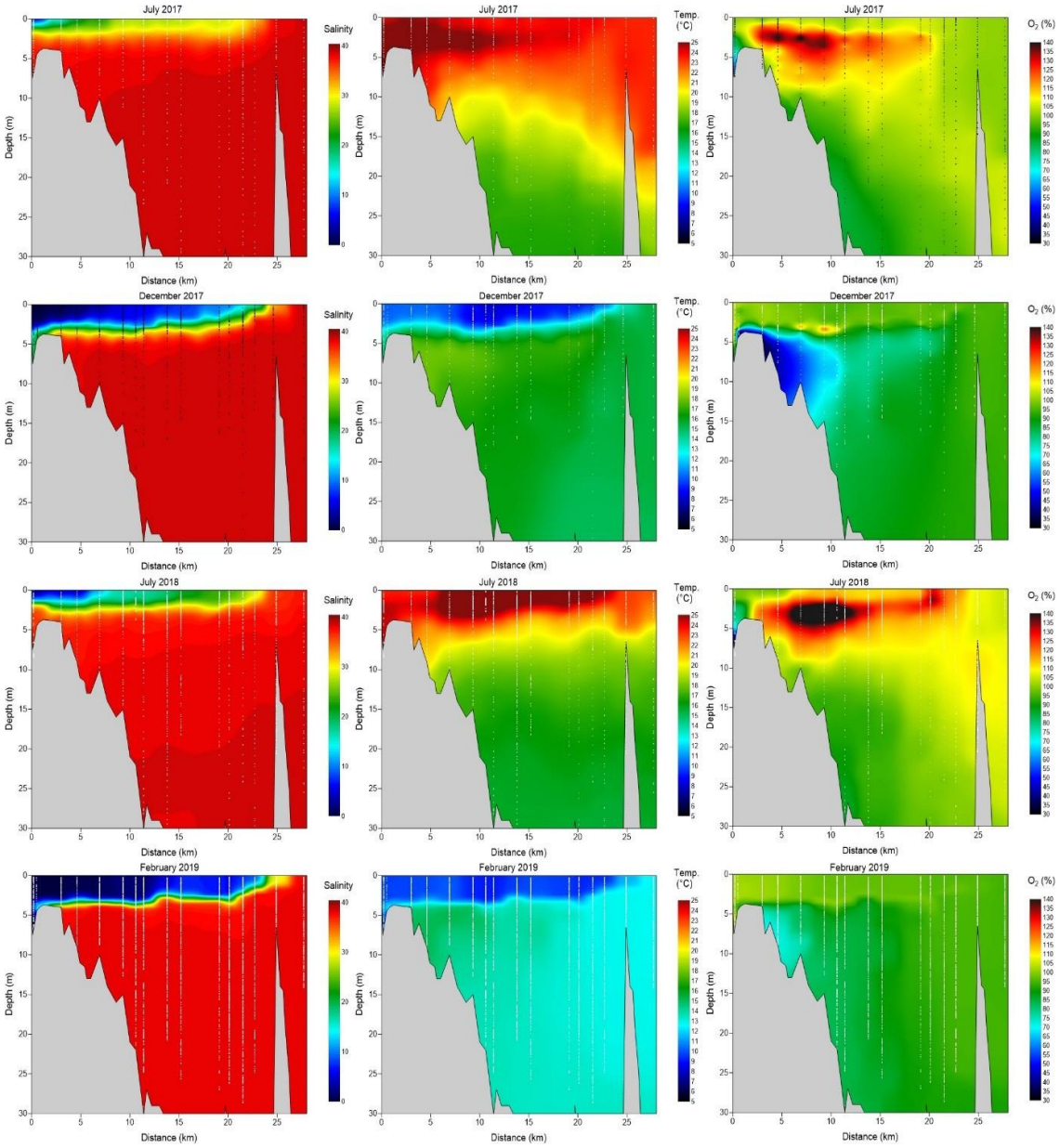

Figure 12. Contour plots of salinity (left panels), temperature (middle) and oxygen saturation (right) in the Krka River estuary for July 2017 (first row), December 2017 (second row), July 2018 (third row), and February 2019 (fourth row). Dashed lines indicate sampling stations.

Salinity profiles exhibited the expected seasonal contrast, with a deeper halocline in winter and a more extensive brackish layer than in summer due to higher freshwater flow. Temperature followed the pattern of salinity, reflecting stratification, whereas oxygen showed substantial variability. Oxygen levels were supersaturated along the halocline, during the summer, reflecting enhanced primary production in this layer (Legović et al., 1994; Marcinek et al., 2020). In contrast, during the winter oxygen levels were much lower, especially in the

deeper upstream sections. In this area oxygen measurements revealed hypoxia in December 2019 resulting from the decomposition of organic matter accumulated during the summer and the long residence time of the seawater layer in that region, consistent with trends reported in other studies from this estuary (Cindrić et al., 2015; Legović et al., 1991; Marcinek et al., 2020). However, hypoxia was not observed in February 2019, due to the water renewal by increased river flow evident from the salinity profile. Dissolved organic carbon also exhibited seasonal and spatial variability. The DOC concentrations were higher in summer (average of ~1.8 mg L^{-1}) than in winter (average of ~1.0 mg L^{-1}), with contrasting patterns at the end-members: in winter, DOC concentration was lower at the freshwater end-member (~0.6 mg L^{-1}) compared to the seawater end-member, whereas in summer it was higher in the freshwater section, most likely due to seasonal phytoplankton bloom in Visovac Lake, which feeds the estuary (Petricioli et al., 1996). These seasonal hydrographic and biogeochemical conditions established the framework for Cr and Pt behaviour along the transect.

Chromium speciation was dominated by Cr(VI) under the oxygenated conditions prevailing in both summer and winter. Interestingly, Cr(III) was minimally particle-bound (< 5%) despite its particle-reactive nature in contrast to Cr(VI), consistent with the generally low suspended particulate matter in the estuary (< 5 mg/L) (Cindrić et al., 2015). Summer transects in the surface layer showed a slight increase of Cr(VI) with salinity, ranging from ~2.5 nmol L^{-1} in the river end-member to 4–5 nmol L^{-1} in the seawater end-member, reflecting non-conservative behaviour (**Fig. 13A**). In winter, Cr(VI) exhibited the opposite trend, with higher concentrations in the freshwater end-member, likely due to enhanced river flow and associated weathering processes, while the seawater end-member remained relatively stable (**Fig. 13B**). This seasonal pattern highlights the sensitivity of Cr(VI) distribution to hydrological conditions and upstream inputs. The bottom-water transect of Cr displayed unusual behaviour compared to other trace metals, which generally increase upstream in bottom waters (Cindrić et al., 2015). Instead, Cr concentrations were lower in the freshwater region and stabilized at ~4.4 nmol L^{-1} downstream of 6 km, following a pattern similar to surface waters (**Fig. 13C**). This trend can be explained by reduction of Cr(VI) to Cr(III) in the hypoxic upstream region, followed by removal of Cr(III) through adsorption onto sinking particles, consistent with the higher particulate flux observed in that section of the estuary (Cindrić et al., 2015). Reduction of Cr(VI) may have been promoted by low-molecular-weight organic matter, as suggested in previous studies (Chen et al., 2011). Photochemical reduction of Cr(VI) to Cr(III), reported in other systems (Achterberg and Van Den Berg, 1997; Kaczynski and Kieber, 1993), did not

appear significant in this estuary, even during summer, suggesting that local light conditions and DOC concentrations limited such reactions. Atmospheric inputs of Cr(III), considered relevant elsewhere (Yu et al., 2014), remain a potential contributing factor but require further seasonal investigation.

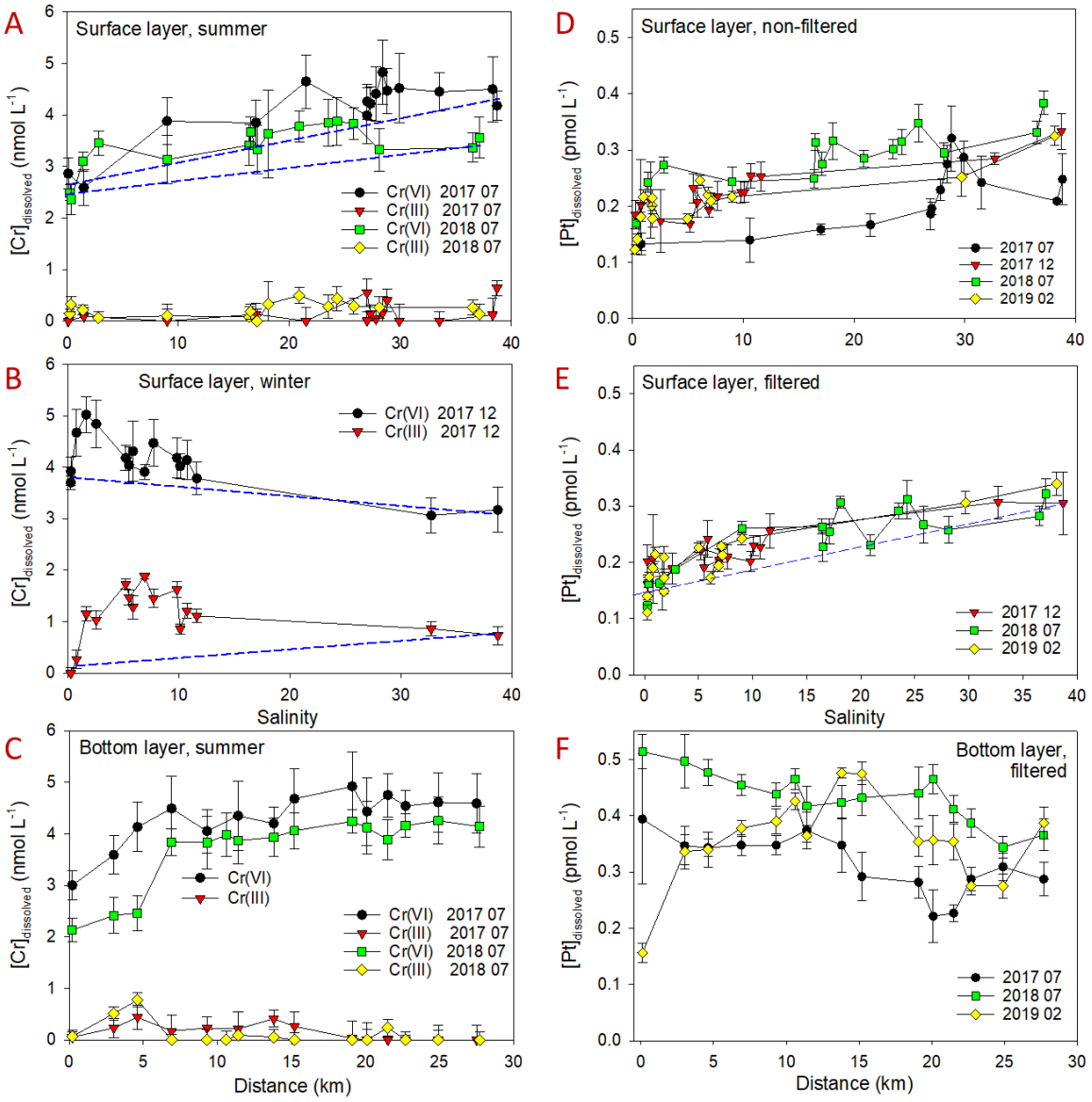

Figure 13. Estuarine distribution of Cr (A-C) and Pt (D–F). Distribution in the surface layer is shown in relation to salinity (A/B/D/E), whereas distribution in the bottom layer in relation to the distance from waterfalls (C/F). Error bars represent 95% confidence interval, and blue dashed lines indicate theoretical conservative behaviour within the salinity gradient. Other details are indicated within the figure.

Platinum, similarly, existed predominantly in the dissolved form (on average 90%), with minor particle associations. In contrast to Cr, Pt concentration was much lower at the river end-member in both seasons, and its dissolved concentrations increased with salinity, from ~0.1 pmol L^{-1} in the freshwater end-member to maximum of ~0.3 pmol L^{-1} (dissolved) and 0.4 pmol L^{-1} (total) at the seawater end-member (**Fig. 13D/E**). Along the surface layer, Pt concentrations displayed a near-conservative behaviour, with a slight positive deviation from the conservative mixing line at low salinities (< 10), likely reflecting desorption of a small fraction of Pt due to changes in redox speciation. Specifically, Pt(II) dominates in freshwater and is more readily adsorbed onto suspended particles, whereas Pt(IV) is more stable in seawater and has weaker interactions with negatively charged particles (Cobelo-García et al., 2013; Turner, 2007). In summer of 2017 a small peak of Pt concentration was observed near Šibenik town possibly reflecting local anthropogenic inputs during periods of heavier traffic, although this was not observed in the following summer campaign, likely due to lower salinity and diluting effect of the riverine water. In the bottom seawater layer, Pt concentrations increased landward in summer, reflecting accumulation while moving upstream (**Fig. 13F**), consistent with observations for other trace metals in this estuary (Cindrić et al., 2015). Sediment porewaters in estuaries, such as the Tagus, can contain higher Pt concentrations than seawater (Almécija et al., 2016), suggesting that sediments may act as a source, although the contribution in the Krka River estuary remains uncertain. Bottom layer transect in February 2019 differed, showing a strong Pt decrease in landward direction, particularly in deeper upstream region. This seasonal difference was primarily controlled by freshwater flow and mixing, with lower concentrations resulting from dilution by extended freshwater intrusion. Vertical profiles collected in Šibenik Bay demonstrated an increase at the halocline, again in accordance with patterns observed for other metals (Cindrić et al., 2015). Concentrations in surface brackish and bottom seawater layers were generally consistent with horizontal transect measurements, and plotting against salinity confirmed a linear trend.

Overall, the comparison of Cr and Pt behaviour in this estuarine system illustrates that while both metals are influenced by hydrographic gradients and particle interactions, their estuarine behaviour differs due to intrinsic chemical properties. Cr exhibits dynamic redox cycling sensitive to oxygen availability and suspended particles, with Cr(VI) prevailing under oxic conditions and Cr(III) formation and removal primarily controlled by reduction processes and particle flux in hypoxic bottom waters, whereas Pt remains near-conservative, with slight influence of redox-driven desorption. These contrasting behaviours highlight metal-specific

chemical processes in controlling the fate and transport of trace elements in estuarine environments, while also underscoring the dominant role of stratification and seasonal freshwater flow in modulating metal concentrations along the estuarine transect.

4.2.2 Copper organic speciation in the organic-rich Arno River estuary

Copper dynamics in the Arno River estuary was strongly influenced by the interplay between salinity gradients, organic matter, and localized anthropogenic inputs. Dissolved Cu exhibited a decrease with increasing salinity in both early spring and late summer campaigns, reflecting the common estuarine pattern of Cu input by the river. This is opposite of what was observed in the Krka River estuary and reflects unusually low trace metal content of the Krka River due to its specific characteristic of self-purification (Cukrov et al., 2008), highlighting the contrast between the two estuarine systems (Cindrić et al., 2015; Marcinek et al., 2025). During late summer, low river discharge was evident from the higher salinity observed at the first three sampling points (**Fig. 14**). At the same time, a non-conservative increase in Cu at mid-salinities suggested a local anthropogenic input (**Fig. 14A**), likely associated with heavily occupied boat anchorage areas, consistent with previous observations in coastal waters (Amara et al., 2018; Carić et al., 2021; Turner, 2007) and studies of Cu release from antifouling paints (Lagerström et al., 2018). In contrast, in spring, when anchoring activity was absent, the salinity-driven Cu removal along the estuarine transect was more pronounced, likely due to flocculation and dilution processes dominating under higher freshwater flow. While Cu concentrations in the river end-member were up to 50 nmol L^{-1}, marine end-member concentrations were comparable to clean coastal Mediterranean waters with ~3 nmol L^{-1} of Cu (Migon et al., 2020), indicating limited background contamination.

Dissolved organic carbon showed a conservative decrease along the salinity gradient (**Fig. 14B**), with higher riverine concentrations in late summer (5.2 mg L^{-1}) than in spring (3.3 mg L^{-1}), reflecting increased seasonal autochthonous production (Retelletti Brogi et al., 2020). Two classes of ligands, L1 (strong; log$K$'=9.6–10.8) and L2 (weak; log$K$'=8.2–9.0), were consistently detected (**Fig. 14C–F**), with total concentrations exceeding total dissolved Cu along the transect, confirming that organic complexation dominates Cu chemistry in estuarine waters (Bruland et al., 2000). Their distribution largely mirrored the DOC patterns, suggesting that organic matter plays a central role in controlling Cu speciation and availability (**Fig. 14H**).

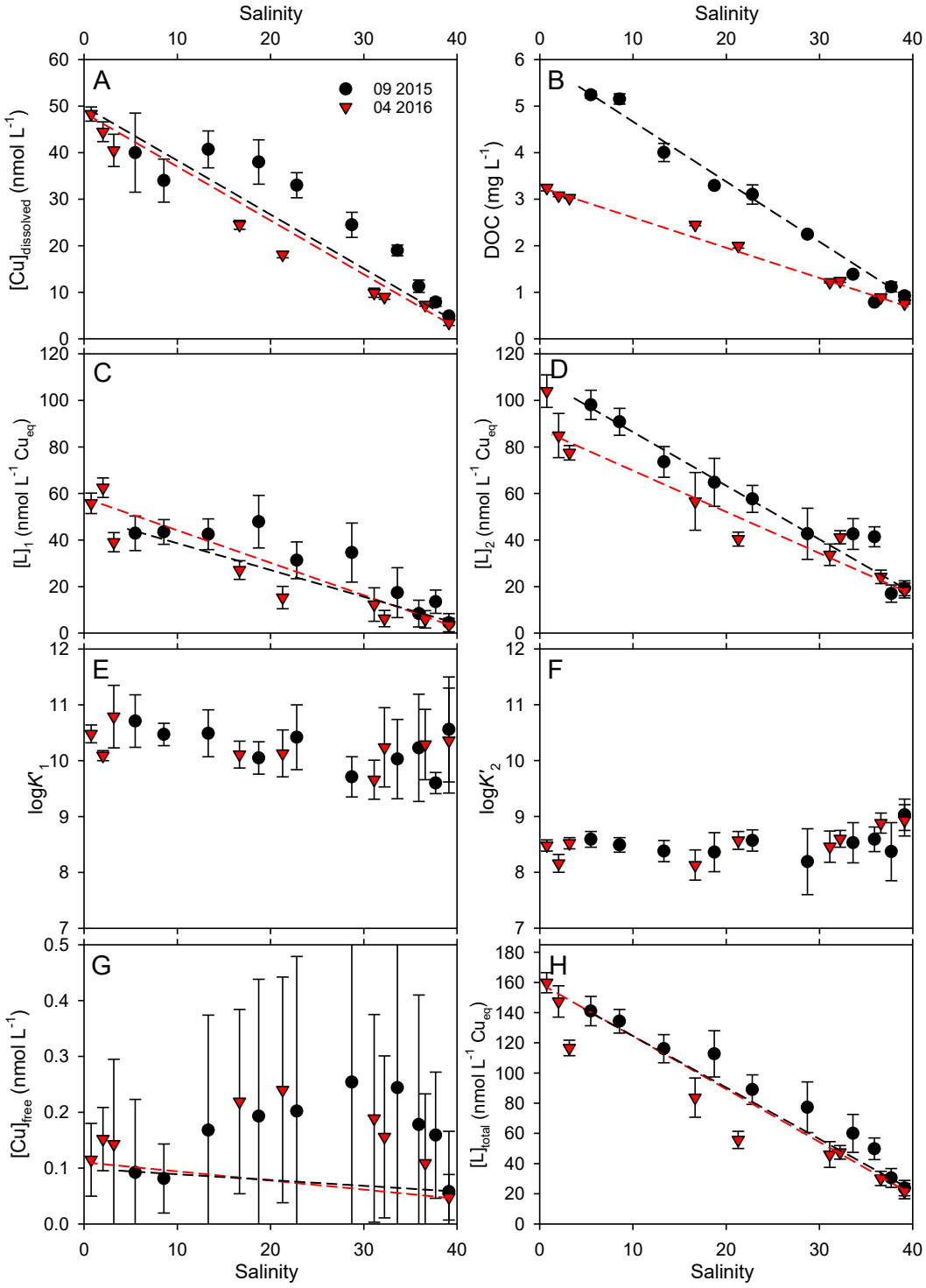

Figure 14. Estuarine distribution of dissolved Cu (A), DOC (B), complexing parameters (B–F), free Cu (G) and total ligand concentration (H) in relation to salinity. Error bars represent 95% confidence interval, and dashed lines represent theoretical conservative estuarine behaviour.

The conditional stability constants were typical for estuarine environments (Karavoltsos et al., 2013; Louis et al., 2009; Plavšić et al., 2009; Strmečki et al., 2024) and did not differ substantially between seasons (**Fig. 14E/F**). As a result, the majority of Cu remained bound to strong ligands, with L1 controlling 55–90% of the total dissolved Cu, while free Cu concentrations remained relatively high (50–250 pmol L^{-1}) (**Fig. 14G**), especially taking into account the fact that the estimated threshold toxicity level is around 10 pmol L^{-1} (Sunda et al., 1990, 1987). However, free Cu was likely overestimated due to methodological limitations of ASV that cannot fully discern between fully labile inorganic Cu species and kinetically labile organic complexes during the deposition step (Bruland et al., 2000; Town, 1997).

The composition and optical characteristics of the DOM were closely linked to ligand distributions. Analysis of fluorescent DOM identified five fluorescent components, dominated by protein-like substances in both seasons, while terrestrial fulvic- and humic-like components were more abundant in spring, corresponding to higher river discharge and terrestrial input (Retelletti Brogi et al., 2020). Late summer samples showed elevated protein+PAH-like component, likely associated with tourist activity and boat traffic, supporting the observed local Cu increase. Fifth component was microbial-humic-like which did not show a change in contribution to the overall DOM pool between seasons. The sum of Cu-binding ligands correlated strongly with DOC and all fluorescence components ($r^2 > 0.9$), suggesting that both terrestrial and autochthonous DOM provide binding sites for Cu throughout the estuary (Dulaquais et al., 2020; Karavoltsos et al., 2013; Marcinek et al., 2025; Voelker and Kogut, 2001; Whitby and van den Berg, 2015; Wong et al., 2019; Yang and van den Berg, 2009). Ratio of total ligand concentrations normalised to DOC was higher in spring than in late summer (average of 37 μmol g^{-1} and 30 μmol g^{-1}, respectively), indicating that increased ligand abundance within the DOM pool under high river flow and increased input of terrestrial humic substances (Retelletti Brogi et al., 2020). In late summer, *in situ* production of protein-like DOM may have enhanced ligand concentrations, potentially in response to Cu release from boat antifouling paints (Sander et al., 2015; Whitby et al., 2017; Zitoun et al., 2019), although, the similar increase in all fluorescent components precludes a definitive attribution solely to Cu-induced biological production.

Overall, Cu behaviour in the Arno River estuary illustrates the combined effects of hydrodynamics, flocculation, organic complexation, and localized anthropogenic inputs. Copper speciation is largely controlled by organic ligands, which drive free Cu concentrations,

while seasonal river discharge and human activities modulate both total Cu and the distribution of ligands.

4.2.3 Trace metal biogeochemistry in contrasting estuarine systems and global context

The Krka River estuary, a stratified karstic system with minimal anthropogenic input, contrasts sharply with the turbid, organic-, and metal-rich Arno River estuary, heavily influenced by industrial, agricultural, and urban activities. Comparing trace metal dynamics in these two systems highlights how intrinsic metal chemistry, estuarine hydrography, and the quantity and composition of organic matter collectively govern metal behaviour under differing seasonal and environmental conditions.

In the relatively pristine Krka River estuary, Cr remained largely in the hexavalent state under oxygenated conditions, and Pt displayed nearly conservative mixing with salinity, suggesting minimal interaction with particles or organic matter. These patterns reflect low anthropogenic pressure, limited suspended particulate matter, as well as pronounced influence of hydrographic features such as halocline depth and seasonal oxygen dynamics. Seasonal hypoxia and increased productivity in Prokljan Lake were sufficient to modulate Cr redox transformations, whereas Pt distributions remained largely unaffected, underlining its inert character in low-contamination systems.

Chromium concentrations in the Krka River estuary (2.2–6.7 nmol L^{-1}; Pađan et al., 2019) fall within the lower range of values reported for natural aquatic systems (**Table 2**). They are comparable to levels found in other relatively unpolluted estuarine and marine environments, such as the Mersey River estuary (2.2–2.8 nmol L^{-1}; Espada-Bellido et al., 2013) and the Baltic Sea (1.5–4.9 nmol L^{-1}; Paulukat et al., 2016). The observed concentrations are also consistent with open ocean values, for example in the Argentine Basin (5.8 nmol L^{-1}; Bonnand et al., 2011) and the Mediterranean Sea (4.6–5.9 nmol L^{-1}; Paulukat et al., 2016), suggesting that the Krka River estuary does not represent a significant source of Cr enrichment to the adjacent coastal sea. By contrast, markedly higher Cr levels have been documented in rivers such as the Paraná River (average 46.8 nmol L^{-1}; Frei et al., 2014) or the Shing Mun River in Hong Kong (32.7–54.8 nmol L^{-1}; Tsoi and Sze-Yin Leung, 2010), as well as in polluted coastal sites. For instance, waters near Porto Romano, Durres Harbour (Albania) exhibit extremely elevated concentrations (645–698 nmol L^{-1}; Lazo, 2009), reflecting severe anthropogenic contamination and their designation as a “Hot Spot Pollution” area by

UNEP/MAP (1992). Such stark contrasts highlight the relatively pristine status of the Krka River estuary with respect to Cr.

Table 2. Global overview of dissolved Cr concentrations in riverine, costal, and open sea waters, with results from this thesis underlined.

Water type	Cr (nmol L^{-1})	Study
Rivers:		
Paraná River, Argentina	avg 46.8	Frei et al., 2014
Shing Mun River, Hing Kong	32.7–54.8	Tsoi and Sze-Yin Leung, 2010
Swiss rivers and lakes	7.8–8.6	Li and Xue, 2001
Coastal sea:		
Durres harbour, Albania	645.2-698.4	Lazo, 2009
Taiwan beaches	34.6–96.2	Lin et al., 2013
Paraná estuary	avg 21.2	Frei et al., 2014
Peruvian coast	2.5–15.4	Bruggmann et al., 2019
Krka River estuary	2.2–6.7	Pađan et al., 2019
Mersey River estuary	2.2–2.8	Espada-Bellido et al., 2013
Open sea:		
North Sea	2.2–9.5	Paulukat et al., 2016
Argentine Basin	avg 5.8	Bonnand et al., 2011
Mediterranean Sea	4.6–5.9	Paulukat et al., 2016
Baltic Sea	1.5–4.9	Paulukat et al., 2016
Southern Ocean	3.7±0.3	Rickli et al., 2019

It is important to note that Cr data for estuarine and marine environments remain relatively scarce, and the limited number of available studies reflects persistent methodological challenges in its determination in seawater. Overall, the low Cr concentrations observed in the Krka River estuary align more closely with background levels typical of uncontaminated coastal and open marine waters, rather than with anthropogenically impacted rivers and harbours. This underscores the value of the Krka River estuary as a reference site for understanding trace metal behaviour under conditions of minimal local anthropogenic input.

For dissolved Pt, its levels in the Krka River estuary (0.1–0.3 pmol L^{-1}) are among the lowest recorded globally, with riverine concentrations lower than the sea and comparable to the pristine rivers and estuaries, such as Lérez River estuary (0.02–0.6 pmol L^{-1}; Cobelo-García et al., 2013) (**Table 3**). Concentrations in the Krka River estuary are even lower than the

baseline values in open-ocean waters (Nort Pacific, Atlantic Ocean with ~0.3 pmol L^{-1}, and North Sea with ~0.9 pmol L^{-1}; Fischer et al., 2018; Hollister et al., 2024; López-Sánchez et al., 2019; Mashio et al., 2016).

Table 3. Global overview of dissolved Pt concentrations in riverine, costal, and open sea waters, with results from this thesis underlined.

Water type	Pt (pmol L^{-1})	Study
Rivers:		
Zenne River, Belgium	15.4–66.6	Abdulbur-Alfakhoury et al., 2021
North German rivers	3.4–6.3	Hollister et al., 2024
Marque River, France	1.0–3.1	Abdulbur-Alfakhoury et al., 2021
East Asia pristine rivers	< 1.4	Soyol-Erdene and Huh, 2012
Coastal sea:		
Tagus estuary, Portugal	0.6–60.0	Monteiro et al., 2021
Tama River estuary, Japan	4.8–35.2	Obata et al., 2006
Ara River estuary, Japan	5.0–13.6	Obata et al., 2006
Ave estuary, Portugal	1.2–10.1	Abdou et al., 2023
North Yellow Sea coast	1.3–4.5	Liu et al., 2018
Boso to Sanriku areas, Japan	0.2–1.5	Mashio et al., 2017
Tokyo and Otsuchy Bay, Japan	0.3–1.5	Mashio et al., 2016
Douro estuary, Portugal	0.4–1.0	Abdou et al., 2023
Gironde estuary, France	0.4–0.8	Cobelo-García et al., 2014a
Lérez estuary, Spain	0.02–0.6	Cobelo-García et al., 2013
Gulf of St. Lawrence, Canada	0.1–0.5	Dang et al., 2022
Krka River estuary	0.1–0.5	Pađan et al., 2020
Open sea:		
Southern North Sea	0.4–2.2	Hollister et al., 2024
Indian Ocean	0.2–1.6	Jacinto and van den Berg, 1989
Atlantic Ocean	0.16–0.3	López-Sánchez et al., 2019
North Pacific	0.19–0.3	Fischer et al., 2018

Coastal waters draining densely urbanized regions, such as the Ave and Tagus estuaries in Portugal, Ara and Tama estuaries in Japan, or inland European rivers, receiving wastewater treatment plant effluents, display markedly elevated Pt concentrations with concentrations up to 66 pmol L^{-1} (Abdou et al., 2023; Abdulbur-Alfakhoury et al., 2021; Monteiro et al., 2021; Obata et al., 2006). These global comparisons highlight the strong influence of anthropogenic pressures, such as wastewater discharges, runoff from roads, and industrial effluents, on Pt

levels in coastal waters. In contrast, natural low-input systems such as the Krka River estuary provide a rare global baseline for sub-picomolar Pt levels, reflecting primarily natural geogenic sources with negligible anthropogenic contributions. Such baselines are essential for assessing Pt enrichment in impacted estuaries and distinguishing natural from human-derived inputs globally.

A direct comparison of the Krka and Arno estuaries is best captured through Cu and DOM dynamics, with Arno estuary data presented in this thesis based on Research paper 3 (Pađan et al., 2021) and Krka River estuary results from other studies (Cindrić et al., 2015; Marcinek et al., 2020, 2025). Copper dynamics between the two estuaries further emphasize the relative cleanliness of Krka River, while also underscoring the central role of DOM in modulating Cu speciation. In the Arno River estuary, Cu concentrations are high in river water and decline with increasing salinity, while in the Krka River estuary an opposite trend was observed with a dilution effect of the river due to the very low Cu content of incoming freshwater (Cindrić et al., 2015). Previous measurements in the Arno River reported very high dissolved Cu concentrations along its course (76.5–140.1 nmol L^{-1}; Legittimo et al., 1991). In contrast, during our campaign the highest concentration detected at the river mouth was 48.3 nmol L^{-1}, suggesting that substantial Cu removal occurs during riverine transport prior to reaching the estuarine zone and it continues along the estuarine transect as it approaches background sea levels. The observed concentrations in the Krka River, on the other hand, can be considered representative of background levels, broadly consistent with values observed in the open ocean (0.5–5 nmol L^{-1}; Arnone et al., 2024; Zitoun et al., 2021), thus winter concentrations within the estuary, in absence of anthropogenic influences, can be compared with relatively pristine estuaries such as Puget Sound (4.1–6.1 nmol L^{-1}; Jacquot et al., 2014) or Otsuchi Bay (2.0–3.6 nmol L^{-1}; Wong et al., 2018). Both estuaries, however, exhibit summer Cu increases in the mid-estuary, linked to boat traffic (Cindrić et al., 2015). More recent works reported Cu dynamics in the Krka River estuary (Crmarić et al., 2024; Marcinek et al., 2025), showing that even in low-input systems such as this one, seasonal anthropogenic inputs linked to touristic boating activities can substantially elevate surface Cu, although maximum dissolved concentrations (up to 25 nmol L^{-1}) still remained lower than in the Arno River estuary or in other similar estuaries like San Francisco Bay, Liverpool Bay, Mersey, Duplin and Loire, or the Amazon River plume with up to 93 nmol L^{-1} (Abualhaija et al., 2015; Buck et al., 2007; Dulaquais et al., 2020; Hollister et al., 2021; Whitby et al., 2017), and well below the extremely elevated levels measured in heavily polluted sites, for example Taiwanese harbours (up to 368.2

nmol L^{-1}; Lin et al., 2013) (**Table 4**). Increased Cu concentrations in the Krka River estuary in summer span a range typical of moderately impacted estuarine systems, highlighting a degree of localized Cu input, though not at levels that would classify the system as severely contaminated.

Table 4. Global overview of dissolved Cu concentrations in riverine, costal, and open sea waters, with results from this thesis underlined.

Water type	Cu (nmol L^{-1})	Study
Rivers:		
Arno River	76.5–140.1	Legittimo et al., 1991
Yangtze River	5.1–26.7	Vasquez et al., 2024
Missouri River, Columbia	avg 47.1	Vance et al., 2008
Coastal sea:		
Taiwan harbour	7.88–368.2	Lin et al., 2013
Taiwan beaches	6.3–34.6	Lin et al., 2013
San Francisco Bay	6.8–93.0	Buck et al., 2007
Loire estuary, France	3.4–80.3	Dulaquais et al., 2020
Duplin River estuary	6.1–65.0	Whitby et al., 2017
Liverpool Bay	11.6–54.5	Abualhaija et al., 2015
Cape Cod, Massachusetts	3.8–54.0	Moffett et al., 1997
Arno River estuary	7.9–48.3	Pađan et al., 2021
Amazon River plume	0.6–35.0	Hollister et al., 2021
Krka River estuary	3.40–25.8	Marcinek et al., 2025
Puget Sound estuary, Washington	4.1–6.1	Jacquot et al., 2014
Otsuchi Bay, Japan	2.0–3.6	Wong et al., 2018
Open sea:		
East China Sea	0.6–4.7	Wong et al., 2019
Pacific Ocean	1.2–3.3	Whitby et al., 2018
Atlantic Ocean	0.5–3.5	Zitoun et al., 2021
Arctic	1.2–5.2	Arnone et al., 2024
Southern Ocean	0.8–2.7	Heller and Croot, 2014

In both Arno and Krka River estuaries, DOM quantity and molecular character exert a primary control on Cu cycling, yet magnitude of their sources differ. The Krka River contributes little DOC to the estuary relative to its marine end-member (Marcinek et al., 2020), whereas the Arno River supplies large amounts of terrestrial organic matter. In the Krka River estuary, winter conditions are characterized by low DOM concentrations but with a strong

terrestrial humic signature and dominance of humic ligands (Marcinek et al., 2020, 2025), driven by high river discharge and limited *in situ* production. This parallels observations in the Arno during spring, a season with higher river influx. In summer, both systems show an increase in autochthonous DOM production, raising overall DOC concentrations, reflected also in increased Cu-binding ligand concentrations. Interestingly, both systems showed contrasting relationships of DOC-normalised ligand concentration with salinity between wet and dry seasons, showing higher Cu-binding site density within the DOM pool at freshwater endmember in the wet season, enriched with terrestrial humic substances (**Fig. 15**). This observation is consistent with studies that suggest that metals preferentially bind to functional groups in close proximity to aromatic rings and that DOM complexing capacity for Cu is correlated with its aromaticity (Kikuchi et al., 2017). Nevertheless, freshly produced sulphur-bearing ligands possess sufficient binding strength to outcompete even highly aromatic ligands, highlighting biologically derived DOM in controlling Cu speciation. This is clearly evident from our study in Arno River estuary, in which strong biogenic ligands were governing 55–90% of organic Cu fraction. However, during periods of low biological production, or when Cu concentrations exceed the capacity of strong biological ligands, humic ligands become important secondary buffers, also evident in the Krka River estuary (Marcinek et al., 2025), where, in winter season, characterized with minimal biological activity, humic-rich DOM pool, although scarce in concentration, was effectively keeping Cu below its toxic threshold.

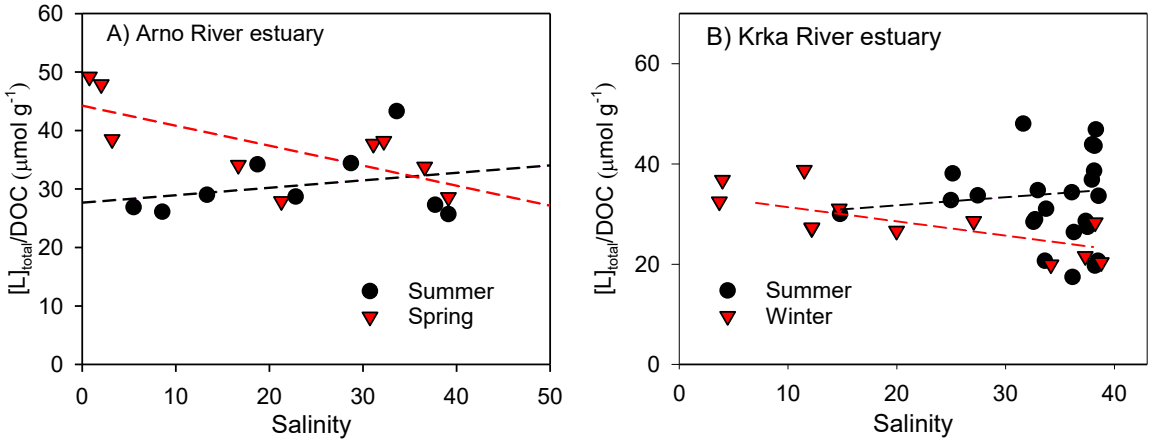

Figure 15. Comparison of DOC-normalised ligand concentration in relation to salinity between Arno River estuary (A) and Krka River estuary (B).

The ecological implications of these dynamics differ between the estuaries. In the Krka River estuary, even low DOM levels in winter are sufficient to buffer Cu from reaching toxic concentrations, although in summer, tourist-related Cu inputs can nearly outpace increased *in situ* ligand production (Marcinek et al., 2025). By contrast, in the Arno River estuary, free Cu concentrations (though potentially overestimated by ASV) remain high enough to warrant ecological concern, in both, wet and dry seasons. These patterns highlight that while Cu-DOM interactions are central in both systems, their relative effectiveness is governed by hydrography, DOM origin, and the magnitude of Cu inputs. Globally, the importance of interplay between Cu and DOM is a recurring theme, emphasizing the influence of seasonal DOM variations on Cu speciation and bioavailability. In systems dominated by terrestrial DOM inputs, such as the Amazon plume, the Strait of Georgia, or the Duplin River estuary, terrestrially sourced humic ligands are extremely abundant and generally efficiently complex even very high Cu concentrations, reducing free ion concentrations and mitigating toxicity (Hollister et al., 2021; Muller et al., 2024; Whitby et al., 2017). In contrast, estuaries with weaker terrestrial influence rely more heavily on *in situ* DOM production, which, because of the high seasonality of this mechanism, leaves them more vulnerable to anthropogenic perturbations (Mellett and Buck, 2020; Sander et al., 2015).

Taken together, these observations illustrate several key principles of estuarine metal cycling: (i) metal-specific chemistry dictates dominant controls, with redox-sensitive Cr responding to oxygen and suspended particulates, Pt behaving conservatively, and Cu strongly regulated by DOM dynamics; (ii) hydrography modulates metal distributions, with stratification preserving vertical gradients and freshwater flow promoting water renewal (important for oxygen levels and Cr chemistry) and driving either dilution (Pt, Cu and DOM in Krka River estuary) or enhanced input (Cu and DOM in Arno River estuary); (iii) anthropogenic pressures related to use of antifouling paints on boats are important contributors of Cu even in clean environments like Krka River estuary; and (iv) natural baselines, exemplified by the Krka River and its estuary, are critical for global comparisons, as they highlight the magnitude of human influence in more urbanized estuaries. Ultimately, the contrasting behaviours of Cr, Pt, and Cu in the Krka and Arno River estuaries demonstrate how hydrography, organic matter, and human activity jointly shape the cycling of trace metals. High-resolution voltammetric methods provide a powerful framework for resolving these dynamics, linking local estuarine processes to global biogeochemical patterns, and refining our understanding of natural versus anthropogenic controls on trace metal fate.

5. CONCLUSION

This doctoral research has contributed to both methodological and environmental understanding of trace metal behaviour in estuarine systems, focusing on Cr, Pt, and Cu. By developing and refining electroanalytical approaches capable of accurate determination of trace metal concentrations and speciation in natural waters, the work addresses critical challenges associated with complex matrices, particularly the interference of SAS. These methodological improvements have enabled high-resolution, reproducible voltammetric measurements under environmentally relevant conditions, providing insights into the interplay between metal chemistry, estuarine hydrography, and organic matter dynamics.

The main achievement of the thesis lies in the optimization of voltammetric protocols for Cr, Pt, and Cu quantification/speciation in natural waters. For Cr, the study demonstrated that selection of highly negative deposition potentials effectively minimizes interference from SAS, preserving peak definition and enabling accurate quantification of Cr(VI) even in organically enriched estuarine samples. The systematic evaluation of deposition potential, combined with derivative transformations of voltamograms, allowed for reliable detection and precise measurement of Cr speciation across salinity gradients, supporting robust assessment of redox cycling under seasonal variations. Similarly, for Pt, the work established that the combination of an optimized deposition potential and a brief desorption step effectively mitigates SAS adsorption, enhancing peak resolution at ultra-trace concentrations. Application of second- and fourth-derivative transformations further improved baseline flattening and detection limits down to the femtomolar range, demonstrating the capacity of the optimized method to resolve Pt dynamics in low-input estuarine systems. For Cu, challenges associated with high DOM content were overcome through the strategic use of a nonionic surfactant, Triton-X-100, combined with deposition potential control and a short desorption step. This approach successfully decoupled electrode surface interactions from bulk Cu speciation, allowing reliable extraction of Cu peak intensities and detailed characterization of organic complexation, while pseudopolarography provided a nuanced view of the heterogeneity of Cu-binding sites on DOM. Collectively, these methodological innovations establish a coherent framework for trace metal analysis in complex natural waters, demonstrating that careful optimization of electrode conditions, surface treatment, and data processing is essential to disentangle genuine chemical signals from interfacial artifacts.

The second contribution of this research is the application of these improved methods to contrasting estuarine systems, providing insights into the environmental behaviour of Cr, Pt, and Cu. In the pristine Krka River estuary, Cr(VI) was shown to dominate under oxic

conditions, with Cr(III) formation and removal occurring primarily in hypoxic bottom waters through reduction and particle interactions. Seasonal variability in freshwater flow and stratification strongly modulated Cr distribution, highlighting the sensitivity of redox cycling to natural hydrographic processes. Platinum exhibited near-conservative behaviour along the salinity gradient, with dissolved concentrations increasing from river to seawater end-members, reflecting its inert nature and minimal anthropogenic influence in low-input estuarine systems. These observations provide a rare global baseline for sub-picomolar Pt levels and underscore the importance of pristine systems for distinguishing natural from human-derived metal inputs.

In the organic-rich Arno River estuary, Cu dynamics were dominated by organic complexation, with two classes of ligands controlling the distribution of dissolved and free Cu. Seasonal differences in river discharge, anthropogenic inputs, and in situ DOM production were shown to modulate both Cu concentrations and ligand availability. While strong ligands controlled most of the Cu speciation under high-DOM conditions, localized anthropogenic inputs, particularly from boat traffic, induced non-conservative increases at mid-salinities, emphasizing the role of human activity even in systems with naturally high organic content. Comparisons between the Krka and Arno estuaries highlight how metal-specific chemistry, DOM composition, hydrography, and anthropogenic pressures interact to govern trace metal cycling in estuarine environments.

Overall, this thesis demonstrates that voltammetric methods, when carefully optimized to account for SAS interferences, provide a powerful tool for studying trace metal biogeochemistry. Methodological refinements have enabled robust determination of concentrations and speciation of Cr, Pt, and Cu, facilitating the identification of metal-specific controls and seasonal variability. Environmental applications reveal the contrasting behaviour of trace metals in low- and high-input estuaries, illustrating the central role of redox processes, particle interactions, and organic complexation, while also highlighting the significance of anthropogenic contributions in shaping Cu dynamics. These findings not only enhance our understanding of estuarine metal cycling but also provide a framework for future studies aimed at linking local hydrology and biogeochemical processes to broader biogeochemical and ecological patterns. By integrating methodological rigor with environmental application, the thesis offers both practical analytical solutions and substantive insight into the factors controlling trace metal behaviour in natural waters.

REFERENCES

Abdou, M., Dutruch, L., Schäfer, J., Zaldibar, B., Medrano, R., Izagirre, U., Gil-Díaz, T., Bossy, C., Catrouillet, C., Hu, R., Coynel, A., Lerat, A., Cobelo-García, A., Blanc, G., Soto, M., 2018. Tracing platinum accumulation kinetics in oyster Crassostrea gigas, a sentinel species in coastal marine environments. Sci. Total Environ. 615, 652–663. https://doi.org/10.1016/j.scitotenv.2017.09.078

Abdou, M., Monteiro, C.E., Brito, P., Neuparth, T., Pinheiro, M., Santos, M., Caetano, M., 2023. Platinum group element distribution in water and marine biota from two impacted estuarine environments (Douro and Ave estuaries, Portugal). Mar. Pollut. Bull. 192, 114990. https://doi.org/10.1016/j.marpolbul.2023.114990

Abdou, M., Schäfer, J., Cobelo-García, A., Neira, P., Petit, J.C.J., Auger, D., Chiffoleau, J.-F., Blanc, G., 2016. Past and present platinum contamination of a major European fluvial–estuarine system: Insights from river sediments and estuarine oysters. Mar. Chem. 185, 104–110. https://doi.org/10.1016/j.marchem.2016.01.006

Abdou, M., Schäfer, J., Gil-Díaz, T., Tercier-Waeber, M.-L., Catrouillet, C., Massa, F., Castellano, M., Magi, E., Povero, P., Blanc, G., 2020. Spatial variability and sources of platinum in a contaminated harbor – tracing coastal urban inputs. Environ. Chem. 17, 105. https://doi.org/10.1071/EN19160

Abdou, M., Schäfer, J., Hu, R., Gil-Díaz, T., Garnier, C., Brach-Papa, C., Chiffoleau, J.-F., Charmasson, S., Giner, F., Dutruch, L., Blanc, G., 2019. Platinum in sediments and mussels from the northwestern Mediterranean coast: Temporal and spatial aspects. Chemosphere 215, 783–792. https://doi.org/10.1016/j.chemosphere.2018.10.011

Abdulbur-Alfakhoury, E., Trommetter, G., Brion, N., Dumoulin, D., Reichstädter, M., Billon, G., Leermakers, M., Baeyens, W., 2021. Distribution of platinum (Pt), palladium (Pd), and rhodium (Rh) in urban tributaries of the Scheldt River assessed by diffusive gradients in thin films technique (DGT). Sci. Total Environ. 784, 147075. https://doi.org/10.1016/j.scitotenv.2021.147075

Abualhaija, M.M., Whitby, H., van den Berg, C.M.G., 2015. Competition between copper and iron for humic ligands in estuarine waters. Mar. Chem. 172, 46–56. https://doi.org/10.1016/j.marchem.2015.03.010

Achterberg, E.P., Van Den Berg, C.M.G., 1997. Chemical speciation of chromium and nickel in the western Mediterranean. Deep Sea Res. Part II Top. Stud. Oceanogr. 44, 693–720. https://doi.org/10.1016/S0967-0645(96)00086-0

Ajjan, F.N., Mecerreyes, D., Inganäs, O., 2019. Enhancing energy storage devices with biomacromolecules in hybrid electrodes. Biotechnol. J. 14. https://doi.org/10.1002/biot.201900062

Alhassan, A.B., Aljahdali, M.O., 2021. Fractionation and distribution of rare earth elements in marine sediment and bioavailability in avicennia marina in central red sea mangrove ecosystems. Plants 10. https://doi.org/10.3390/plants10061233

Almécija, C., Cobelo-García, A., Santos-Echeandía, J., Caetano, M., 2016. Platinum in salt marsh sediments: Behavior and plant uptake. Mar. Chem. 185, 91–103. https://doi.org/10.1016/j.marchem.2016.05.009

Amara, I., Miled, W., Slama, R. Ben, Ladhari, N., 2018. Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. Environ. Toxicol. Pharmacol. 57, 115–130. https://doi.org/10.1016/j.etap.2017.12.001

Amin, S.A., Moffett, J.W., Martens-Habbena, W., Jacquot, J.E., Han, Y., Devol, A., Ingalls, A.E., Stahl, D.A., Armbrust, E.V., 2013. Copper requirements of the ammonia-oxidizing archaeon Nitrosopumilus maritimus SCM1 and implications for nitrification in the marine environment. Limnol. Oceanogr. 58, 2037–2045. https://doi.org/10.4319/lo.2013.58.6.2037

Ao, M., Zhou, R., Meng, R., Bonaventure, L.A., Luo, B., Qian, X., Xu, X., Wang, S., Wu, P., 2025. Chromium transformations and biological impacts in aquatic systems: From sediment-water interfaces to food web complexity. Ecotoxicol. Environ. Saf. 303, 118850.

https://doi.org/10.1016/j.ecoenv.2025.118850
Arnone, V., Santana-Casiano, J.M., González-Dávila, M., Sarthou, G., Krisch, S., Lodeiro, P., Achterberg, E.P., González, A.G., 2024. Distribution of copper-binding ligands in Fram Strait and influences from the Greenland Shelf (GEOTRACES GN05). Sci. Total Environ. 909, 168162. https://doi.org/10.1016/j.scitotenv.2023.168162
Artelt, S., Kock, H., König, H.P., Levsen, K., Rosner, G., 1999. Engine dynamometer experiments: platinum emissions from differently aged three-way catalytic converters. Atmos. Environ. 33, 3559–3567. https://doi.org/10.1016/S1352-2310(99)00109-0
Auger, Y., Bodineau, L., Leclercq, S., Wartel, M., 1999. Some aspects of vanadium and chromium chemistry in the English Channel. Cont. Shelf Res. 19, 2003–2018. https://doi.org/10.1016/S0278-4343(99)00050-3
Barber-Lluch, E., Nieto-Cid, M., Santos-Echeandía, J., Sánchez-Marín, P., 2023. Effect of dissolved organic matter on copper bioavailability to a coastal dinoflagellate at environmentally relevant concentrations. Sci. Total Environ. 901. https://doi.org/10.1016/j.scitotenv.2023.165989
Berezhnaya, E.D., Dubinin, A. V., 2024. Platinum group element geochemistry in the ocean. Geochemistry Int. 62, 327–343. https://doi.org/10.1134/S0016702923700106
Bi, Z., Salaün, P., van den Berg, C.M.G., 2013. The speciation of lead in seawater by pseudopolarography using a vibrating silver amalgam microwire electrode. Mar. Chem. 151, 1–12. https://doi.org/10.1016/j.marchem.2013.02.003
Bielicka, A., Bojanowska, I., Wisniewski, A., 2005. Two Faces of Chromium-Pollutant and Bioelement. Polish J. Environ. Stud. 14.
Blossom, N., 2007. Copper in the ocean environment. Am Chemet Corp. pp. 1–8.
Bobrowski, A., Królicka, A., Zarębski, J., 2009. Characteristics of voltammetric determination and speciation of chromium – A review. Electroanalysis 21, 1449–1458. https://doi.org/10.1002/elan.200904582
Bonnand, P., Parkinson, I.J., James, R.H., Karjalainen, A.-M., Fehr, M.A., 2011. Accurate and precise determination of stable Cr isotope compositions in carbonates by double spike MC-ICP-MS. J. Anal. At. Spectrom. 26, 528. https://doi.org/10.1039/c0ja00167h
Borrill, A.J., Reily, N.E., Macpherson, J. V., 2019. Addressing the practicalities of anodic stripping voltammetry for heavy metal detection: a tutorial review. Analyst 144, 6834–6849. https://doi.org/10.1039/C9AN01437C
Boussemart, M., van den Berg, C.M.G., Ghaddaf, M., 1992. The determination of the chromium speciation in sea water using catalytic cathodic stripping voltammetry. Anal. Chim. Acta 262, 103–115. https://doi.org/10.1016/0003-2670(92)80014-X
Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. J. Exp. Mar. Bio. Ecol. 96, 225–250. https://doi.org/10.1016/0022-0981(86)90205-4
Branica, G., Lovrić, M., 1997. Pseudopolarography of totally irreversible redox reactions. Electrochim. Acta 42, 1247–1251. https://doi.org/10.1016/S0013-4686(96)00297-6
Branica, M., Novak, D.M., Bubić, S., 1977. Application of anodic stripping voltammetry to determination of the state of complexation of traces of metal ions at low concentration levels. Croat. Chem. Acta 49, 539–547.
Bruggmann, S., Scholz, F., Klaebe, R.M., Canfield, D.E., Frei, R., 2019. Chromium isotope cycling in the water column and sediments of the Peruvian continental margin. Geochim. Cosmochim. Acta 257, 224–242. https://doi.org/10.1016/j.gca.2019.05.001
Bruland, K.W., Lohan, M.C., 2003. Controls of trace metals in seawater, in: Turekian, K.K., Holland, H.D. (Eds.), Treatise on Geochemistry. Elsevier, pp. 23–47.
Bruland, K.W., Rue, E.L., Donat, J.R., Skrabal, S.A., Moffett, J.W., 2000. Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. Anal. Chim. Acta 405, 99–113. https://doi.org/10.1016/S0003-2670(99)00675-3
Bucci, R., Magri, A.L., Napoli, A., 1991. Chromium(III) complexes with diethylentriaminepentaacetic acid. J. Coord. Chem. 24, 169–175. https://doi.org/10.1080/00958979109409459
Buck, K.N., Bruland, K.W., 2005. Copper speciation in San Francisco Bay: A novel approach using

multiple analytical windows. Mar. Chem. 96, 185–198. https://doi.org/10.1016/j.marchem.2005.01.001
Buck, K.N., Ross, J.R.M., Russell Flegal, A., Bruland, K.W., 2007. A review of total dissolved copper and its chemical speciation in San Francisco Bay, California. Environ. Res. 105, 5–19. https://doi.org/10.1016/j.envres.2006.07.006
Buerge-Weirich, D., Sulzberger, B., 2004. Formation of Cu(I) in estuarine and marine waters: Application of a new solid-phase extraction method to measure Cu(I). Environ. Sci. Technol. 38, 1843–1848. https://doi.org/10.1021/es034845x
Bužančić, M., Ninčević Gladan, Ž., Marasović, I., Kušpilić, G., Grbec, B., 2016. Eutrophication influence on phytoplankton community composition in three bays on the eastern Adriatic coast. Oceanologia 58, 302–316. https://doi.org/10.1016/j.oceano.2016.05.003
Campbell, A.L., Mangan, S., Ellis, R.P., Lewis, C., 2014. Ocean acidification increases copper toxicity to the early life history stages of the polychaete Arenicola marina in artificial seawater. Environ. Sci. Technol. 48, 9745–9753. https://doi.org/10.1021/es502739m
Carić, H., Cukrov, N., Omanović, D., 2021. Nautical tourism in marine protected areas (Mpas): Evaluating an impact of copper emission from antifouling coating. Sustain. 13, 11897. https://doi.org/10.3390/su132111897
Cauwet, G., 1991. Carbon inputs and biogeochemical processes at the halocline in a stratified estuary: Krka River, Yugoslavia. Mar. Chem. 32, 269–283. https://doi.org/10.1016/0304-4203(91)90043-V
Chen, S.Y., Huang, S.W., Chiang, P.N., Liu, J.C., Kuan, W.H., Huang, J.H., Hung, J.T., Tzou, Y.M., Chen, C.C., Wang, M.K., 2011. Influence of chemical compositions and molecular weights of humic acids on Cr(VI) photo-reduction. J. Hazard. Mater. 197, 337–344. https://doi.org/10.1016/j.jhazmat.2011.09.091
Cindrić, A.-M., Garnier, C., Oursel, B., Pižeta, I., Omanović, D., 2015. Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: The Krka River estuary (Adriatic, Croatia). Mar. Pollut. Bull. 94, 199–216. https://doi.org/10.1016/j.marpolbul.2015.02.029
Cindrić, A.-M.M., Marcinek, S., Garnier, C., Salaün, P., Cukrov, N., Oursel, B., Lenoble, V., Omanović, D., 2020. Evaluation of diffusive gradients in thin films (DGT) technique for speciation of trace metals in estuarine waters - A multimethodological approach. Sci. Total Environ. 721, 137784. https://doi.org/10.1016/j.scitotenv.2020.137784
Cobelo-García, A., López-Sánchez, D.E., Almécija, C., Santos-Echeandía, J., 2013. Behavior of platinum during estuarine mixing (Pontevedra Ria, NW Iberian Peninsula). Mar. Chem. 150, 11–18. https://doi.org/10.1016/j.marchem.2013.01.005
Cobelo-García, A., López-Sánchez, D.E., Schäfer, J., Petit, J.C.J., Blanc, G., Turner, A., 2014a. Behavior and fluxes of Pt in the macrotidal Gironde Estuary (SW France). Mar. Chem. 167, 93–101. https://doi.org/10.1016/j.marchem.2014.07.006
Cobelo-García, A., Santos-Echeandía, J., López-Sánchez, D.E., Almécija, C., Omanović, D., 2014b. Improving the voltammetric quantification of lll-defined peaks using second derivative signal transformation: Example of the determination of platinum in water and sediments. Anal. Chem. 86, 2308–2313. https://doi.org/10.1021/ac403558y
Cobelo-García, A., Turner, A., Millward, G.E., 2008. Fractionation and reactivity of platinum group elements during estuarine mixing. Environ. Sci. Technol. 42, 1096–1101. https://doi.org/10.1021/es0712118
Colodner, D.C., Boyle, E.A., Edmond, J.M., 1993. Determination of rhenium and platinum in natural waters and sediments, and iridium in sediments by flow injection isotope dilution inductively coupled plasma mass spectrometry. Anal. Chem. 65, 1419–1425. https://doi.org/10.1021/ac00058a019
Connelly, D.P., Statham, P.J., Knap, A.H., 2006. Seasonal changes in speciation of dissolved chromium in the surface Sargasso Sea. Deep. Res. Part I Oceanogr. Res. Pap. 53, 1975–1988. https://doi.org/10.1016/j.dsr.2006.09.005
Cortecci, G., Boschetti, T., Dinelli, E., Cidu, R., Podda, F., Doveri, M., 2009. Geochemistry of trace elements in surface waters of the Arno River Basin, northern Tuscany, Italy. Appl. Geochemistry 24, 1005–1022. https://doi.org/10.1016/j.apgeochem.2009.03.002

Cortecci, G., Dinelli, E., Bencini, A., Adorni-Braccesi, A., La Ruffa, G., 2002. Natural and anthropogenic SO4 sources in the Arno river catchment, northern Tuscany, Italy: a chemical and isotopic reconnaissance. Appl. Geochemistry 17, 79–92. https://doi.org/10.1016/S0883-2927(01)00100-7

Costa, M., Klein, C.B., 2006. Toxicity and carcinogenicity of chromium compounds in humans. Crit. Rev. Toxicol. 36, 155–163. https://doi.org/10.1080/10408440500534032

Crmarić, D., Marcinek, S., Cindrić, A.-M., Omanović, DarioBura-Nakić, E., 2024. Redox speciation of copper in the estuarine environment: towards better understanding of copper water chemistry. Mar. Chem. 265–266, under review.

Croot, P.L., Moffett, J.W., Brand, L.E., 2000. Production of extracellular Cu complexing ligands by eucaryotic phytoplankton in response to Cu stress. Limnol. Oceanogr. 45, 619–627. https://doi.org/10.4319/lo.2000.45.3.0619

Cuéllar, M., Vettorelo, S.N., Ortiz, P.I., Garay, F., 2016. Theoretical and experimental study of the catalytic cathodic stripping square-wave voltammetry of chromium species. J. Solid State Electrochem. 20, 3279–3286. https://doi.org/10.1007/s10008-016-3307-3

Cukrov, N., Cmuk, P., Mlakar, M., Omanović, D., 2008. Spatial distribution of trace metals in the Krka River, Croatia: An example of the self-purification. Chemosphere 72, 1559–1566. https://doi.org/10.1016/j.chemosphere.2008.04.038

Cutter, G., Casciotti, K., Croot, P.L., Geibert, W., Heimbürger, L.-E., Lohan, M.C., Planquette, H., van de Flierdt, T., 2017. Sampling and sample-handling protocols for GEOTRACES cruises. Version 3. GEOTRACES International Project Office, Toulouse, France, 139pp. & Appendices pp. https://doi.org/10.25607/OBP-2

Dahlheimer, S.R., Neal, C.R., Fein, J.B., 2007. Potential mobilization of platinum-group elements by siderophores in surface environments. Environ. Sci. Technol. 41, 870–875. https://doi.org/10.1021/es0614666

Dang, D.H., Omanović, D., Mucci, A., Wang, W., Sikma, A., Chatzis, A., 2022. The winter estuarine geochemistry of platinum in the Estuary and Gulf of St. Lawrence. Mar. Chem. 242, 104123. https://doi.org/10.1016/j.marchem.2022.104123

DeLoughery, Z., Luczak, M.W., Zhitkovich, A., 2014. Monitoring Cr intermediates and reactive oxygen species with fluorescent probes during chromate reduction. Chem. Res. Toxicol. 27, 843–851. https://doi.org/10.1021/tx500028x

Dinelli, E., Cortecci, G., Lucchini, F., Zantedeschi, E., 2005. Sources of major and trace elements in the stream sediments of the Arno river catchment (northern Tuscany, Italy). Geochem. J. 39, 531–545. https://doi.org/10.2343/geochemj.39.531

Dulaquais, G., Waeles, M., Breitenstein, J., Knoery, J., Riso, R., 2020. Links between size fractionation, chemical speciation of dissolved copper and chemical speciation of dissolved organic matter in the Loire estuary. Environ. Chem. 17, 385–399. https://doi.org/10.1071/EN19137

Elderfield, H., 1970. Chromium speciation in sea water. Earth Planet. Sci. Lett. 9, 10–16. https://doi.org/10.1016/0012-821X(70)90017-8

Espada-Bellido, E., Bi, Z., van den Berg, C.M.G., 2013. Determination of chromium in estuarine waters by catalytic cathodic stripping voltammetry using a vibrating silver amalgam microwire electrode. Talanta 105, 287–291. https://doi.org/10.1016/j.talanta.2012.10.031

Essumang, D., 2008. Bioaccumulation of platinum group metals in dolphins, Stenella sp., caught off Ghana. African J. Aquat. Sci. 33, 255–259. https://doi.org/10.2989/AJAS.2008.33.3.8.620

Fischer, L., Smith, G., Hann, S., Bruland, K.W., 2018. Ultra-trace analysis of silver and platinum in seawater by ICP-SFMS after off-line matrix separation and pre-concentration. Mar. Chem. 199, 44–52. https://doi.org/10.1016/j.marchem.2018.01.006

Frache, R., Abelmoschi, M.L., Grotti, M., Ianni, C., Magi, E., Soggia, F., Capodaglio, G., Turetta, C., Barbante, C., 2001. Effects of ice melting on Cu, Cd and Pb profiles in Ross Sea waters (Antarctica). Int. J. Environ. Anal. Chem. 79, 301–313. https://doi.org/10.1080/03067310108044391

Frei, R., Poiré, D., Frei, K.M., 2014. Weathering on land and transport of chromium to the ocean in a subtropical region (Misiones, NW Argentina): A chromium stable isotope perspective. Chem. Geol. 381, 110–124. https://doi.org/10.1016/j.chemgeo.2014.05.015

Gagnon, Z.E., Newkirk, C., Hicks, S., 2006. Impact of platinum group metals on the environment: A toxicological, genotoxic and analytical chemistry study. J. Environ. Sci. Heal. Part A 41, 397–414. https://doi.org/10.1080/10934520500423592
Gibbon-Walsh, K., Salaün, P., van den Berg, C.M.G., 2012. Pseudopolarography of copper complexes in seawater using a vibrating gold microwire electrode. J. Phys. Chem. A 116, 6609–6620. https://doi.org/10.1021/jp3019155
Glass, J.B., Orphan, V.J., 2012. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. Front. Microbiol. 3. https://doi.org/10.3389/fmicb.2012.00061
Gledhill, M., Buck, K., 2012. The organic complexation of iron in the marine environment: a review. Front. Microbiol. 3. https://doi.org/10.3389/fmicb.2012.00069
Goldberg, E.D., Hodge, V., Kay, P., Stallard, M., Koide, M., 1986. Some comparative marine chemistries of platinum and iridium. Appl. Geochemistry 1, 227–232. https://doi.org/10.1016/0883-2927(86)90006-5
Grabarczyk, M., Korolczuk, M., 2003. Modification of catalytic adsorptive stripping voltammetric method of hexavalent chromium determination in the presence of DTPA and nitrate. Anal. Bioanal. Chem. 376, 1115–1118. https://doi.org/10.1007/s00216-003-2026-7
Guo, J., Annett, A.L., Taylor, R.L., Lapi, S., Ruth, T.J., Maldonado, M.T., 2010. Copper-uptake kinetics of coastal and oceanic diatoms. J. Phycol. 46, 1218–1228. https://doi.org/10.1111/j.1529-8817.2010.00911.x
Hakemian, A.S., Tinberg, C.E., Kondapalli, K.C., Telser, J., Hoffman, B.M., Stemmler, T.L., Rosenzweig, A.C., 2005. The copper chelator methanobactin from methylosinus t richosporium OB3b binds copper(I). J. Am. Chem. Soc. 127, 17142–17143. https://doi.org/10.1021/ja0558140
Han, H., Pan, D., 2021. Voltammetric methods for speciation analysis of trace metals in natural waters. Trends Environ. Anal. Chem. 29, e00119. https://doi.org/10.1016/j.teac.2021.e00119
Hartland, A., Zitoun, R., Middag, R., Sander, S., Laferriere, A., Saeed, H., De Luca, S., Ross, P.M., 2019. Aqueous copper bioavailability linked to shipwreck-contaminated reef sediments. Sci. Rep. 9, 9573. https://doi.org/10.1038/s41598-019-45911-8
Heller, M.I., Croot, P.L., 2014. Copper speciation and distribution in the Atlantic sector of the Southern Ocean. Mar. Chem. 173, 253–268. https://doi.org/10.1016/j.marchem.2014.09.017
Hirata, S., Honda, K., Shikino, O., Maekawa, N., Aihara, M., 2000. Determination of chromium(III) and total chromium in seawater by on-line column preconcentration inductively coupled plasma mass spectrometry. Spectrochim. Acta Part B At. Spectrosc. 55, 1089–1099. https://doi.org/10.1016/S0584-8547(00)00169-5
Hodge, V., Stallard, M., Koide, M., Goldberg, E.D., 1986. Determination of platinum and iridium in marine waters, sediments, and organisms. Anal. Chem. 58, 616–620. https://doi.org/10.1021/ac00294a029
Hollister, A., Marcinek, S., Schmidt, K., Omanović, D., Schulte, M.B., Koschinsky, A., 2024. First indication of platinum input into the southern North Sea via German Rivers. Mar. Chem. 265–266. https://doi.org/10.1016/j.marchem.2024.104439
Hollister, A.P., Whitby, H., Seidel, M., Lodeiro, P., Gledhill, M., Koschinsky, A., 2021. Dissolved concentrations and organic speciation of copper in the Amazon River estuary and mixing plume. Mar. Chem. 234, 104005. https://doi.org/10.1016/j.marchem.2021.104005
Jacinto, G.S., van den Berg, C.M.G., 1989. Different behaviour of platinum in the Indian and Pacific Oceans. Nature 338, 332–334. https://doi.org/10.1038/338332a0
Jacquot, J.E., Horak, R.E.A., Amin, S.A., Devol, A.H., Ingalls, A.E., Armbrust, E.V., Stahl, D.A., Moffett, J.W., 2014. Assessment of the potential for copper limitation of ammonia oxidation by Archaea in a dynamic estuary. Mar. Chem. 162, 37–49. https://doi.org/10.1016/j.marchem.2014.02.002
Jacquot, J.E., Moffett, J.W., 2015. Copper distribution and speciation across the International GEOTRACES Section GA03. Deep Sea Res. Part II Top. Stud. Oceanogr. 116, 187–207. https://doi.org/10.1016/j.dsr2.2014.11.013
Jakimska, A., Konieczka, P., Skóra, K., Namieśnik, J., 2011. Bioaccumulation of metals in tissues of marine animals, Part II: metal concentrations in animal tissues. Polish J. Environ. Stud. 20.
Jeong, H., Ali, W., Zinck, P., Souissi, S., Lee, J.-S., 2024. Toxicity of methylmercury in aquatic

organisms and interaction with environmental factors and coexisting pollutants: A review. Sci. Total Environ. 943, 173574. https://doi.org/10.1016/j.scitotenv.2024.173574
Kaczynski, S.E., Kieber, R.J., 1993. Aqueous trivalent chromium photoproduction in natural waters. Environ. Sci. Technol. 27, 1572–1576. https://doi.org/10.1021/es00045a011
Karavoltsos, S., Sakellari, A., Strmečki, S., Plavšić, M., Ioannou, E., Roussis, V., Dassenakis, M., Scoullos, M., 2013. Copper complexing properties of exudates and metabolites of macroalgae from the Aegean Sea. Chemosphere 91, 1590–1595. https://doi.org/10.1016/j.chemosphere.2012.12.053
Karbassi, A.R., Bassam, S.S., Ardestani, M., 2013. Flocculation of Cu, Mn, Ni, Pb, and Zn during estuarine mixing (Caspian Sea). Int. J. Environ. Res. 7, 917–924. https://doi.org/10.22059/ijer.2013.674
Kiaune, L., Singhasemanon, N., 2011. Pesticidal copper (I) oxide: Environmental fate and aquatic toxicity. pp. 1–26. https://doi.org/10.1007/978-1-4419-9860-6_1
Kikuchi, T., Fujii, M., Terao, K., Jiwei, R., Lee, Y.P., Yoshimura, C., 2017. Correlations between aromaticity of dissolved organic matter and trace metal concentrations in natural and effluent waters: A case study in the Sagami River Basin, Japan. Sci. Total Environ. 576, 36–45. https://doi.org/10.1016/j.scitotenv.2016.10.068
Kim, H.J., Graham, D.W., DiSpirito, A.A., Alterman, M.A., Galeva, N., Larive, C.K., Asunskis, D., Sherwood, P.M.A., 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. Science (80-.). 305, 1612–1615. https://doi.org/10.1126/science.1098322
Kogut, M.B., Voelker, B.M., 2001. Strong copper-binding behavior of terrestrial humic substances in seawater. Environ. Sci. Technol. 35, 1149–1156. https://doi.org/10.1021/es0014584
Komorsky-Lovrić, Š., Lovrić, M., Branica, M., 1986. Application of asv for trace metal speciation. J. Electroanal. Chem. Interfacial Electrochem. 214, 37–50. https://doi.org/10.1016/0022-0728(86)80084-5
Korolczuk, M., 2000. Voltammetric determination of traces of Cr(VI) in the presence of Cr(III) and humic acid. Anal. Chim. Acta 414, 165–171. https://doi.org/10.1016/S0003-2670(00)00793-5
Korolczuk, M., Grabarczyk, M., 1999. Voltammetric determination of Cr(VI) in a flow system in the presence of diethylenetriaminepentaacetic acid (DTPA) following its deposition in the metallic state. Anal. Chim. Acta 387, 97–102. https://doi.org/10.1016/S0003-2670(99)00013-6
Krznarić, D., Plavšić, M., Ćosović, B., 1994. Voltammetric investigations of the effect of triton X-100 on copper processes in the presence of oxygen. Electroanalysis 6, 131–137. https://doi.org/10.1002/elan.1140060210
Lagerström, M., Lindgren, J.F., Holmqvist, A., Dahlström, M., Ytreberg, E., 2018. In situ release rates of Cu and Zn from commercial antifouling paints at different salinities. Mar. Pollut. Bull. 127, 289–296. https://doi.org/10.1016/j.marpolbul.2017.12.027
Lazo, P., 2009. Determination of Cr (VI) in environmental samples evaluating Cr (VI) impact in a contaminated area. J. Int. Environ. Appl. Sci. 4, 207–213.
Legittimo, P.C., Panta, S. Del, Pantani, F., 1991. Assessment of the water quality of the river Arno in the area of Florence, Italy. Chem. Ecol. 5, 185–195. https://doi.org/10.1080/02757549108035249
Legović, T., Petricioli, D., Žutić, V., 1991. Hypoxia in a pristine stratified estuary (Krka, Adriatic Sea). Mar. Chem. 32, 347–359. https://doi.org/10.1016/0304-4203(91)90048-2
Legović, T., Žutić, V., Gržetić, Z., Cauwet, G., Precali, R., Viličić, D., 1994. Eutrophication in the Krka estuary. Mar. Chem. 46, 203–215. https://doi.org/10.1016/0304-4203(94)90056-6
Lewis, B.L., Luther, G.W., Lane, H., Church, T.M., 1995. Determination of metal–organic complexation in natural waters by SWASV with pseudopolarograms. Electroanalysis 7, 166–177. https://doi.org/10.1002/elan.1140070213
Li, S.X., Zheng, F.Y., Hong, H.S., Deng, N. sheng, Lin, L.X., 2009. Influence of marine phytoplankton, transition metals and sunlight on the species distribution of chromium in surface seawater. Mar. Environ. Res. 67, 199–206. https://doi.org/10.1016/j.marenvres.2009.02.001
Li, Y., Xue, H., 2001. Determination of Cr(III) and Cr(VI) species in natural waters by catalytic cathodic stripping voltammetry. Anal. Chim. Acta 448, 121–134. https://doi.org/10.1016/S0003-2670(01)01314-9
Liang, J., Huang, X., Yan, J., Li, Y., Zhao, Z., Liu, Y., Ye, J., Wei, Y., 2021. A review of the

formation of Cr(VI) via Cr(III) oxidation in soils and groundwater. Sci. Total Environ. 774, 145762. https://doi.org/10.1016/j.scitotenv.2021.145762
Liang, J., Ma, C.-W., Kim, K.-B., 2024. Comparing the environmental impacts of pollution from two types of industrial zones on the coast. Front. Mar. Sci. 11. https://doi.org/10.3389/fmars.2024.1433536
Lin, Yung-Chang, Chang-Chien, G.-P., Chiang, P.-C., Chen, W.-H., Lin, Yuan-Chung, 2013. Multivariate analysis of heavy metal contaminations in seawater and sediments from a heavily industrialized harbor in Southern Taiwan. Mar. Pollut. Bull. 76, 266–275. https://doi.org/10.1016/j.marpolbul.2013.08.027
Little, S.H., Vance, D., Walker-Brown, C., Landing, W.M., 2014. The oceanic mass balance of copper and zinc isotopes, investigated by analysis of their inputs, and outputs to ferromanganese oxide sediments. Geochim. Cosmochim. Acta 125, 673–693. https://doi.org/10.1016/j.gca.2013.07.046
Liu, K., Gao, X., Li, L., Chen, C.-T.A., Xing, Q., 2018. Determination of ultra-trace Pt, Pd and Rh in seawater using an off-line pre-concentration method and inductively coupled plasma mass spectrometry. Chemosphere 212, 429–437. https://doi.org/10.1016/j.chemosphere.2018.08.098
Locatelli, C., 2005. Platinum, Rhodium, Palladium and Lead: Elements Linked to Vehicle Emissions. Their Simultaneous Voltammetric Determination in Superficial Water. Electroanalysis 17, 140–147. https://doi.org/10.1002/elan.200403082
López-Sánchez, D.E., Cobelo-García, A., Rijkenberg, M.J.A., Gerringa, L.J.A., de Baar, H.J.W., 2019. New insights on the dissolved platinum behavior in the Atlantic Ocean. Chem. Geol. 511, 204–211. https://doi.org/10.1016/j.chemgeo.2019.01.003
Louis, Y., Cmuk, P., Omanović, D., Garnier, C., Lenoble, V., Mounier, S., Pižeta, I., 2008. Speciation of trace metals in natural waters: The influence of an adsorbed layer of natural organic matter (NOM) on voltammetric behaviour of copper. Anal. Chim. Acta 606, 37–44. https://doi.org/10.1016/j.aca.2007.11.011
Louis, Y., Garnier, C., Lenoble, V., Mounier, S., Cukrov, N., Omanović, D., Pižeta, I., 2009. Kinetic and equilibrium studies of copper-dissolved organic matter complexation in water column of the stratified Krka River estuary (Croatia). Mar. Chem. 114, 110–119. https://doi.org/10.1016/j.marchem.2009.04.006
Lovrić, M., Branica, M., 1980. Application of ASV for trace metal speciation II. Digital simulation of neopolarogram using hanging-mercury-drop electrode. Croat. Chem. Acta 53, 477–483.
Luther, G.W., Swartz, C.B., Ullman, W.J., 1988. Direct determination of iodide in seawater by cathodic stripping square wave voltammetry. Anal. Chem. 60, 1721–1724. https://doi.org/10.1021/ac00168a017
Mann, E.L., Ahlgren, N., Moffett, J.W., Chisholm, S.W., 2002. Copper toxicity and cyanobacteria ecology in the Sargasso Sea. Limnol. Oceanogr. 47, 976–988. https://doi.org/10.4319/lo.2002.47.4.0976
Marcinek, S., Chapoulie, A., Salaün, P., Smith, S., Omanović, D., 2021. Revised application of copper ion selective electrode (Cu-ISE) in marine waters: A new meta-calibration approach. Talanta 226. https://doi.org/10.1016/j.talanta.2021.122170
Marcinek, S., Cindrić, A.M., Omanović, D., 2025. Influence of seasonal changes in organic matter pool on copper bioavailability in a stratified estuary. Mar. Chem. 270. https://doi.org/10.1016/j.marchem.2025.104513
Marcinek, S., Cindrić, A.M., Padan, J., Omanović, D., Pađan, J., Omanović, D., Padan, J., Omanović, D., 2022. Trace metal partitioning in the salinity gradient of the highly stratified estuary: A case study in the Krka River estuary (Croatia). Appl. Sci. 12. https://doi.org/10.3390/app12125816
Marcinek, S., Galceran, J., Ciglenečki, I., Omanović, D., 2023. A new tool for the determination of humic substances in natural waters: Pulsed voltammetry approach. Talanta 259. https://doi.org/10.1016/j.talanta.2023.124547
Marcinek, S., Santinelli, C., Cindrić, A.M., Evangelista, V., Gonnelli, M., Layglon, N., Mounier, S., Lenoble, V., Omanović, D., 2020. Dissolved organic matter dynamics in the pristine Krka River estuary (Croatia). Mar. Chem. 225. https://doi.org/10.1016/j.marchem.2020.103848
Markiewicz, B., Komorowicz, I., Sajnóg, A., Belter, M., Barałkiewicz, D., 2015. Chromium and its speciation in water samples by HPLC/ICP-MS – technique establishing metrological traceability: A review since 2000. Talanta 132, 814–828.

https://doi.org/10.1016/j.talanta.2014.10.002
Mashio, A.S., Obata, H., Gamo, T., 2017. Dissolved platinum concentrations in coastal seawater: Boso to Sanriku areas, Japan. Arch. Environ. Contam. Toxicol. 73, 240–246. https://doi.org/10.1007/s00244-017-0373-1
Mashio, A.S., Obata, H., Tazoe, H., Tsutsumi, M., Ferrer i Santos, A., Gamo, T., 2016. Dissolved platinum in rainwater, river water and seawater around Tokyo Bay and Otsuchi Bay in Japan. Estuar. Coast. Shelf Sci. 180, 160–167. https://doi.org/10.1016/j.ecss.2016.07.002
Mason, R.P., 2013. Trace metals in aquatic systems. John Wiley & sons, New York.
Mellett, T., Buck, K.N., 2020. Spatial and temporal variability of trace metals (Fe, Cu, Mn, Zn, Co, Ni, Cd, Pb), iron and copper speciation, and electroactive Fe-binding humic substances in surface waters of the eastern Gulf of Mexico. Mar. Chem. 227, 103891. https://doi.org/10.1016/j.marchem.2020.103891
Migon, C., Heimbürger-Boavida, L.-E., Dufour, A., Chiffoleau, J.-F., Cossa, D., 2020. Temporal variability of dissolved trace metals at the DYFAMED time-series station, Northwestern Mediterranean. Mar. Chem. 225, 103846. https://doi.org/10.1016/j.marchem.2020.103846
Moffett, J.W., Brand, L.E., 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnol. Oceanogr. 41, 388–395. https://doi.org/10.4319/lo.1996.41.3.0388
Moffett, J.W., Brand, L.E., Croot, P.L., Barbeau, K.A., 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnol. Oceanogr. 42, 789–799. https://doi.org/10.4319/lo.1997.42.5.0789
Monteiro, C.E., Cobelo-García, A., Correia dos Santos, M.M., Caetano, M., 2021. Drivers of Rh and Pt variability in the water column of a hydrodynamic estuary: Effects of contrasting environments. Sci. Total Environ. 760, 143909. https://doi.org/10.1016/j.scitotenv.2020.143909
Muller, F.L.L., Tankéré-Muller, S.P.C., Tang, C.H., 2024. Terrigenous humic substances regulate the concentrations of dissolved Fe and Cu (but not Al, Mn, Ni or Zn) in the Gaoping River plume. Sci. Total Environ. 906. https://doi.org/10.1016/j.scitotenv.2023.167374
Neira, P., Cobelo-García, A., Besada, V., Santos-Echeandía, J., Bellas, J., 2015. Evidence of increased anthropogenic emissions of platinum: Time-series analysis of mussels (1991–2011) of an urban beach. Sci. Total Environ. 514, 366–370. https://doi.org/10.1016/j.scitotenv.2015.02.016
Niewiara, E., Baś, B., Kubiak, W.W., 2007. Elimination of SAS interferences in catalytic adsorptive stripping voltammetric determination of Cr(VI) by means of fumed silica. Electroanalysis 19, 2185–2193. https://doi.org/10.1002/elan.200703949
Nurmi, J.T., Tratnyek, P.G., 2002. Electrochemical properties of natural organic matter (NOM), fractions of NOM, and model biogeochemical electron shuttles. Environ. Sci. Technol. 36, 617–624. https://doi.org/10.1021/es0110731
Obata, H., Yoshida, T., Ogawa, H., 2006. Determination of picomolar levels of platinum in estuarine waters: A comparison of cathodic stripping voltammetry and isotope dilution-inductively coupled plasma mass spectrometry. Anal. Chim. Acta 580, 32–38. https://doi.org/10.1016/j.aca.2006.07.044
Omanović, D., Branica, M., 2004. Pseudopolarography of trace metals. Part II. The comparison of the reversible, quasireversible and irreversible electrode reactions. J. Electroanal. Chem. 565, 37–48. https://doi.org/10.1016/j.jelechem.2003.09.026
Omanović, D., Garnier, C., Pižeta, I., 2015. ProMCC: An all-in-one tool for trace metal complexation studies. Mar. Chem. https://doi.org/10.1016/j.marchem.2014.10.011
Omanović, D., Pižeta, I., Peharec, Ž., Branica, M., 1996. Voltammetric determination of the metal complexing capacity in model solutions. Mar. Chem. 53, 121–129. https://doi.org/10.1016/0304-4203(96)00018-7
Oursel, B., Garnier, C., Durrieu, G., Mounier, S., Omanović, D., Lucas, Y., 2013. Dynamics and fates of trace metals chronically input in a Mediterranean coastal zone impacted by a large urban area. Mar. Pollut. Bull. 69, 137–149. https://doi.org/10.1016/j.marpolbul.2013.01.023
Pađan, J., Marcinek, S., Cindrić, A.-M.M., Layglon, N., Garnier, C., Salaün, P., Cobelo-García, A., Omanović, D., 2020. Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology. Environ. Chem. 17, 77. https://doi.org/10.1071/EN19157

Pađan, J., Marcinek, S., Cindrić, A.-M.M., Layglon, N., Lenoble, V., Salaün, P., Garnier, C., Omanović, D., 2019. Improved voltammetric methodology for chromium redox speciation in estuarine waters. Anal. Chim. Acta 1089, 40–47. https://doi.org/10.1016/j.aca.2019.09.014
Pađan, J., Marcinek, S., Cindrić, A.M., Santinelli, C., Retelletti Brogi, S., Radakovitch, O., Garnier, C., Omanović, D., 2021. Organic copper speciation by anodic stripping voltammetry in estuarine waters with high dissolved organic matter. Front. Chem. 8, 1–16. https://doi.org/10.3389/fchem.2020.628749
Panagopoulos, A., Giannika, V., 2024. A comprehensive assessment of the economic and technical viability of a zero liquid discharge (ZLD) hybrid desalination system for water and salt recovery. J. Environ. Manage. 359, 121057. https://doi.org/10.1016/j.jenvman.2024.121057
Paulukat, C., Gilleaudeau, G.J., Chernyavskiy, P., Frei, R., 2016. The Cr-isotope signature of surface seawater — A global perspective. Chem. Geol. 444, 101–109. https://doi.org/10.1016/j.chemgeo.2016.10.004
Peers, G., Price, N.M., 2006. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 441, 341–344. https://doi.org/10.1038/nature04630
Peers, G., Quesnel, S.-A., Price, N.M., 2005. Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. Limnol. Oceanogr. 50, 1149–1158. https://doi.org/10.4319/lo.2005.50.4.1149
Pérez, P., Beiras, R., Fernández, E., 2010. Monitoring copper toxicity in natural phytoplankton assemblages: application of Fast Repetition Rate fluorometry. Ecotoxicol. Environ. Saf. 73, 1292–1303. https://doi.org/10.1016/j.ecoenv.2010.06.008
Petricioli, D., Bakran-Petricioli, T., Viličič, D., Požar-Domac, A., 1996. Freshwater Phytoplankton Bloom in Visovac Lake - A Possible Cause of Benthic Mortality in Krka Estuary (Adriatic Sea, Croatia). Mar. Ecol. 17, 373–382. https://doi.org/10.1111/j.1439-0485.1996.tb00515.x
Pinheiro, J.P., Galceran, J., Rotureau, E., Companys, E., Puy, J., 2020. Full wave analysis of stripping chronopotentiometry at scanned deposition potential (SSCP): Obtaining binding curves in labile heterogeneous macromolecular systems for any metal-to-ligand ratio. J. Electroanal. Chem. 873, 114436. https://doi.org/10.1016/j.jelechem.2020.114436
Pižeta, I., Sander, S.G., Hudson, R.J.M., Omanović, D., Baars, O., Barbeau, K.A., Buck, K.N., Bundy, R.M., Carrasco, G., Croot, P.L., Garnier, C., Gerringa, L.J.A., Gledhill, M., Hirose, K., Kondo, Y., Laglera, L.M., Nuester, J., Rijkenberg, M.J.A., Takeda, S., Twining, B.S., Wells, M., 2015. Interpretation of complexometric titration data: An intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. Mar. Chem. 173, 3–24. https://doi.org/10.1016/j.marchem.2015.03.006
Plavšić, M., Krznarić, D., Branica, M., 1982. Determination of the apparent copper complexing capacity of seawater by anodic stripping voltammetry. Mar. Chem. 11, 17–31. https://doi.org/10.1016/0304-4203(82)90045-7
Plavšić, M., Krznarić, D., Ćosović, B., 1994. The electrochemical processes of copper in the presence of triton X-100. Electroanalysis 6, 469–474. https://doi.org/10.1002/elan.1140060518
Plavšić, M., Kwokal, Ž., Strmečki, S., Peharec, Ž., Omanović, D., Branica, M., 2009. Determination of the copper complexing ligands in the Krka River estuary. Fresenius Environ. Bull. 18, 327–334.
Qambrani, N.A., Hwang, J.-H., Oh, S.-E., 2016. Comparison of chromium III and VI toxicities in water using sulfur-oxidizing bacterial bioassays. Chemosphere 160, 342–348. https://doi.org/10.1016/j.chemosphere.2016.06.090
Ravindra, K., Bencs, L., Van Grieken, R., 2004. Platinum group elements in the environment and their health risk. Sci. Total Environ. 318, 1–43. https://doi.org/10.1016/S0048-9697(03)00372-3
Retelletti Brogi, S., Balestra, C., Casotti, R., Cossarini, G., Galletti, Y., Gonnelli, M., Vestri, S., Santinelli, C., 2020. Time resolved data unveils the complex DOM dynamics in a Mediterranean river. Sci. Total Environ. 733, 139212. https://doi.org/10.1016/j.scitotenv.2020.139212
Rickli, J., Janssen, D.J., Hassler, C., Ellwood, M.J., Jaccard, S.L., 2019. Chromium biogeochemistry and stable isotope distribution in the Southern Ocean. Geochim. Cosmochim. Acta 262, 188–206. https://doi.org/10.1016/j.gca.2019.07.033
Rowe, D.R., Abdel-Magid, I.M., 2020. Handbook of wastewater reclamation and reuse. CRC Press. https://doi.org/10.1201/9780138752514

Roy, C., 2000. Marine Geochemistry. Blackwell Science Ltd.
Ruacho, A., Richon, C., Whitby, H., Bundy, R.M., 2022. Sources, sinks, and cycling of dissolved organic copper binding ligands in the ocean. Commun. Earth Environ. 3, 1–19. https://doi.org/10.1038/s43247-022-00597-1
Ruchter, N., Zimmermann, S., Sures, B., 2015. Field studies on PGE in aquatic ecosystems, in: Zereini, F., Wiseman, C.L.S. (Eds.), Platinum Metals in the Environment. pp. 351–360. https://doi.org/10.1007/978-3-662-44559-4_22
Rudnick, R.L., Gao, S., 2003. Composition of the Continental Crust, in: Rudnick, R.L. (Ed.), Treatise on Geochemistry. Elsevier, Amsterdam, pp. 1–64. https://doi.org/10.1016/B0-08-043751-6/03016-4
Sánchez-Marín, P., 2020. A review of chemical speciation techniques used for predicting dissolved copper bioavailability in seawater. Environ. Chem. 17, 469–478. https://doi.org/10.1071/EN19266
Sander, S., Koschinsky, A., 2000. Onboard-ship redox speciation of chromium in diffuse hydrothermal fluids from the North Fiji Basin. Mar. Chem. 71, 83–102. https://doi.org/10.1016/S0304-4203(00)00042-6
Sander, S., Koschinsky, A., Halbach, P., 2003a. Redox speciation of chromium in the oceanic water column of the Lesser Antilles and offshore Otago Peninsula, New Zealand. Mar. Freshw. Res. 54, 745. https://doi.org/10.1071/MF02074
Sander, S., Navrátil, T., Novotný, L., 2003b. Study of the complexation, adsorption and electrode reaction mechanisms of chromium(VI) and (III) with DTPA under adsorptive stripping voltammetric conditions. Electroanalysis 15, 1513–1521. https://doi.org/10.1002/elan.200302735
Sander, S.G., Buck, K.N., Wells, M., 2015. The effect of natural organic ligands on trace metal speciation in San Francisco Bay: Implications for water quality criteria. Mar. Chem. 173, 269–281. https://doi.org/10.1016/j.marchem.2014.09.015
Sander, S.G., Koschinsky, A., 2011. Metal flux from hydrothermal vents increased by organic complexation. Nat. Geosci. 4, 145–150. https://doi.org/10.1038/ngeo1088
Santos, L., Pinto, A., Filipe, O., Cunha, Â., Santos, E.B.H., Almeida, A., 2016. Insights on the optical properties of estuarine DOM - Hydrological and biological influences. PLoS One 11, 1–26. https://doi.org/10.1371/journal.pone.0154519
Schiff, K., Brown, J., Diehl, D., Greenstein, D., 2007. Extent and magnitude of copper contamination in marinas of the San Diego region, California, USA. Mar. Pollut. Bull. 54, 322–328. https://doi.org/10.1016/j.marpolbul.2006.10.013
Schlosser, C., Streu, P., Croot, P.L., 2013. Vivaspin ultrafiltration: A new approach for high resolution measurements of colloidal and soluble iron species. Limnol. Oceanogr. Methods 11, 187–201. https://doi.org/10.4319/lom.2013.11.187
Scholz, F., 2015. Voltammetric techniques of analysis: the essentials. ChemTexts 1, 17. https://doi.org/10.1007/s40828-015-0016-y
Semeniuk, D.M., Maldonado, M.T., Jaccard, S.L., 2016. Chromium uptake and adsorption in marine phytoplankton - Implications for the marine chromium cycle. Geochim. Cosmochim. Acta 184, 41–54. https://doi.org/10.1016/j.gca.2016.04.021
Sen, I.S., Peucker-Ehrenbrink, B., 2012. Anthropogenic disturbance of element cycles at the Earth's surface. Environ. Sci. Technol. 46, 8601–8609. https://doi.org/10.1021/es301261x
Serrano, N., Díaz-Cruz, J.M., Ariño, C., Esteban, M., Puy, J., Companys, E., Galceran, J., Cecilia, J., 2007. Full-wave analysis of stripping chronopotentiograms at scanned deposition potential (SSCP) as a tool for heavy metal speciation: Theoretical development and application to Cd(II)-phthalate and Cd(II)-iodide systems. J. Electroanal. Chem. 600, 275–284. https://doi.org/10.1016/j.jelechem.2006.10.007
Singh, V., Abhishek, K., Nand Rai, S., Singh, S.K., Vamanu, E., Kumar, A., 2023. Source of Cr(VI) in the aquatic ecosystem, its genotoxic effects and microbial removal from contaminated water. Green Chem. Lett. Rev. 16. https://doi.org/10.1080/17518253.2023.2267079
Šipoš, L., Valenta, P., Nurnberg, H.W., Branica, M., 1977. Applications of polarography and voltammetry to marine and aquatic chemistry. J. Electroanal. Chem. Interfacial Electrochem. 77, 263–266. https://doi.org/10.1016/S0022-0728(77)80479-8

Soyol-Erdene, T.-O.O., Huh, Y., Hong, S., Hur, S. Do, 2011. A 50-year record of platinum, iridium, and rhodium in Antarctic snow: Volcanic and anthropogenic sources. Environ. Sci. Technol. 45, 5929–5935. https://doi.org/10.1021/es2005732
Soyol-Erdene, T., Huh, Y., 2012. Dissolved platinum in major rivers of East Asia: Implications for the oceanic budget. Geochemistry, Geophys. Geosystems 13. https://doi.org/10.1029/2012GC004102
Strivens, J., Hayman, N., Johnston, R., Rosen, G., 2019. Effects of dissolved organic carbon on copper toxicity to embryos of Mytilus galloprovincialis as measured by diffusive gradient in thin films. Environ. Toxicol. Chem. 38, 1029–1034. https://doi.org/10.1002/etc.4404
Strmečki, S., Dešpoja, I., Penezić, A., Milinković, A., Alempijević, S.B., Kiss, G., Hoffer, A., Mitić, B., Hruševar, D., Frka, S., 2024. How do certain atmospheric aerosols affect Cu-binding organic ligands in the oligotrophic coastal sea surface microlayer? Environ. Sci. Process. Impacts 26, 119–135. https://doi.org/10.1039/D3EM00415E
Sunda, W.G., Tester, P.A., Huntsman, S.A., 1990. Toxicity of trace metals to Acartia tonsa in the Elizabeth River and southern Chesapeake Bay. Estuar. Coast. Shelf Sci. 30, 207–221. https://doi.org/10.1016/0272-7714(90)90048-V
Sunda, W.G., Tester, P.A., Huntsman, S.A., 1987. Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. Mar. Biol. 94, 203–210. https://doi.org/10.1007/BF00392932
Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J., Parker, D., Verity, R., Webb, S.M., 2004. Biogenic manganese oxides: Properties and mechanisms of formation. Annu. Rev. Earth Planet. Sci. 32, 287–328. https://doi.org/10.1146/annurev.earth.32.101802.120213
Templeton, D.M., Fujishiro, H., 2017. Terminology of elemental speciation – An IUPAC perspective. Coord. Chem. Rev. 352, 424–431. https://doi.org/10.1016/j.ccr.2017.02.002
Tercier Waeber, M.-L., Stoll, S., Slaveykova, V., 2012. Trace metal behavior in surface waters: emphasis on dynamic speciation, sorption processes and bioavailability. Arch. des Sci. 65, 119–142.
Town, R.M., 1997. Potentiometric stripping analysis and anodic stripping voltammetry for measurement of copper(<scp>II</scp>) and lead(<scp>II</scp>) complexation by fulvic acid: A comparative study. Electroanalysis 9, 407–415. https://doi.org/10.1002/elan.1140090511
Town, R.M., Filella, M., 2000. A comprehensive systematic compilation of complexation parameters reported for trace metals in natural waters. Aquat. Sci. 62, 252–295. https://doi.org/10.1007/PL00001335
Town, R.M., van Leeuwen, H.P., 2019. Stripping chronopotentiometry at scanned deposition potential (SSCP): An effective methodology for dynamic speciation analysis of nanoparticulate metal complexes. J. Electroanal. Chem. 853, 113530. https://doi.org/10.1016/j.jelechem.2019.113530
Tsoi, Y.-K., Sze-Yin Leung, K., 2010. Simultaneous determination of seven elemental species in estuarine waters by LC-ICP-DRC-MS. J. Anal. At. Spectrom. 25, 880. https://doi.org/10.1039/b923343a
Turetta, C., Cozzi, G., Varga, A., Barbante, C., Capodaglio, G., Cescon, P., 2003. Platinum group elements determination in seawater by ICP-SFMS: Initial results. J. Phys. IV 107, 1321–1324. https://doi.org/10.1051/jp4:20030544
Turner, A., 2007. Particle–water interactions of platinum group elements under estuarine conditions. Mar. Chem. 103, 103–111. https://doi.org/10.1016/j.marchem.2006.08.002
Turner, A., 1996. Trace-metal partitioning in estuaries: Importance of salinity and particle concentration. Mar. Chem. 54, 27–39. https://doi.org/10.1016/0304-4203(96)00025-4
Turner, A., Lewis, M.S., Shams, L., Brown, M.T., 2007. Uptake of platinum group elements by the marine macroalga, Ulva lactuca. Mar. Chem. 105, 271–280. https://doi.org/10.1016/j.marchem.2007.02.009
Turner, D.., Whitfield, M., Dickson, A.., 1981. The equilibrium speciation of dissolved components in freshwater and sea water at 25°C and 1 atm pressure. Geochim. Cosmochim. Acta 45, 855–881. https://doi.org/10.1016/0016-7037(81)90115-0
Umbría-Salinas, K., Valero, A., Martins, S.E., Wallner-Kersanach, M., 2021. Copper ecological risk assessment using DGT technique and PNEC: A case study in the Brazilian coast. J. Hazard. Mater. 403, 123918. https://doi.org/10.1016/j.jhazmat.2020.123918

Vaiopoulou, E., Gikas, P., 2020. Regulations for chromium emissions to the aquatic environment in Europe and elsewhere. Chemosphere 254, 126876. https://doi.org/10.1016/j.chemosphere.2020.126876
Van Den Berg, C.M.G., 1984. Organic and inorganic speciation of copper in the Irish Sea. Mar. Chem. 14, 201–212. https://doi.org/10.1016/0304-4203(84)90042-2
van den Berg, C.M.G., Jacinto, G.S., 1988. The determination of platinum in sea water by adsorptive cathodic stripping voltammetry. Anal. Chim. Acta 211, 129–139. https://doi.org/10.1016/S0003-2670(00)83675-2
Vance, D., Archer, C., Bermin, J., Perkins, J., Statham, P.J., Lohan, M.C., Ellwood, M.J., Mills, R.A., 2008. The copper isotope geochemistry of rivers and the oceans. Earth Planet. Sci. Lett. 274, 204–213. https://doi.org/10.1016/j.epsl.2008.07.026
Vasquez, A.C., He, Z., Guo, J., Yang, S., 2024. Spatial variation of dissolved copper isotope systematics in the Changjiang (Yangtze) River: Response to weathering processes and anthropogenic impacts. Chem. Geol. 649, 121977. https://doi.org/10.1016/j.chemgeo.2024.121977
Velma, V., Tchounwou, P.B., 2010. Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, carassius auratus. Mutat. Res. Toxicol. Environ. Mutagen. 698, 43–51. https://doi.org/10.1016/j.mrgentox.2010.03.014
Vercoutere, K., Cornelis, R., Mees, L., Quevauviller, P., 1998. Certification of the contents of the chromium(III) and chromium(VI) species and total chromium in a lyophilised solution (CRM 544)†. Analyst 123, 965–969. https://doi.org/10.1039/a705040b
Viličić, D., Legović, T., Žutić, V., 1989. Vertical distribution of phytoplankton in a stratified estuary. Aquat. Sci. 51, 31–46. https://doi.org/10.1007/BF00877779
Voelker, B.M., Kogut, M.B., 2001. Interpretation of metal speciation data in coastal waters: The effects of humic substances on copper binding as a test case. Mar. Chem. 74, 303–318. https://doi.org/10.1016/S0304-4203(01)00022-6
Vraspir, J.M., Butler, A., 2009. Chemistry of marine ligands and siderophores. Ann. Rev. Mar. Sci. 1, 43–63. https://doi.org/10.1146/annurev.marine.010908.163712
Vyas, N., Turner, A., Sewell, G., 2014. Platinum-based anticancer drugs in waste waters of a major UK hospital and predicted concentrations in recipient surface waters. Sci. Total Environ. 493, 324–329. https://doi.org/10.1016/j.scitotenv.2014.05.127
Wang, C., Cheng, T., Zhang, D., Pan, X., 2023. Electrochemical properties of humic acid and its novel applications: A tip of the iceberg. Sci. Total Environ. 863, 160755. https://doi.org/10.1016/j.scitotenv.2022.160755
Wasiński, K., Walkowiak, M., Lota, G., 2014. Humic acids as pseudocapacitive electrolyte additive for electrochemical double layer capacitors. J. Power Sources 255, 230–234. https://doi.org/10.1016/j.jpowsour.2013.12.140
Wells, M.L., 2002. Marine colloids and trace metals, in: Carlson, C.A., Hansell, D.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 367-404.
Whitby, H., 2016. Identifying the factors affecting copper speciation in estuarine, coastal and open ocean waters. Dr. Thesis. University of Liverpool.
Whitby, H., Hollibaugh, J.T., van den Berg, C.M.G., 2017. Chemical speciation of copper in a salt marsh estuary and bioavailability to Thaumarchaeota. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00178
Whitby, H., Posacka, A.M., Maldonado, M.T., van den Berg, C.M.G., 2018. Copper-binding ligands in the NE Pacific. Mar. Chem. 204, 36–48. https://doi.org/10.1016/j.marchem.2018.05.008
Whitby, H., van den Berg, C.M.G., 2015. Evidence for copper-binding humic substances in seawater. Mar. Chem. 173, 282–290. https://doi.org/10.1016/j.marchem.2014.09.011
White, E.M., Vaughan, P.P., Zepp, R.G., 2003. Role of the photo-Fenton reaction in the production of hydroxyl radicals and photobleaching of colored dissolved organic matter in a coastal river of the southeastern United States. Aquat. Sci. - Res. Across Boundaries 65, 402–414. https://doi.org/10.1007/s00027-003-0675-4
Wong, K.H., Obata, H., Kim, T., Mashio, A.S., Fukuda, H., Ogawa, H., 2018. Organic complexation of copper in estuarine waters: An assessment of the multi-detection window approach. Mar. Chem. 204, 144–151. https://doi.org/10.1016/j.marchem.2018.07.001

Wong, K.H., Obata, H., Kim, T., Wakuta, Y., Takeda, S., 2019. Distribution and speciation of copper and its relationship with FDOM in the East China Sea. Mar. Chem. 212, 96–107. https://doi.org/10.1016/j.marchem.2019.04.005

Wu, Y.-S., Osman, A.I., Hosny, M., Elgarahy, A.M., Eltaweil, A.S., Rooney, D.W., Chen, Z., Rahim, N.S., Sekar, M., Gopinath, S.C.B., Mat Rani, N.N.I., Batumalaie, K., Yap, P.-S., 2024. The toxicity of mercury and its chemical compounds: molecular mechanisms and environmental and human health implications: A comprehensive review. ACS Omega 9, 5100–5126. https://doi.org/10.1021/acsomega.3c07047

Yamashita, Y., Jaffé, R., Maie, N., Tanoue, E., 2008. Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). Limnol. Oceanogr. 53, 1900–1908. https://doi.org/10.4319/lo.2008.53.5.1900

Yang, R., van den Berg, C.M.G.G., 2009. Metal complexation by humic substances in seawater. Environ. Sci. Technol. 43, 7192–7197. https://doi.org/10.1021/es900173w

Yu, C.H., Huang, L., Shin, J.Y., Artigas, F., Fan, Z. (Tina), 2014. Characterization of concentration, particle size distribution, and contributing factors to ambient hexavalent chromium in an area with multiple emission sources. Atmos. Environ. 94, 701–708. https://doi.org/10.1016/j.atmosenv.2014.06.004

Yu, X., Liu, J., Qiu, T., Cao, L., Dou, S., 2023. Ocean acidification induces tissue-specific interactions with copper toxicity on antioxidant defences in viscera and gills of Asiatic hard clam Meretrix petechialis (Lamarck, 1818). Sci. Total Environ. 875, 162634. https://doi.org/10.1016/j.scitotenv.2023.162634

Zhang, H., 2000. Light and iron(III)-induced oxidation of chromium(III) in the presence of organic acids and manganese(II) in simulated atmospheric water. Atmos. Environ. 34, 1633–1640. https://doi.org/10.1016/S1352-2310(99)00384-2

Zhang, Y., Yang, K., Du, J., Zhang, F., Dong, Y., Li, W., 2018. Chemical characterization of fractions of dissolved humic substances from a marginal sea—a case from the Southern Yellow Sea. J. Oceanol. Limnol. 36, 238–248. https://doi.org/10.1007/s00343-017-6202-6

Zhang, Z., Wu, F., Deng, N., 2002. Photochemical reduction of Cr(VI) in aqueous solutions containing Fe(III)-hydroxy complexes. Toxicol. Environ. Chem. 82, 129–137. https://doi.org/10.1080/716067224

Zhao, Z., Freiser, H., 1986. Differential pulse polarographic determination of trace levels of platinum. Anal. Chem. 58, 1498−1501.

Žic, V., Truesdale, V.W., Garnier, C., Cukrov, N., 2012. The distribution of iodine in the Croatian marine lake, Mir – The missing iodate. Estuar. Coast. Shelf Sci. 115, 377–387. https://doi.org/10.1016/j.ecss.2012.07.026

Zimmermann, S., Sures, B., Ruchter, N., 2015. Laboratory studies on the uptake and bioaccumulation of PGE by aquatic plants and animals, in: Zereini, F., Wiseman, C.L.S. (Eds.), Platinum Metals in the Environment. pp. 361–381. https://doi.org/10.1007/978-3-662-44559-4_23

Zitoun, R., Achterberg, E.P., Browning, T.J., Hoffmann, L.J., Krisch, S., Sander, S.G., Koschinsky, A., 2021. The complex provenance of Cu-binding ligands in the South-East Atlantic. Mar. Chem. 237, 104047. https://doi.org/10.1016/j.marchem.2021.104047

Zitoun, R., Clearwater, S.J., Hassler, C., Thompson, K.J., Albert, A., Sander, S.G., 2019. Copper toxicity to blue mussel embryos (Mytilus galloprovincialis): The effect of natural dissolved organic matter on copper toxicity in estuarine waters. Sci. Total Environ. 653, 300–314. https://doi.org/10.1016/j.scitotenv.2018.10.263

RESEARCH PAPERS

Research paper 1

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Improved voltammetric methodology for chromium redox speciation in estuarine waters

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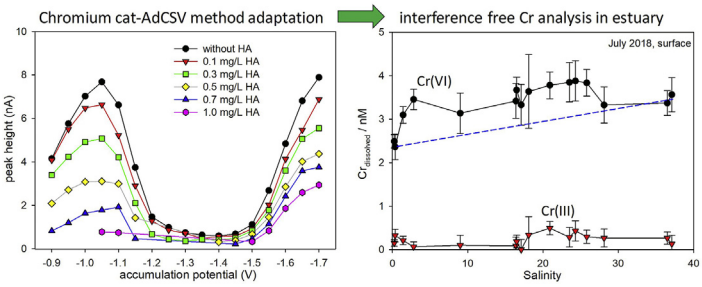
HIGHLIGHTS

- Method for elimination of SAS influence on voltammetric Cr speciation.
- Same protocol for Cr determination of freshwater and seawater.
- Cr(VI) dominate in estuarine samples.
- Non-conservative behaviour of Cr(VI) in salinity gradient.
- Cr present mainly in the dissolved form.

GRAPHICAL ABSTRACT



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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.

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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 CrO_4^{2-}-and $Cr_2O_7^{2-}$, and the complexation with organic and inorganic ligands is thought to be insignificant. In freshwaters, predominant inorganic forms of Cr(III) are hydro complexes ($Cr(OH)^{2+}$, $Cr(OH)_3$), while in the presence of chloride (seawater) it forms hexaaquo complex ($[Cr(H_2O)_6]Cl_3$). 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 (H_2S) 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 minimise 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 pH < 2 (if only the dissolved concentration has to be determined), or at natural pH (if speciation is of primary interest). However, this storage scheme is not adequate for Cr. On one hand, at natural pH of ~8, Cr(VI) is stable (especially under a CO_2 blanket [13]) but Cr(III) is rapidly (minutes to hours) removed from the solution due to adsorption on the container walls. On the other hand, in acidic conditions, Cr(VI) could be reduced to Cr(III) by the oxidation of organic matter. In addition to these storage issues, the relatively low Cr concentration encountered in natural waters (0.1–16 nM in seawater, 0.5–100 nM in freshwater) is also presenting an analytical challenge.

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

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

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

2. Study site

Krka River and its estuary are part of National Park Krka, which is situated on the eastern coast of Adriatic Sea (Croatia).The river is characterized by numerous lakes formed by tufa barriers, each finishing with waterfalls. Measured flow in Krka River over the last 50 years range from 5 to 450 m^3s^{-1}, while in the period from 2001 to 2013, the average annual flow spans from 40 to 60 m^3s^{-} [25]. This highly stratified estuary is restricted between the last and largest waterfall (Skradinski buk) and Šibenik Channel, measuring in total of ~23 km. The map of the estuary with marked sampling locations is presented in Fig. 1. The Krka River estuary is a typical, highly stratified salt-wedge estuary. Its vertical gradient is characterized by three layers: (1) surface fresh/brackish layer (FWL), (2) freshwater-seawater interface (FSI) and (3) seawater layer (SWL). While FWL flows downstream (seaward), the bottom SWL flows in opposite direction, upstream (landward). The halocline is usually positioned between 1.5 and 3 m, and its "thickness" varies between few cm only to 1 m. Due to numerous tufa barriers preceding the estuary and the absence of significant anthropogenic sources, the terrigenous material, nutrients and trace metal river input [26] are very low.

3. Sampling and storage

Sampling was performed using FEP Nalgene bottles which were previously cleaned with 10% HNO_3 (suprapur) and thoroughly rinsed with MQ water (18.2 MΩ, Millipore, USA). Samples were collected using a van Dorn horizontal acrylic sampler or by using grab sampling with 1 L FEP bottle at 16 sites along the whole estuary (Fig. 1). Three sampling campaigns were conducted: summer 2017 and 2018 and winter 2017. For the summer campaigns (summer 2017/2018), both surface (~0.2 m below the surface) and bottom seawater samples were collected, whereas for the winter campaign, due to logistic difficulties, only surface samples were taken. Samples were filtered either immediately onboard or in the laboratory within few hours by using precleaned (MQ + sample) syringe filters 0.22 μm (cellulose-acetate, Minisart, Sartorius). All samples were stored at natural pH at +4 °C until analysis in 125 mL FEP bottles which were previously washed using trace metals clean procedure. For total chromium determination, samples were UV-irradiated at 254 nm directly in the FEP bottle for 24 h prior to measurement. Concentrations of Cr(VI) in estuarine samples were always determined within two days of sampling. Repeated analyses on the same filtered samples stored for up to 5 days in the dark and at +4 °C did not show any significant differences (within experimental uncertainty, i.e. 10% [12]). This result indicates that adsorption of Cr(III) on the container walls did not occurr, in contrast to previously reported [10], possibly because fluorinated (FEP) bottles were used in this study. Vertical profiles of physicochemical parameters (S, T, O_2, pH and Chl-a) were recorded using the EXO2 multiparameter CTD probe (YSI).

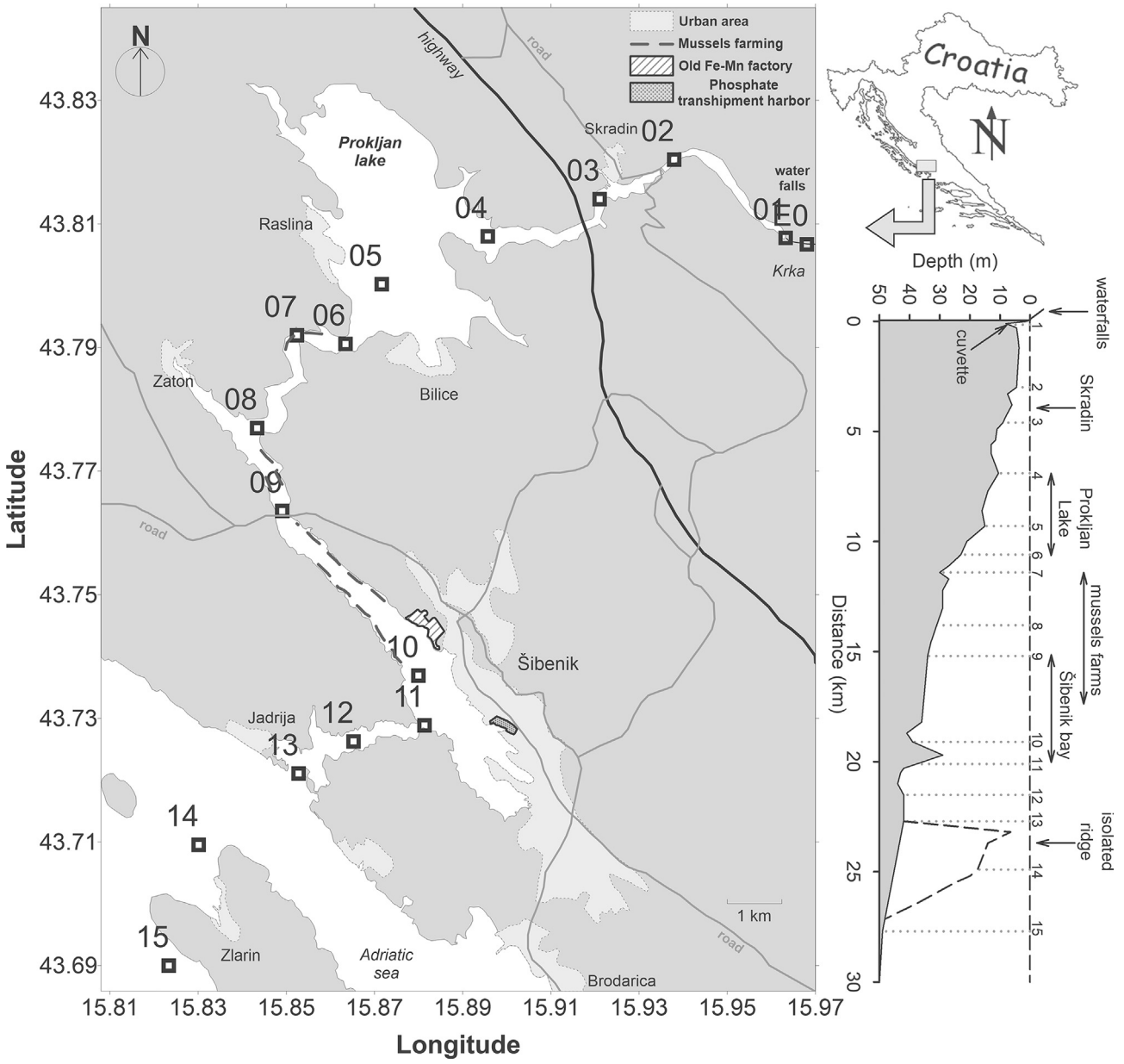

Fig. 1. Left: Map of the Krka River estuary with indicated locations of sampling sites (open diamonds). Right: horizontal bottom depth profile with positions of sampling sites and specific regions along the estuary.

4. Equipment and chemicals

Voltammetric measurements were performed using a μAutolabIII (EcoChemie) potentiostat coupled with a three-electrode cell (663 VA Stand, Metrohm) with a static mercury drop (SMDE), Ag|AgCl|sat. NaCl and Pt wire as the working, reference and auxiliary electrodes respectively. A home-made sample-changer, five Cavro XE 1000 syringe pumps and home-made software VoltAA were used in conjunction with the potentiostat allowing fully automated measurements to be performed.

Sodium nitrate (3 M stock solution) was prepared by mixing HNO_3 (*suprapur* Merck) and NaOH (*suprapur* Merck), Diethylenetriaminepentaacetic acid (DTPA; 0.25 M stock solution prepared) was purchased from Fluka (analytical grade) and 2-(N-morpholino) ethanesulfonic acid (MES; 1 M stock solution prepared) was purchased from VWR (ultrapure). Stock solutions of Cr(VI) were prepared by appropriate dissolution of K_2CrO_4 (Fluka). Humic acid (HA) was from Sigma-Aldrich. All laboratory solutions were stored in polyethylene bottles, while all the samples were stored in acid-washed FEP (Nalgene) bottles.

5. Basics of Cat-AdCSV method for Cr analysis

The method for chromium determination in freshwater and seawater is based on the *in-situ* formation and adsorption of the Cr(III)-DTPA complex (from Cr(VI) reduction which exists in the solution at potentials more negative than −0.05 V) during the accumulation step. During the stripping step, this complex is further reduced to Cr(II) which is immediately oxidised back to Cr(III) by nitrate, resulting in an enhancement of the signal due to this catalytic effect [10,18,19,27]. Since the original description of this method [28], numerous authors reported on using AdCSV to

measure trace levels of chromium in different matrices and using different working electrodes [10,18,21,22,29–31]. Even though the method was widely used, its analytical application was limited until the mechanism of reaction, formation, adsorption and electrode reaction of Cr(III)-DTPA complex were fully studied [27,30]. The critical step of the methodology is that the Cr(III) originally present in the solution slowly forms an electro-inactive complex with DTPA. At room temperature, the kinetics of this complexation is believed to take 30 min, allowing then the sole determination of Cr(VI) (increasing the temperature decreases this time [32]).

In this work, the determination of Cr concentrations was performed in buffered samples (MES, pH 5.5) using the following fully automated procedure: rinsing of the cell between samples by acidified MQ (pH 2, 10 mM HCl), sample exchange, addition of reagents (DTPA, $NaNO_3$) and Cr(VI) standard using syringe burettes. Adequate volume of Cr standard was determined by using predefined sensitivity. Prepared samples were measured ~1 h after the addition of reagents when all originally present Cr(III) was transferred into an electroinactive complex. Total Cr is determined after 24 h UV-irradiation of the sample at neutral or slightly acidic pH (to convert the existing Cr(III) to Cr(VI)). Cr(III) concentration is calculated as the difference between total Cr and Cr(VI). Typical voltammetric conditions were: 3 min initial purging, accumulation at −1.65 V for 60 s, stripping from −0.95 to −1.35 V using differential pulse mode (2 mV potential step, 0.1 s interval time, 0.040 s pulse time, 10 or 40 mV amplitude).

6. Results and discussion

6.1. Optimization of the analytical procedure

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

Initial tests in estuarine samples revealed that the sensitivity and the shape of voltammograms were changing from sample to sample when using a deposition potential of −1.0 V. In contrast, we obtained much better stability and better-shaped peaks when using a more negative deposition potential (e.g. −1.65 V). This is also corroborated by previous studies: a low deposition potential was found optimal by Korolczuk et al. [21,22] when used with a medium exchange procedure and an increase of the Cr peak was also reported at low deposition potentials (−1.8 V) at a vibrating silver amalgam microwire electrode [20], although that potential was not suggested as the optimum one. The same study reported that Cr(VI) determination in samples without UV-irradiation step was not possible due to interference by dissolved organic matter (DOM), although no further study was performed by the authors. Surface active substances (SAS), naturally present in aqueous samples, were identified to have interference in the determination of chromium by AdCSV [23] and were removed using a fumed silica column. It is thus likely that the application of a low deposition potential minimise the interference from SAS, similar to what was observed for Cu complexation studies [34] or for the determination of platinum [35].

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

To identify if the decrease of the signal is due to SAS interference on the voltammetric signal or is due to complexation by HA, we carried out analytical determinations of Cr by the method of standard addition at both potentials (−1.0 and −1.65 V) at each HA concentration (calibrations not shown). At low HA concentrations, below 0.7 mg/L, despite a strong decrease of signal intensity, the accuracy of Cr determination is not significantly impacted, at both potentials. At higher HA concentrations, analysis of Cr using −1.0 V deposition potential was not possible, whereas using deposition at −1.65 V a reproducible and accurate determination of Cr was

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[a]	−1.65
Deposition time/s	60	60	60	60	60
DTPA/c (mM)	2.5	5	1.25	10	1.25
NO_3^-/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

[a] - with matrix exchange.

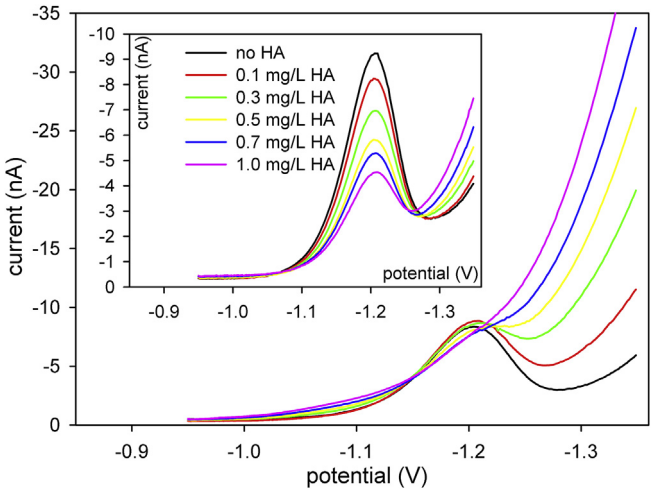

Fig. 2. Voltammograms recorded in UV-irradiated seawater spiked with 6 nM Cr(VI) at varying concentrations of HA using −1.0 V (main plot) and −1.65 V (inset) as accumulation potentials.

always obtained (recovery ~100%), suggesting that complexation of Cr(VI) with HA did not occur within the time frame of the experiment. The only problem that was sometimes observed when using an accumulation potential of −1.65 V was the dislodgement of the Hg-drop. However, as the accumulation time for most of the samples measured in natural water is only 60 s, this problem did not impact Cr determination because duplicate or triplicate measurements were always performed (for the sample and for each addition of Cr standard).

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

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

6.2. Voltammogram shape and baseline elimination

The most common way of expressing sensitivity is in terms of nA/nM. However, in cases where the signal is positioned at the steep part of the baseline (as for Cr), the signal to baseline shape is much more important than just pure (and high) sensitivity expressed in nA/nM. A typical example is given in Fig. 4: while the peaks obtained at −1.0 and −1.65 V are of the same absolute intensity (~4.5 nA; determined using curvature baseline), the shape of the latter peak is much better resolved than the former (see also voltammograms in Fig. 2). Thus, when optimising any voltammetric procedures, both the absolute intensity and the shape of the voltammogram should be improved. This is especially important at low signal amplitude, where the steep baseline could mask the analyte signal. In such cases, the application of derivative transformations is beneficial: it eliminates the curvature of the baseline (see inset of Fig. 4) and consequently lower detection limits [35,37]. We used here the peak height of the 2nd derivative transformation for quantification of Cr concentration.

6.3. Hydrography of the estuary

Physico-chemical parameters for the sampling periods are presented in Fig. S3. The vertical and horizontal profiles of salinity are typical of two contrasting sampling periods (summer and winter): the halocline is deeper and the low salinity brackish layer

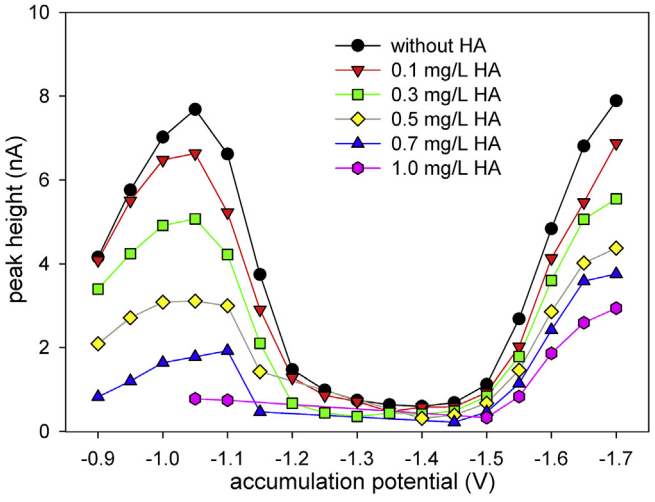

Fig. 3. Variation of peak area on the accumulation potential without and with increasing concentration of HA in UV-irradiated seawater (UVSW) with total Cr concentration of 6 nM.

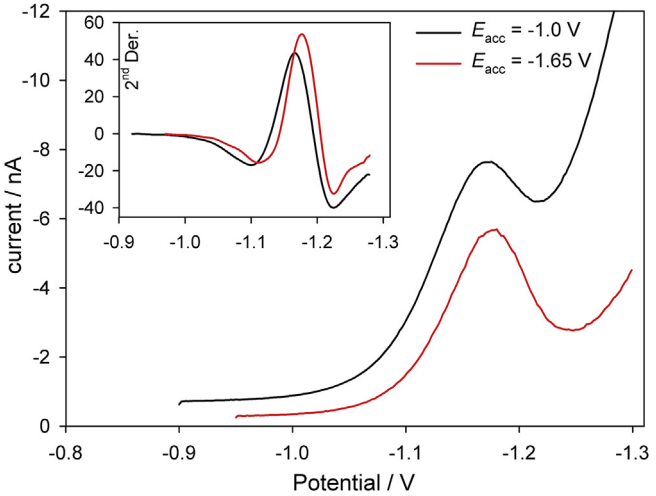

Fig. 4. Voltammograms obtained at −1.0 V and −1.65 V accumulation potential in seawater sample ([Cr] = 4.5 nM). Inset: 2nd derivative vs potentials.

extends more within the estuary in winter than in summer. The pH of samples (not shown) was between 8.4 (at the freshwater part) and 8.2 (at the seawater). Higher pH in freshwater part is related to CO_2 removal at waterfalls which precede the estuarine transect [25]. Temperature profiles follow a similar trend as those of salinity for both periods, whereas oxygen profiles were the most variable. A clear increase of oxygen levels below the halocline (reaching value up to 140% of oxygen saturation) between 3rd and 20th kilometre were observed in summer, due to high productivity occurring in this lacustrine part of the estuary (Prokljan lake) [38]. On the other hand, hypoxic conditions were found in the deeper regions of the upstream part of the estuary during winter. This is due to progressive degradation of organic matter produced during summer period, associated with the high residence time of the seawater layer in that upper part of the estuary. Fig. S4 presents typical profiles of dissolved organic carbon (DOC) for the winter and summer periods. DOC concentrations were higher in summer (up to ~150 μM) than in winter (up to ~80 μM). Typically, DOC was lower (~50 μM) in winter in the freshwater end-member compared to the seawater end-member, whereas for the summer period it could be the opposite, due to developed biological productivity in the freshwater Visovac Lake, that is located before the waterfalls and the estuary [39].

6.4. Chromium distribution along the Krka River estuary

The distributions of dissolved Cr(VI) and Cr(III) along the estuarine transect are presented in Fig. 5 as a function of salinity. Due to the fully oxygenated samples, Cr(VI) predominates both in summer and winter. Although the suspended particulate matter is generally low in the Krka River estuary (<5 mg/L) [25], some portion of Cr(III), which is particle-reactive in contract to Cr(VI), could be adsorbed on the particulate matter. Thus, higher concentrations of Cr(III) in non-filtered samples were expected but these were not found. In unfiltered samples, slightly higher (<5%) concentration of Cr(III) were found compared to the dissolved ones (Fig. S5). One of the known challenge in Cr determination is that Cr(III) is strongly adsorbed on the bottle walls [10]. In cases when UV-irradiation of sample to remove organic matter is performed in separate, usually quartz tubes [20], the adsorbed Cr(III) is not recoverable because the sample is transferred from storage bottle to the UV-digestion vessel. In our work, Teflon (FEP) bottles were used to store the samples and UV-irradiation was performed directly in these bottles (FEP is UV transparent), with adaptation of samples pH to around 5. In this way, any adsorbed Cr(III) is expected to be recovered and reliably quantified.

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

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

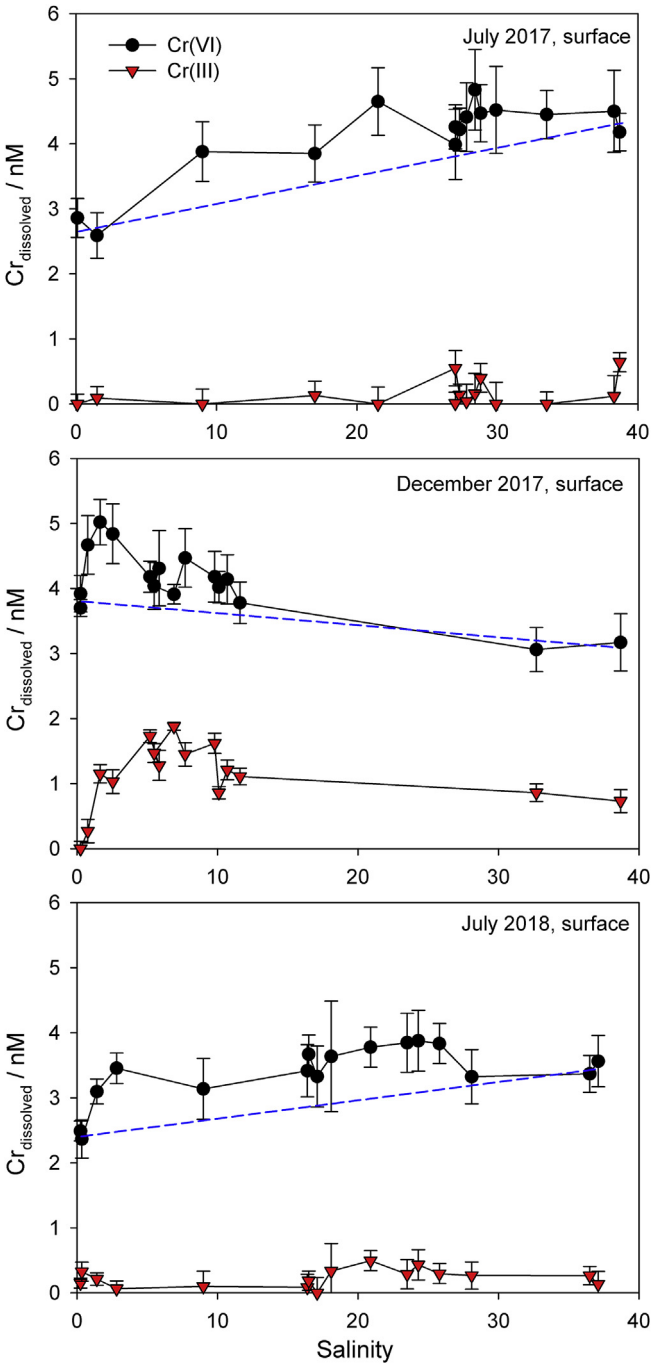

Fig. 5. Distribution of dissolved chromium along the salinity gradient of the Krka River estuary in surface layer. Blue dotted line corresponds to conservative mixing line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the hypothesis that atmospheric/aerosol inputs of Cr(III) is regulating its concentration in the surface layer of the estuary and that Cr(III) is photo-oxidised to Cr(VI).

The transect of chromium in the bottom water is unusual. While an upstream increase of trace metals was recorded for many metals in the bottom layer [25], Cr concentrations here follow an opposite trend, similar to that observed in surface water. A lower concentration is detected on the freshwater side compared to those measured lower in the estuary (see Fig. S6), reaching a stable value of c.a. 4.4 nM from 6 km downstream. This unusual behaviour can be explained by a reduction of Cr(VI) to Cr(III) in the upstream part, followed by removal of Cr(III) through adsorption onto sinking particulate matter, which is known to be increased in that part of the estuary [25]. Reduction of Cr(VI) to Cr(III) can be favoured by the hypoxic conditions in that section of the estuary (Fig. S3) and/or through reduction by low molecular weight organic matter, as previously suggested [43].

7. Conclusions

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jasmin Pađan: Formal analysis, Methodology, Validation, Writing - original draft. **Saša Marcinek:** Formal analysis, Methodology, Validation, Writing - original draft. **Ana-Marija Cindrić:** Investigation, Resources, Visualization. **Nicolas Layglon:** Formal analysis, Investigation, Resources, Visualization. **Veronique Lenoble:** Conceptualization, Supervision, Writing - review & editing. **Pascal Salaün:** Conceptualization, Writing - review & editing. **Cédric Garnier:** Conceptualization, Supervision. **Dario Omanović:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.09.014.

References

[1] H. Elderfield, Chromium speciation in sea water, Earth Planet. Sci. Lett. 9 (1970) 10–16.
[2] J. Kotas, Z. Stasicka, Chromium occurrence in the environment and methods of its speciation, Environ. Pollut. 107 (2000) 263–283.
[3] A.K. Shanker, C. Cervantes, H. Loza-Tavera, S. Avudainayagam, Chromium toxicity in plants, Environ. Int. 31 (2005) 739–753.
[4] A.D. Dayan, A.J. Paine, Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000, Hum. Exp. Toxicol. 20 (2001) 439–451.
[5] F.T. Stanin, The transport and fate of chromium (VI) in the environment, in: J. Guertin, J.A. Jacobs, C.P. Avakian (Eds.), Chromium(VI) Handbook, CRC Press, Florida, USA, 2005, pp. 165–214.
[6] J. Gorny, G. Billon, C. Noiriel, D. Dumoulin, L. Lesven, B. Made, Chromium behavior in aquatic environments: a review, Environ. Rev. 24 (2016) 503–516.
[7] J.P. Gustafsson, I. Persson, A.G. Oromieh, J.W.J. van Schaik, C. Sjostedt, D.B. Kleja, Chromium(III) complexation to natural organic matter: mechanisms and modeling, Environ. Sci. Technol. 48 (2014) 1753–1761.
[8] S. Fendorf, B.W. Wielinga, C.M. Hansel, Chromium transformations in natural environments: the role of biological and abiological. processes in chromium(VI) reduction, Int. Geol. Rev. 42 (2000) 691–701.
[9] M. Sadiq, Chromium in Marine Environments, Toxic Metal Chemistry in Marine Environments, Marcel Dekker, New York, 1992, pp. 154–197.
[10] M. Boussemart, C.M.G. Van Den Berg, M. Ghaddaf, The determination of the chromium speciation in sea-water using catalytic cathodic stripping voltammetry, Anal. Chim. Acta 262 (1992) 103–115.
[11] C. Jeandel, J.F. Minster, Isotope-dilution measurement of inorganic chromium(iii) and total chromium in seawater, Mar. Chem. 14 (1984) 347–364.
[12] S. Comber, M. Gardner, Chromium redox speciation in natural waters, J. Environ. Monit. 5 (2003) 410–413.
[13] K. Vercoutere, R. Cornelis, L. Mees, P. Quevauviller, Certification of the contents of the chromium(III) and chromium(VI) species and total chromium in a lyophilised solution (CRM 544), Analyst 123 (1998) 965–969.
[14] S. Sander, A. Koschinsky, Onboard-ship redox speciation of chromium in diffuse hydrothermal fluids from the North Fiji Basin, Mar. Chem. 71 (2000) 83–102.
[15] S. Sander, A. Koschinsky, P. Halbach, Redox speciation of chromium in the oceanic water column of the Lesser Antilles and offshore Otago Peninsula, New Zealand, Mar. Freshw. Res. 54 (2003) 745–754.
[16] D.M. Semeniuk, M.T. Maldonado, S.L. Jaccard, Chromium uptake and adsorption in marine phytoplankton - implications for the marine chromium cycle, Geochem. Cosmochim. Acta 184 (2016) 41–54.
[17] B. Markiewicz, I. Komorowicz, A. Sajnog, M. Belter, D. Baralkiewicz, Chromium and its speciation in water samples by HPLC/ICP-MS - technique establishing metrological traceability: a review since 2000, Talanta 132 (2015) 814–828.
[18] A. Bobrowski, A. Krolicka, J. Zarebski, Characteristics of voltammetric determination and speciation of chromium - a review, Electroanalysis 21 (2009) 1449–1458.
[19] M. Cuellar, S.N. Vettorelo, P.I. Ortiz, F. Garay, Theoretical and experimental study of the catalytic cathodic stripping square-wave voltammetry of chromium species, J. Solid State Electrochem. 20 (2016) 3279–3286.
[20] E. Espada-Bellido, Z.S. Bi, C.M.G. van den Berg, Determination of chromium in estuarine waters by catalytic cathodic stripping voltammetry using a vibrating silver amalgam microwire electrode, Talanta 105 (2013) 287–291.
[21] M. Korolczuk, Voltammetric determination of traces of Cr(VI) in the presence of Cr(III) and humic acid, Anal. Chim. Acta 414 (2000) 165–171.
[22] M. Korolczuk, M. Grabarczyk, Voltammetric determination of Cr(VI) in a flow system in the presence of diethylenetriaminepentaacetic acid (DTPA) following its deposition in the metallic state, Anal. Chim. Acta 387 (1999) 97–102.
[23] E. Niewiara, B. Bas, W.W. Kubiak, Elimination of SAS interferences in catalytic adsorptive stripping voltammetric determination of Cr(VI) by means of fumed silica, Electroanalysis 19 (2007) 2185–2193.
[24] A.R. Borges, L.F. Niencheski, I.C.B. Milani, M.R. Milani, Optimisation and application of the voltammetric technique for speciation of chromium in the Patos Lagoon Estuary-Brazil, Environ. Monit. Assess. 184 (2012) 5553–5562.
[25] A.M. Cindrić, C. Garnier, B. Oursel, I. Pižeta, D. Omanović, Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: the Krka River estuary (Adriatic, Croatia), Mar. Pollut. Bull. 94 (2015) 199–216.
[26] N. Cukrov, P. Cmuk, M. Mlakar, D. Omanović, Spatial distribution of trace metals in the Krka River, Croatia: an example of the self-purification, Chemosphere 72 (2008) 1559–1566.
[27] S. Sander, T. Navratil, L. Novotny, Study of the complexation, adsorption and electrode reaction mechanisms of chromium(VI) and (III) with DTPA under adsorptive stripping voltammetric conditions, Electroanalysis 15 (2003) 1513–1521.

[28] J. Zarebski, Alternating-current, normal and differential pulse polarographic studies of chromium Edta, Cdta and Dtpa complexes for application to determination of chromium in trace amounts, Chem. Anal. (Warsaw) 22 (1977) 1037–1048.
[29] E.P. Achterberg, C.M.G. Van Den Berg, Automated voltammetric system for shipboard determination of metal speciation in sea-water, Anal. Chim. Acta 284 (1994) 463–471.
[30] Y.J. Li, H.B. Xue, Determination of Cr(III) and Cr(VI) species in natural waters by catalytic cathodic stripping voltammetry, Anal. Chim. Acta 448 (2001) 121–134.
[31] E.M. deSouza, A.D.L.R. Wagener, P. Farias, Voltammetric determination of Cr(III) and Cr(VI) in tropical estuarine waters: advantages and limitations, Croat. Chem. Acta 70 (1997) 259–269.
[32] M. Grabarczyk, M. Korolczuk, Modification of catalytic adsorptive stripping voltammetric method of hexavalent chromium determination in the presence of DTPA and nitrate, Anal. Bioanal. Chem. 376 (2003) 1115–1118.
[33] M. Korolczuk, How faster and cheaper to determine chromium by adsorptive cathodic stripping voltammetry in the presence of DTPA and nitrate, Fresenius J. Anal. Chem. 367 (2000) 761–762.
[34] Y. Louis, P. Cmuk, D. Omanović, C. Garnier, V. Lenoble, S. Mounier, I. Pižeta, Speciation of trace metals in natural waters: the influence of an adsorbed layer of natural organic matter (NOM) on voltammetric behaviour of copper, Anal. Chim. Acta 606 (2008) 37–44.
[35] J. Paḍan, S. Marcinek, A.-M. Cindrić, N. Layglon, C. Garnier, A. Cobelo-García, D. Omanović, Determination of sub-pico-molar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology, Environ. Chem. (2019), EN19157. https://www.publish.csiro.au/EN/justaccepted/EN19157. in press.
[36] H. Whitby, C.M.G. van den Berg, Evidence for copper-binding humic substances in seawater, Mar. Chem. 173 (2015) 282–290.
[37] A. Cobelo-Garcia, J. Santos-Echeandia, D.E. Lopez-Sanchez, C. Almecija, D. Omanovic, Improving the voltammetric quantification of ill-defined peaks using second derivative signal transformation: example of the determination of platinum in water and sediments, Anal. Chem. 86 (2014) 2308–2313.
[38] T. Legović, D. Petricioli, V. Žutić, Hypoxia in a pristine stratified estuary (Krka, Adriatic Sea), Mar. Chem. 32 (1991) 347–359.
[39] D. Petricioli, T. BakranPetricioli, D. Viličić, A. PožarDomac, Freshwater phytoplankton bloom in Visovac lake - a possible cause of benthic mortality in Krka estuary (Adriatic sea, Croatia), PSZNI Mar. Ecol. 17 (1996) 373–382.
[40] S.E. Kaczynski, R.J. Kleber, Aqueous trivalent chromium photoproduction in natural-waters, Environ. Sci. Technol. 27 (1993) 1572–1576.
[41] E.P. Achterberg, C.M.G. van den Berg, Chemical speciation of chromium and nickel in the western Mediterranean, Deep-Sea Res Pt Ii 44 (1997) 693–720.
[42] C.H. Yu, L.H. Huang, J.Y. Shin, F. Artigas, Z.H. Fan, Characterization of concentration, particle size distribution, and contributing factors to ambient hexavalent chromium in an area with multiple emission sources, Atmos. Environ. 94 (2014) 701–708.
[43] S.Y. Chen, S.W. Huang, P.N. Chiang, J.C. Liu, W.H. Kuan, J.H. Huang, J.T. Hung, Y.M. Tzou, C.C. Chen, M.K. Wang, Influence of chemical compositions and molecular weights of humic acids on Cr(VI) photo-reduction, J. Hazard Mater. 197 (2011) 337–344.

Supplementary document to Research paper 1

Improved voltammetric methodology for chromium redox speciation in estuarine waters

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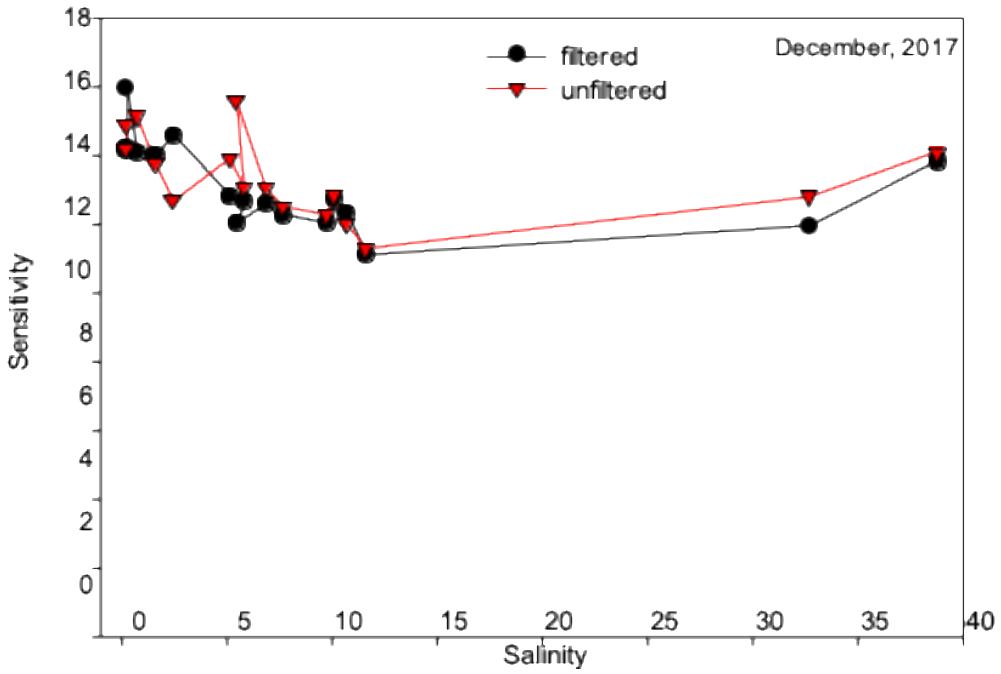

Figure S1. Variation of sensitivity (2nd derivative peak height/nM) on the salinity for dissolved (filtered) and unfiltered samples.

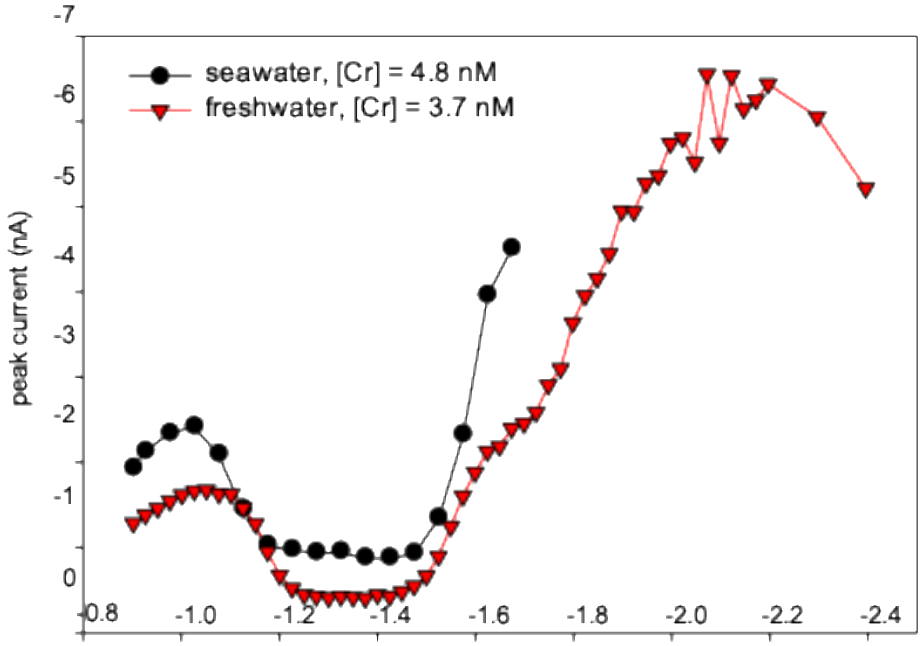

Figure S2. Variation of peak height as a function of the accumulation potentials in seawater and freshwater.

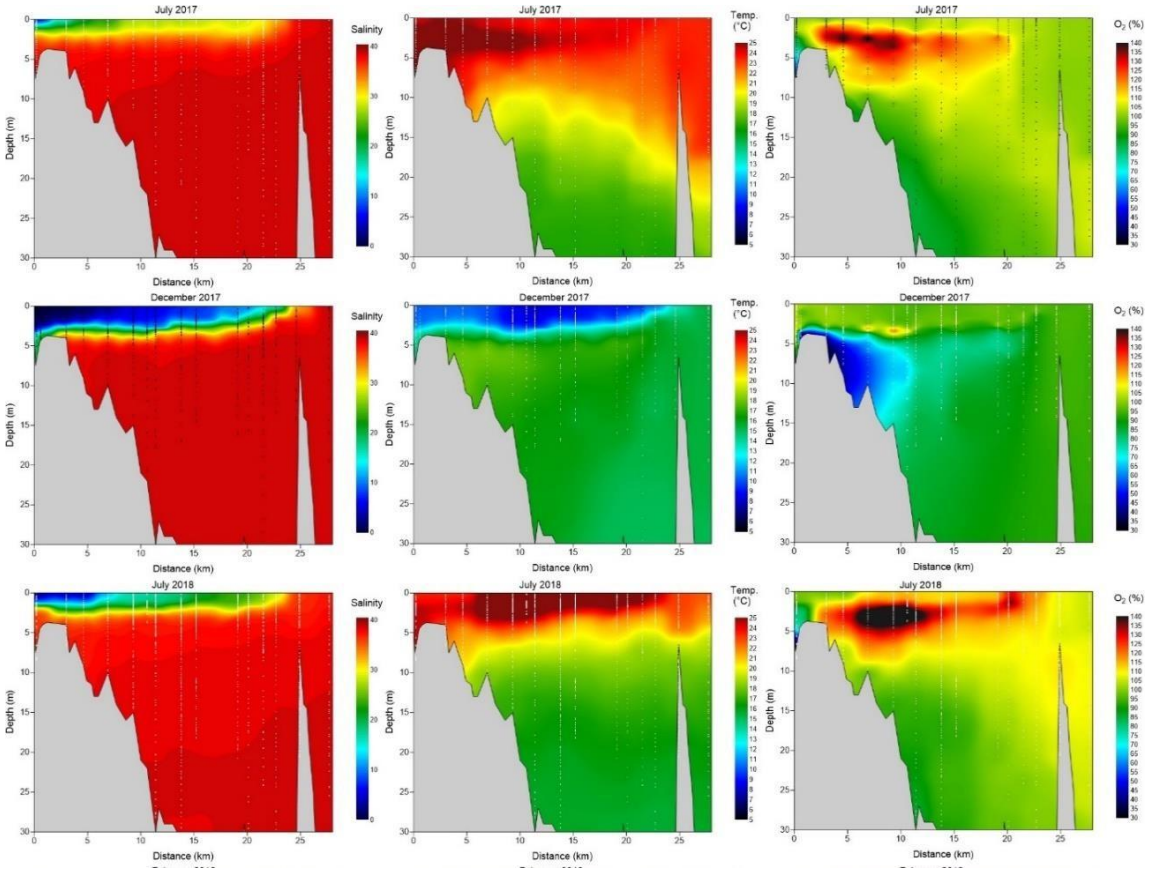

Figure S3. Contour plots of salinity (left panels), temperature (middle) and oxygen saturation (right) for July 2017 (top panels), December 2017 (middle) and July 2018 (bottom).

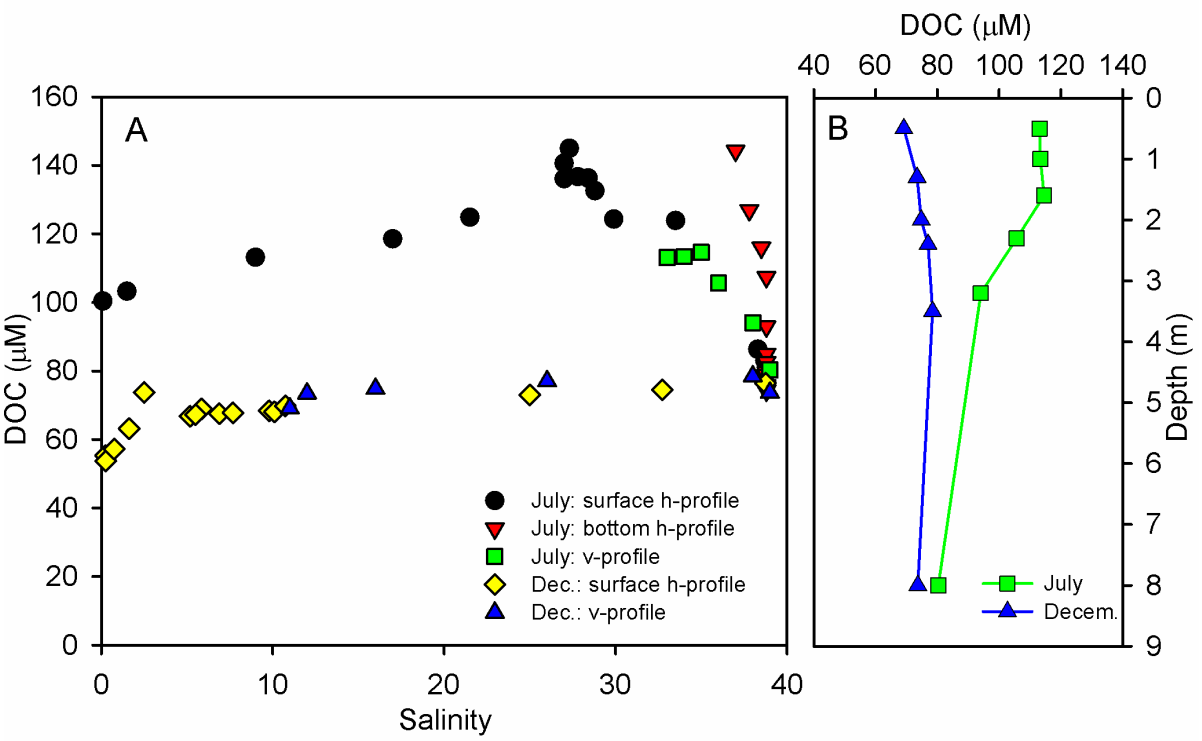

Figure S4. Typical horizontal and vertical profiles of dissolved organic carbon (DOC) for winter and summer period in the Krka River estuary ("h" - horizontal; "v" - vertical).

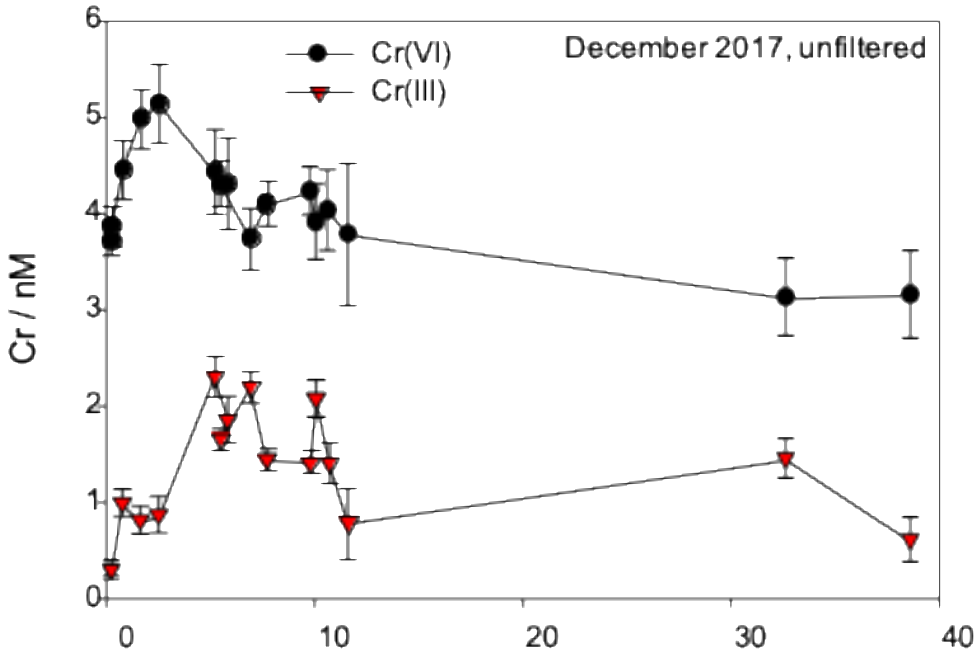

Figure S5. Distribution of chromium in unfiltered surface samples along the salinity gradient of the Krka River estuary in December 2017.

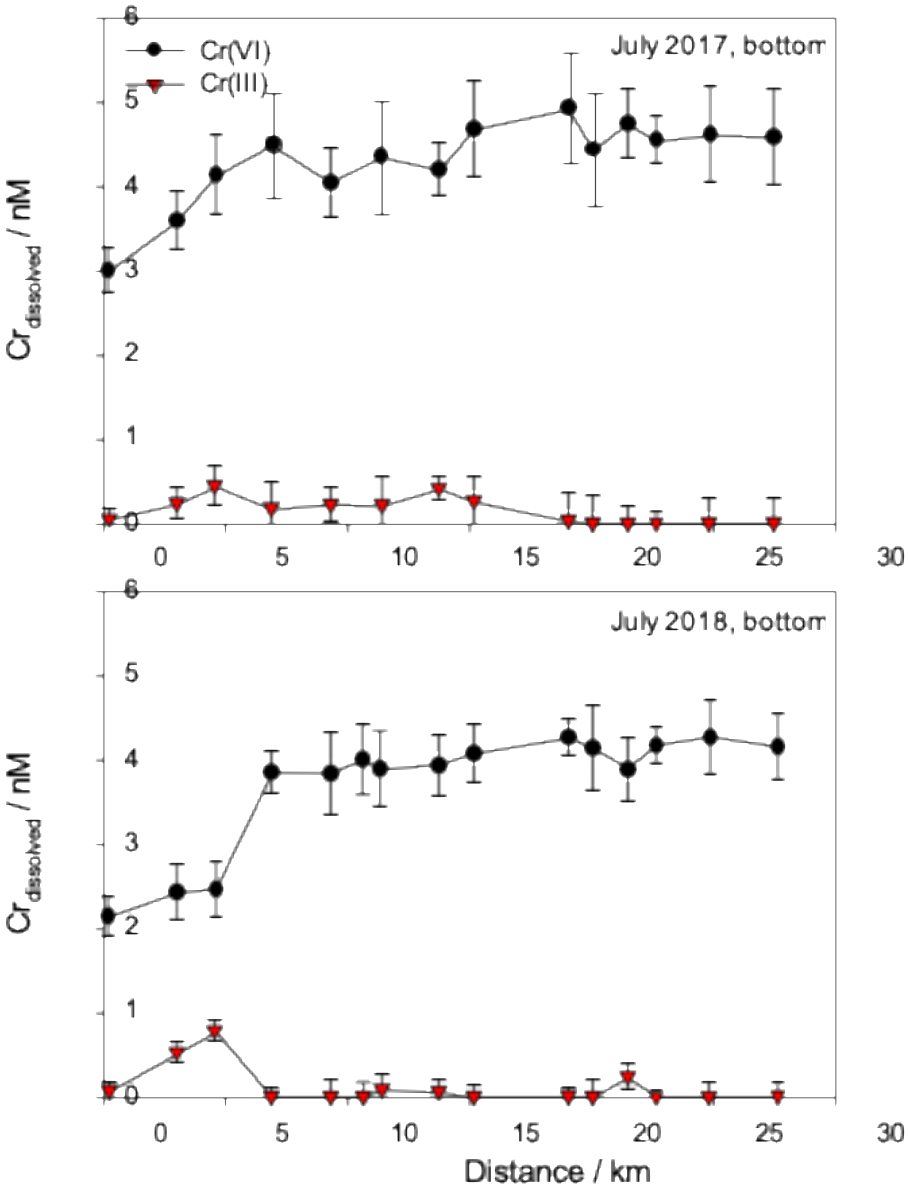

Figure S6. Distribution of dissolved chromium in the bottom seawater layer as a function of the distance from the freshwater Krka end-member.

Research paper 2

Pađan, J., Marcinek, S., Cindrić, A.M., Layglon, N., Garnier, C., Salaün, P., Cobelo-García, A., Omanović, D. (2020) Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology. *Environmental chemistry*, **17**, 2; 74-84. doi: 10.1071/EN19157

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Research Paper

Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology

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Environmental context. Platinum concentrations in natural waters such as oceans, rivers and lakes are extremely low, hindering studies of Pt distributions and biogeochemical cycles. An improved electrochemical method was used to reliably determine Pt in estuarine conditions at trace concentrations. Platinum displayed a near-conservative behaviour along the salinity gradient of the estuary, with about 90 % remaining in the dissolved form.

Abstract. Extremely low concentrations of platinum in natural waters require very sensitive analytical techniques, with adsorptive cathodic stripping voltammetry (AdCSV) being one of the most frequently used techniques. A 'fine tuning' of the voltammetric parameters, along with advanced data treatment based on derivative transformations, allowed us to determine reliably Pt levels down to 50 fM (0.05 pM). By using short modulation and interval times of the differential pulse stripping waveform, and applying a 4th derivative transformation to the voltammograms, the limit of detection (LOD) was lowered down to 10 fM. Although very small concentrations of surface-active substances (e.g. 0.025 mg L^{-1} fulvic acid) strongly influenced the method sensitivity, recoveries of spiked samples were not impacted (~100 %). The application of a desorption step ($E_{ds} = -1.35$ V; $t_{ds} = 2$ s) at the end of the accumulation significantly improved the sensitivity, presumably through the removal of adsorbed surface-active substances. Using this optimised methodology, we determined the Pt distribution in the pristine Krka River estuary in the winter and summer periods by performing both horizontal transects and vertical profiles (salinity ~1 to 39). In surface waters, dissolved Pt concentrations gradually increased towards the seawater end-member (from ~0.15 to ~0.3 pM). A small deviation from the conservative mixing line was observed at salinities below 10, which may reflect changes in Pt redox speciation. In bottom waters, the trend was opposite with dissolved Pt concentrations increasing towards the freshwater end-member, probably owing to progressive accumulation related to seawater residence time. On average, 90 % of Pt was present in the dissolved form.

Additional keywords: catalytic stripping voltammetry, derivative transformation, estuaries, salinity gradient.

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Introduction

Platinum (Pt) abundance and distribution on the Earth's surface is impacted by anthropogenic activities, which accounts for ~80 % of Pt fluxes, with ~45 % of produced Pt being used in catalytic converters for cars (Sen and Peucker-Ehrenbrink 2012). Insufficient data on Pt distribution, behaviour and role in the aquatic environment have placed Pt on the list of potentially eco-toxic elements (Mashio et al. 2017). Platinum is a technology critical element (TCE) and thus there is an emerging need for its assessment of distribution and impacts in different environments (Cobelo-Garcia et al. 2015). The most extensive application of Pt is in catalytic converters for vehicles, which is the most demanding Pt need (Matthey 2018). Pt emissions from vehicle exhausts over the decades have increased the Pt concentration in many environmental compartments, especially in the atmosphere, urban soils, sediments and natural waters (Schafer et al. 1999; Rauch et al. 2005; Obata et al. 2006; Soyol-Erdene et al. 2011; Abdou et al. 2019). Despite growing efforts

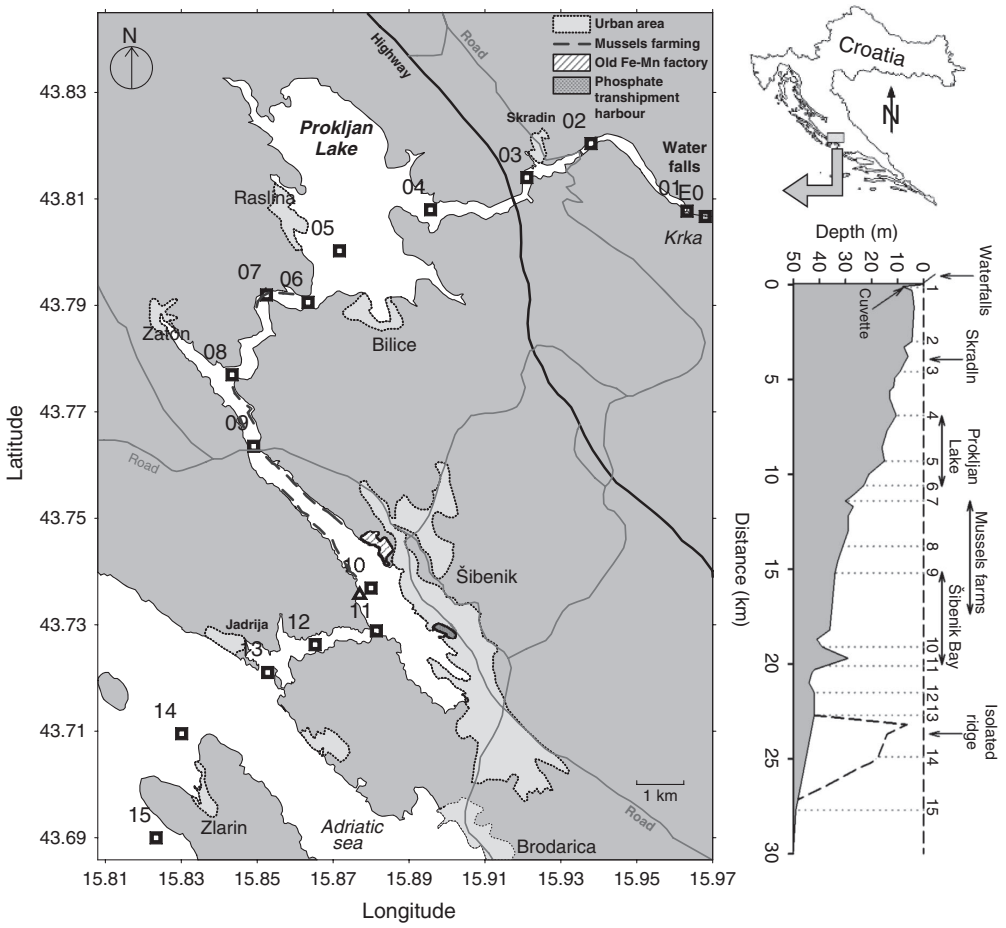

Fig. 1. Left: Map of the Krka River estuary with indicated locations of sampling sites (open squares). Open triangle is the location where the water column was sampled. Right: horizontal bottom depth profile with positions of sampling sites and specific regions along the estuary.

to improve the understanding of biogeochemical Pt cycles, there is still a lack of relevant environmental data. One of the reasons for this scarcity of Pt data is the extremely low concentrations of Pt (<1 pM) that are encountered in natural waters (van den Berg and Jacinto 1988; Cobelo-Garcia et al. 2013; Mashio et al. 2017; Fischer et al. 2018). There are only a few studies that have reported on the Pt distribution and behaviour in coastal environments (van den Berg and Jacinto 1988; Obata et al. 2006; Cobelo-Garcia et al. 2011; Cobelo-Garcia et al. 2013; Cobelo-Garcia et al. 2014*a*; Mashio et al. 2017; Fischer et al. 2018; Abdou et al. 2019). The concentration of Pt in natural waters ranges from below 0.1 pM to several pM (van den Berg and Jacinto 1988; Cobelo-Garcia et al. 2013; Mashio et al. 2017; Fischer et al. 2018). Higher concentrations are found in coastal regions impacted by urban pressure, while in open marine water, the concentration is usually below 0.5 pM. To date, only a few studies have been conducted in estuarine environments (Obata et al. 2006; Cobelo-Garcia et al. 2011; Cobelo-Garcia et al. 2013; Cobelo-Garcia et al. 2014*a*), with a non-conservative behaviour reported during estuarine mixing (Cobelo-Garcia et al. 2013).

Because of such low concentrations, the determination of Pt in natural water require extremely sensitive analytical techniques. Inductively coupled plasma mass spectrometry (ICP-MS) with a pre-concentration step (Turetta et al. 2003; Mashio et al. 2017; Fischer et al. 2018) and catalytic stripping voltammetry (van den Berg and Jacinto 1988; Locatelli 2005; Obata et al. 2006; Cobelo-Garcia et al. 2013) are the most widely used techniques. The limit of detection (LOD) of both methods is low and suitable for studying Pt behaviour in unpolluted natural waters: a LOD of 30 fM has been reported for the voltammetric method (Cobelo-Garcia et al. 2014*b*) and 15–20 pM for the more complex ICP-MS pre-concentration technique (Mashio et al. 2017; Fischer et al. 2018).

In this work, we focussed on (1) optimising the analytical protocol to improve the reliability of Pt determination by voltammetry (which consists in the fine adjustment of the voltammetric procedure and the treatment of the voltammograms); (2) assessing and removing the interference of surface-active substances on the voltammetric signal and (3) evaluating the level, distribution and behaviour of Pt under estuarine mixing of the pristine Krka River estuary.

Experimental

Study site

The Krka River estuary is located on the eastern coast of the Adriatic Sea (Croatia). It is a typical, highly stratified estuary along its entire length of 22 km. The halocline depth and the extension of the low salinity surface brackish water is mainly regulated by the Krka River flow, with an average flow of around 40 $m^3\ s^{-1}$ (over the last 50 years, it has ranged from 5 to 450 $m^3\ s^{-1}$ (Prohic and Kniewald 1987; Cindric et al. 2015). Fig. 1 presents a map of the sampling area with the location of

the 16 sampling sites used in the transect as well as an extra site near the Marine station where a vertical profile was obtained (open triangle in Fig. 1). The potential anthropogenic sources of Pt along the estuary are motorways with heavy traffic during the summer period and the Šibenik harbour.

Sampling

At each sampling site, samples were collected using a van Dorn horizontal acrylic sampler for bottom waters or by using grab sampling with a 1-L FEP bottle (previously cleaned with 1 % HNO_3 (Suprapur) and MilliQ water) for collection of surface waters (~0.3 m below the surface). Four sampling campaigns were conducted: summer and winter 2017, summer 2018 and winter 2019. Surface and bottom waters (filtered and unfiltered) were always collected except for the winter 2017 campaign where only surface water was sampled. Sampling for the vertical profile was performed using a horizontal acrylic sampler at all depths, which included the surface. Samples were filtered either immediately onboard or in the laboratory (within a few hours) using syringe filters with a pore size of 0.22 µm (Minisart, Sartoris). Both filtered and unfiltered samples were collected and stored in 125-mL FEP bottles which were previously washed using the trace metal clean procedure. Samples were acidified to 0.2 % v/v conc. Suprapur HCl (Merck) and UV-digested for 24 h using 250 W high pressure Hg-lamp to decompose organic matter and release Pt from potential Pt-organic complexes. We used hydrochloric acid to acidify the samples because the acidification by nitric acid led to a higher baseline current and a decrease of the sensitivity. Vertical profiles of physico-chemical parameters (S, T, O_2, pH and Chl-a) were recorded using an EXO2 multiparameter CTD probe (YSI).

Because of the extremely low concentrations of Pt, obtaining reliable 'blanks' and eliminating any contamination was a challenge. During the first sampling in summer 2017, high and uniform Pt concentrations were obtained in the filtered samples, with values slightly above the level expected for the coastal sea. We found that this arose from contaminations caused by the release of Pt from the silicone ring on the syringe plunger used for filtration (Pt or peroxide was used in the silicon production process ('Platinum- or Peroxide-cured Silicone')). The release of Pt from the silicone ring generated a Pt concentration in the sample of between 0.5 and 0.7 pM. Thereafter, all samples were filtered with syringes free of any silicone ring. Thus, for the July 2017 campaign, only unfiltered concentrations were reported.

Equipment and analysis

All measurements were performed using µAutolabIII or PGSTAT12N potentiostats (EcoChemie) coupled with a three-electrode cell (663 VA Stand, Metrohm) with a static mercury drop working electrode (SMDE), Ag|AgCl|sat. NaCl electrode as the reference electrode and a glassy carbon rod as the auxiliary electrode. Measurements were performed by coupling the potentiostat with a home-made fully automated system, which consisted of a sample-changer, five Cavro XE 1000 syringe pumps and home-made software (*VoltAA*: https://sites.google.com/site/daromasoft/home/voltaa).

Platinum was analysed using catalytic adsorptive cathodic stripping voltammetry (Cat-AdCSV) according to the method proposed by van den Berg and Jacinto (1988). UV-digestion was an important step here, as this method is sensitive to the presence of organic matter (Obata et al. 2006; Cobelo-Garcia et al. 2013). Prior to the measurement, 30 mL of sample was added to acid-cleaned 50-mL PP vials, and sulfuric acid was added to a final concentration of ~0.6 M (Trace Select, Fluka). This solution was then poured into a Teflon or quartz voltammetric cell and additions of formaldehyde (final concentration 3.5 mM; Riedel-de-Haen) and hydrazine sulfate (final concentration 0.45 mM; Fluka) were performed using automated burettes, which led to a total dilution factor of 1.04.

Detection was conducted by differential pulse anodic stripping voltammetry (DPASV) using a deposition potential of −0.65 V and deposition times of 5 or 7.5 min, which were found to be sufficient for reliable measurements. The parameters of the differential pulse voltammetric (DPV) technique were varied to determine the optimal conditions where the Pt peak was well expressed.

Before each set of measurements, a blank of MilliQ and the chemicals was checked. The blank level was below 30 fM, which was found to originate mainly from the MilliQ water and not from the chemicals used. Thus, the blank was not used to correct any measured Pt concentrations.

Results and discussion

Optimisation of the voltammetric procedure

Considering the expected low Pt concentrations (<1 pM), our first aims were to optimise the voltammetric procedure and data treatment, to increase the reliability of the measurement and to lower the limit of detection. The concentrations of formaldehyde, hydrazine and sulfuric acid have already been optimised by other authors (van den Berg and Jacinto 1988; Obata et al. 2006; Cobelo-Garcia et al. 2013), and we focussed first on the optimisation of the stripping parameters (interval time, modulation time and amplitude) and deposition potential(s). The purpose was not to obtain a higher peak intensity, but to obtain a well-resolved Pt peak in relation to the steep baseline current that exists at that peak position. A small shoulder, which represented no more than 20 % of the Pt analytical signal, was overlapping the Pt peak. Its position varied depending on the voltammetric procedure and its intensity was independent of the deposition time. In addition, owing to the very acidic conditions, a current of 100 nA had to be used (in place of the usual 10 nA), which led to a higher current noise and consequently led us to increase the differential pulse (DP) amplitude to 40 mV so as to maintain a good signal-to-noise ratio. Finally, we found that lowering the pulse time and interval time resulted in a better resolved Pt peak (see example in Fig. S1, Supplementary Material). The deposition potential used to accumulate the Pt-formazone complex has varied between authors: −0.3 V (Cobelo-Garcia et al. 2013), −0.7 V (Obata et al. 2006) and −0.95 V (van den Berg and Jacinto 1988). This latter study reported a stable response when using deposition potentials above −0.8 V, a sharp increase in the peak height between −0.85 and −1.0 V, and a sharp decrease at more negative potentials. In our experiment, the peak intensity increased gradually up to approximately −0.7 V, followed by a sharp decrease at more negative potentials (Fig. 2). From such a response, a deposition potential of −0.65 V was chosen as the optimal potential.

The voltammetric determination of Pt is very sensitive to the presence of even small concentrations of organic matter. This interference effect has been attributed to the adsorption of electroinactive surface-active compounds on the electrode

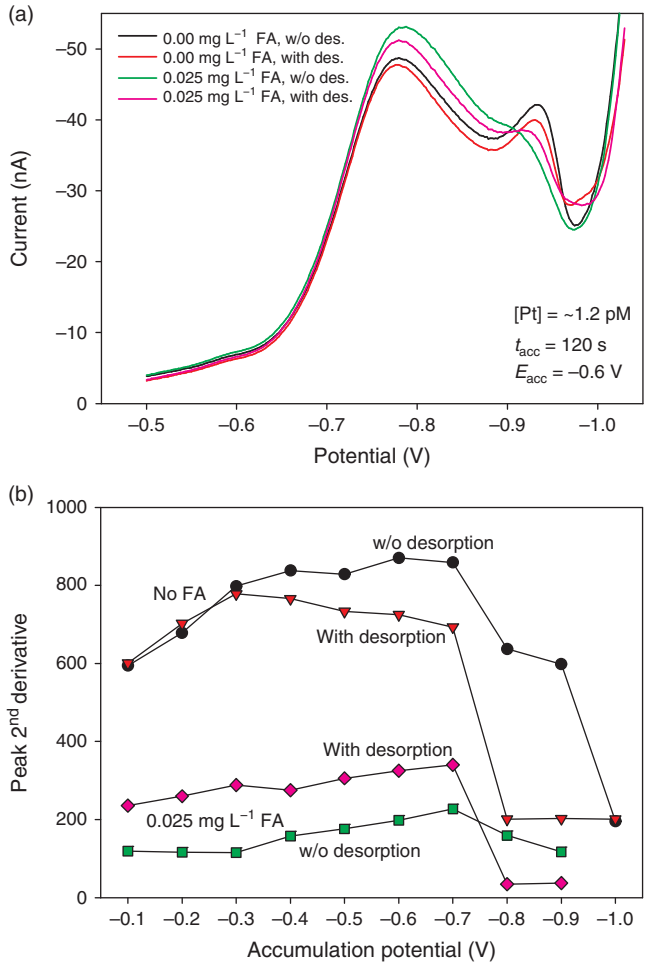

Fig. 2. (a) Voltammograms and (b) relationship of the peak intensity (2nd derivative) on the adsorption potential for ~1.2 pM Pt in seawater obtained with and without added fulvic acid (FA) and with and without desorption step (DS).

surface that inhibit the catalytic reaction of hydrogen evolution that occurs during Pt analysis (van den Berg and Jacinto 1988). Other studies have found that full Pt recovery could not be achieved if the samples were not UV irradiated, which suggested the presence of electroinactive Pt-organic complexes (Obata et al. 2006; Cobelo-Garcia et al. 2013). Here, we investigated the effect of small concentrations of fulvic acid (FA = 0.025 mg L^{-1}) in UV-digested estuarine water, spiked with Pt, on its voltammetric peak. Two different voltammetric procedures were used: with and without a short 2-s desorption step (DS) at −1.35 V (Fig. 2). This short negative desorption step was previously found to assist in removing adsorbed organic surface-active substances from the electrode surface, which provided significantly better shaped voltammograms for Cu analysis (Louis et al. 2008). In the case of Pt, in the UV digested sample without FA additions, slightly higher signals were obtained without DS. Upon addition of FA, the Pt peak intensity for both voltammetric procedures strongly decreased but the Pt peaks obtained with DS were twice as intense as those without DS, which showed clear evidence of its beneficial effect. It was however not clear if this significant decrease arose from competitive adsorption at the electrode surface owing to the surface activity of this low level of FA (0.025 mg L^{-1}, equivalent to ~0.0125 mg L^{-1} DOC) or if strong complexation of Pt^{II} with organic matter could occur, even in these acidic conditions. van den Berg and Jacinto (1988) noted that in seawater, Pt^{II} could be complexed by natural organic ligands. Thermodynamic calculations of Cobelo-Garcia et al. (2013) showed that in seawater, Pt^{IV} predominates over Pt^{II}, in agreement with the observation of a rapid oxidation of spiked Pt^{II} to Pt^{IV} in the presence of the macroalgae *Ulva lactuca L* (Cosden et al. 2003), and agreed also with the results of another study (Mashio et al. 2017). In contrast, in freshwater, speciation calculation suggested that Pt^{II} should predominate over Pt^{IV}. To date, there have been no reported studies that have focussed on the interaction of Pt with natural organic ligands. The extension of our experiment with added FA and with two voltammetric procedures (with and without DS) included testing whether the recovery of Pt would be impacted by the addition of a small concentration of FA (having a strong influence on peak intensity or shape).

Experiments performed with spiked Pt in MilliQ (0.85 pM) showed that under these experimental conditions, the analytical determination was not compromised. The obtained recoveries were ~103 % for both voltammetric procedures (Fig. S2, Supplementary Material). However, the sensitivities differed strongly depending on the conditions. While the sensitivity (2nd derivative, 5 min accumulation time) without added FA was 606 pM^{-1}, addition of FA decreased it down to 345 pM^{-1} and 219 pM^{-1} with and without DS respectively. For seawater samples, no peak was detected in the samples that were not UV irradiated if DS was not applied, whereas a barely noticeable peak was detected if DS was applied. However, owing to the much higher concentrations of organic matter in the natural samples, the analytical determination of Pt could be unreliable if the water sample is not UV irradiated, despite using DS. Therefore, applying both procedures (with and without DS) on the same sample could provide a clear answer concerning if the UV-irradiation was complete or if some surface active organic matter still remained in the sample (test method). The test performed on real samples (UV-irradiated) showed that the difference in peak intensity between two applied procedures was within 10 %, which was within the expected experimental uncertainty.

In conclusion, our optimised procedure was: deposition potential of −0.3 or −0.65 V, deposition time of 300–450 s, a short DS (−1.35 V for 2 s, in some cases), interval time = 0.1 s, modulation time = 0.025 s and amplitude = 40 mV. Typical catalytic voltammograms at increasing concentrations of Pt obtained from a seawater sample, together with the associated standard addition plot, are presented in Fig. S3 (Supplementary Material). The Pt concentration in that sample was measured at 0.29 ± 0.02 pM with an obtained LOD of 15 fM. Even lower LODs down to below 10 fM were obtained for low Pt concentration samples.

Voltammograms treatment

We wanted here to explore whether higher derivative transformations could improve the determination of low levels of Pt in comparison with the 2nd derivative treatment, as shown previously (Cobelo-Garcia et al. 2014*b*). Fig. 3 shows the typical raw DP voltammograms, along with the corresponding 2nd and 4th derivative transformations. For small Pt peaks, the use of the 4th derivative yielded a slightly better peak than that with the 2nd derivative because it provided a flatter baseline. Owing to the relatively low noise and smooth voltammograms

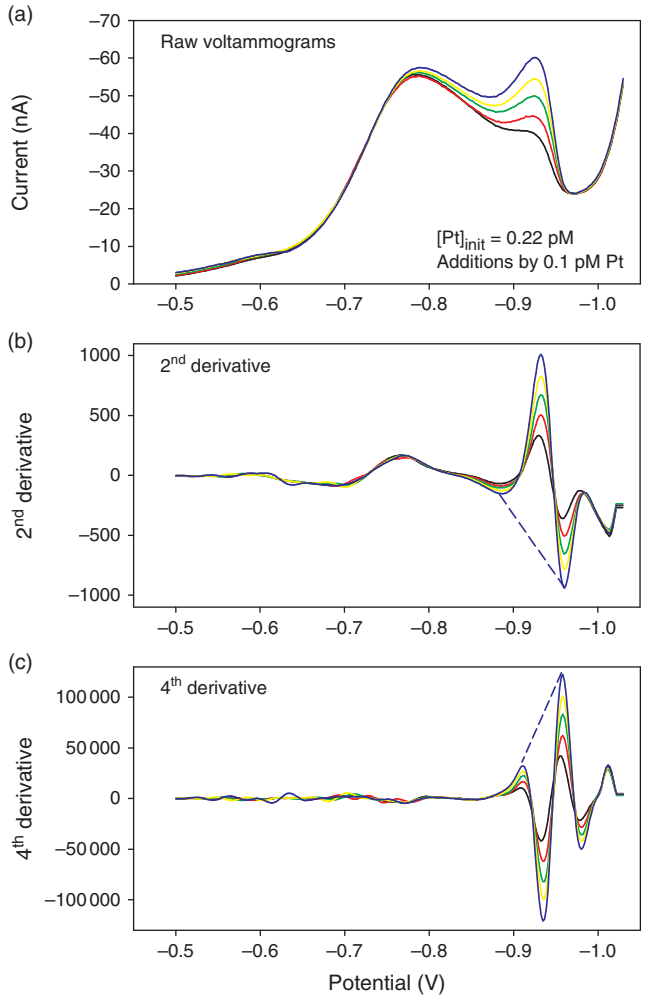

Fig. 3. (a) Raw DP voltammograms and corresponding (b) 2nd and (c) 4th derivative transformations. Blue dashed line approximates the 'tangent fit' line used for peak determination.

(no unexpected 'shoulders'), both the 2nd and 4th derivative peaks were well expressed. In our study, we used a DP as the stripping technique, but square wave voltammetry (SW) (Locatelli 2005) or linear scan voltammetry (LSV) (van den Berg and Jacinto 1988) can also be used. Given that the DP forward current scan is analogous to that of LSV, it could also be used for quantification of Pt. Although the peak at low concentrations was not well expressed in the raw forward current voltammogram, the 2nd and 4th derivatives both provided clear peaks (Fig. S4, Supplementary Material) and the same concentrations (within 10 %) were determined using either of them. In this study, we used the 4th derivative transformation of the DP voltammograms because of the flatter baseline that should lead to lower detection limits.

Hydrography of the estuary

Physico-chemical parameters for the sampling period are presented by the contour plots in Fig. S5 (Supplementary Material). The vertical and horizontal salinity profiles were typical for those two sampling periods (summer and winter). The halocline was positioned deeper in the winter than in the summer, with a low salinity upper layer extending more towards the lower part of the estuary, owing to the higher freshwater flow. The variation of pH (not shown) was minimal, starting at 8.4 in the upper estuary (freshwater side) and finishing at around 8.2 in the seawater end-member. The slightly higher pH in the freshwater part was related to the degassing (removal of CO_2) that occurred at the waterfalls which preceded the estuarine transect (Cindric et al. 2015). The suspended particulate matter (SPM) level was not measured in this work, but previous studies found relatively low concentrations, not exceeding 5 mg L^{-1}. Dissolved organic carbon (DOC) concentrations were higher in summer (up to ~150 µM) than in winter (up to ~80 µM). Typically, DOC was lower (~50 µM) in winter at the freshwater end-member than at the seawater end-member, whereas for the summer period, an opposite trend was observed owing to the high biological activity that occurs in the freshwater Visovac Lake preceding the estuary (Petricioli et al. 1996) (Fig. 1). Typical DOC profiles (Fig S5, Supplementary Material) for the Krka estuary were obtained for both seasons; these were rather unusual compared with other estuaries that show a decreasing trend in the seaward direction. Temperature profiles followed those of salinity for both seasons. The oxygen saturation profile was the most variable; in summer, high oxygen levels (up to 140 % saturation) were found below the halocline between Station P3 and Station P11 (Fig. 1), while very low levels were observed in the upper part of the estuary in winter. Such trends have already been reported in a previous study (Legović et al. 1991). The former case was related to the high productivity at the lacustrine part of the estuary (Prokljan Lake). The low oxygen levels in winter arose from the degradation of organic matter produced during the summer period associated with the high residence time of the bottom seawater layer in that upper part of the estuary.

Distribution of platinum along the estuarine transect

Horizontal distributions of dissolved and/or total Pt concentrations in the surface layer along the estuarine horizontal transect are presented in Fig. 4a (in relation to salinity) and Fig. 4b (in relation to distance from the waterfalls, station E0). In most cases, Pt concentrations increased with salinity, reaching a maximum value of ~0.3 pM at the seawater end-member, similar to those reported in the North Pacific (~0.3 pM) (Fischer et al. 2018) or in the Venice Lagoon (Turetta et al. 2003). The concentrations of Pt in the freshwater end-member were low (down to 0.11 pM), which were higher than those measured in Lérez River (~0.05 pM) (Cobelo-Garcia et al. 2013), but significantly lower than those reported by Obata et al. (2006) in Ara and Tama Rivers (Japan) or in the urban Como channel (Italy) (Monticelli et al. 2010). Dissolved Pt concentration measured in major rivers of East Asia ranged between <0.1 and 5.8 pM, with median values mainly lower than 0.5 pM, with some samples having a concentration below 0.1 pM (Soyol-Erdene and Huh 2012). In the Gironde estuary, a decreasing trend of dissolved Pt concentrations with salinity was observed, with Pt concentrations on the level of ~0.55 pM at the freshwater part and ~0.25 pM in the seawater end-member (Cobelo-Garcia et al. 2014*a*). In studies of Gironde and Lérez estuaries (Cobelo-Garcia et al. 2013; Cobelo-Garcia et al. 2014*a*), as well as Tokyo Bay's estuaries (Obata et al. 2006; Mashio et al. 2017) a non-conservative behaviour was found for dissolved Pt. Our results suggest a near-conservative behaviour. A slight positive deviation from the conservative mixing line of dissolved Pt at salinities below ~10 was observed, followed by a clear linear increase towards higher salinities. Taking into account that Pt^{II}

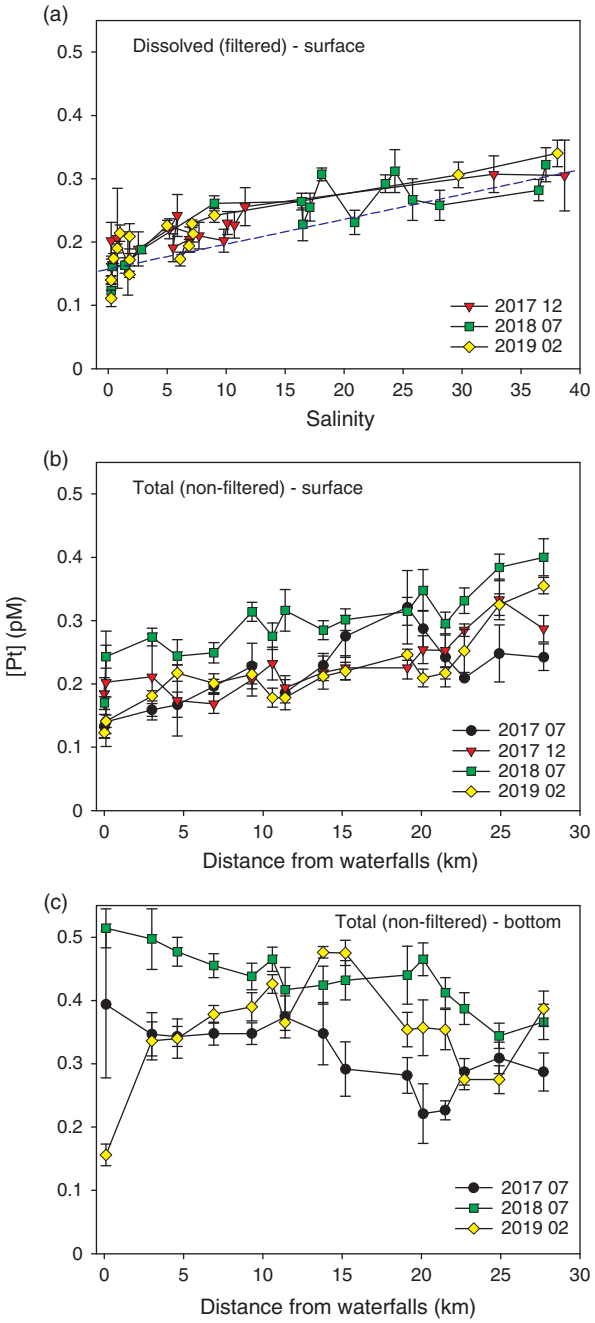

Fig. 4. Dissolved (a) and 'total' (b) Pt profiles in surface layer in relation to salinity (a) and distance from waterfalls (b), and 'total' Pt profiles in seawater layer (c). Error bars correspond to 95 % CI of each Pt determination.

is predicted to dominate Pt^{IV} in freshwater, while the latter is the dominant species in seawater (Cobelo-Garcia et al. 2013), this slight positive deviation from the conservative line might arise from a change in Pt redox speciation, which favours the desorption of a small amount of Pt from the particulate matter. Indeed, Pt^{IV} has a low affinity at higher salinities owing to the weak electrostatic attraction between inorganic Pt^{IV} complexes and the negatively charged suspended particles (Turner 2007). In contrast, in freshwater conditions, Pt^{II} is easily adsorbed on the suspended particles (Turner et al. 2006). No systematic differences in the level of dissolved Pt concentrations between the summer and winter periods were found.

Our results show that Pt exists predominantly in the dissolved form, with no evident Pt partitioning trend regardless of salinity. Fig. 4b presents Pt levels measured in unfiltered surface samples as a function of distance from E0. Linear increases were obtained for all campaigns. In summer 2017, a slight increase was observed in the region of the town of Šibenik (distance of ~18 km), which suggested an anthropogenic source connected to the heavier traffic during the summer season. However, this was not observed in summer 2018, perhaps owing to the lower salinity which is characterised to have generally lower concentrations of trace metals in the bay (Cindric et al. 2015).

On average, ~90 % Pt was present in the dissolved form (min/max = 54 %/112 %), similar to previously reported level in the Lérez River estuary (Cobelo-Garcia et al. 2013). In a few samples, Pt concentrations in the filtered samples were slightly higher (up to ~10 %) than those in the unfiltered samples. This was not attributed to contamination issues but more to the uncertainly of the analytical technique (10–15 %; error bars in Fig. 4).

Along with the horizontal surface profiles shown in Fig. 4a, b, Pt concentration profiles for the unfiltered samples in the bottom seawater layer are plotted in Fig. 4c. In this case, an increasing trend in the landward direction was evident in both summer 2017 and 2018. Such an increase arose from the progressive accumulation occurring in that bottom layer while moving upstream, slowly collecting particles from the upper layer, which might explain why the upstream bottom water had the highest concentrations (Cindric et al. 2015). This trend was not unique to Pt and has been observed for a few other trace metals (Cindric et al. 2015). Another possibility is sediment input. Pt concentrations in the range of 2.8–40 μg kg^{-1} were observed in estuarine sediments of the Tagus Estuary with dissolved porewaters concentrations of 0.7–3.6 pM (Almecija et al. 2016). These concentrations are higher than that of seawater, which implies that sediments and interstitial waters can also be a source of Pt in the bottom waters. As the Pt concentrations in the Krka River sediments and porewaters are not know, the contribution of the sediment as a source of Pt to the bottom layer remains an open question.

In winter 2019, the profile showed a different behaviour with an increase low in the estuary and a strong decrease higher in the estuary. Such a trend might be explained by the low Pt levels in the Krka river (~0.11 pM) and its relatively high flow during that period. Indeed, in summer, the seawater layer was extended all along the estuary up to the freshwater end, while in winter, the freshwater layer extended ~3 km down the estuary. Such a strong freshwater flow caused a mixing of the bottom layer, thus diluting this bottom layer and reducing its Pt concentration levels.

A vertical profile was also collected at a site located in Šibenik Bay, ~10 m from the shore (Fig. 1). Fig. 5 shows the Pt concentrations as a function of depth measured in summer 2017 and 2018 as well in winter 2019. A significant increase was observed at the position of the halocline, as has been observed for many other metals (Cindric et al. 2015). Concentrations in the surface (brackish) and bottom (seawater) layers were in general agreement with those of the horizontal transect for that particular location. Small differences were attributed to the different locations, as observed for other metals in that bay (Cindric et al. 2015). If plotted against salinity, the concentrations followed a linear trend (Fig. S6, Supplementary Material), similar to those reported for the transect. For comparison purposes, a typical level of the procedural MilliQ blank was plotted, which showed no practical influence on the measured Pt concentrations in the samples.

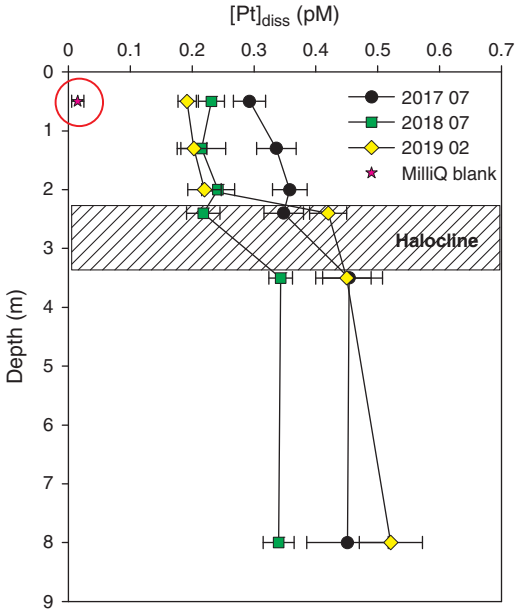

Fig. 5. Vertical profiles of dissolved Pt obtained in summer 2017/2018 and winter 2019 in Šibenik Bay.

Conclusions

The very low concentrations of Pt in marine or estuarine waters require extremely sensitive analytical methods. In our work, we used a well-known adsorptive cathodic stripping voltammetry, with the addition of formaldehyde, hydrazine and sulfuric acid. To obtain well resolved voltammograms at low Pt concentrations (e.g. ~0.1 pM), we tested different combinations of the voltammetric parameters. An optimal signal-to-noise ratio was obtained using short interval and modulation times of the differential pulse stripping waveform (0.1 s and 0.025 s respectively), along with a relatively high pulse amplitude (40 mV). Data treatment was conducted using a 4th derivative transformation that provided a slightly better resolution than the 2nd derivative that is normally applied (Cobelo-Garcia et al. 2014*b*). Using the optimised voltammetric procedure, LODs were lowered down to 10 pM (LOD based on the standard addition method). The presence of very small concentrations of surface-active substances from natural organic matter was found to strongly impact the sensitivity of the method. However, in model solutions, the obtained recoveries were ~100 %, which implied that under our acidic experimental conditions, Pt was not complexed to the added fulvic acid (0.025 mg L^{-1} FA). Further studies would be needed to resolve whether the strong decrease in the measurement sensitivity in natural samples arises from the complexation of Pt with organic ligands or from the adsorption of these organic substances on the electrode surface that interfere with the stripping signal. Here, UV-irradiation was required to be able to obtain a signal.

We applied our optimised methodology to study the distribution and behaviour of Pt along the salinity gradient of the stratified Krka River estuary. We found that Pt was mostly present in the dissolved form and total concentrations (unfiltered) ranged between 0.10 and 0.5 pM, with, on average, ~50 % higher concentrations in the bottom seawater layer than in the surface layer. In the latter, Pt concentrations gradually increased towards the sea end-member, following a near-conservative behaviour, with a slight increase in the dissolved Pt at salinities below ~10. Speciation modelling predicts a change in the redox speciation of Pt, from Pt^{II} in freshwater (with higher affinity to particles), to Pt^{IV} in seawater in the form of $PtCl_5(OH)^{2-}$. There is a clear increase of Pt concentrations in the bottom seawater layer when moving upstream; this increase might arise from the progressive accumulation of Pt, which is consistent with the residence time of that bottom layer in the estuary and/or from the sediment (Mashio et al. 2017). This question remains open and will require further studies.

Supplementary material

The supplementary material contains additional figures which complement the research study performed and could be useful to readers for comparison purposes.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Abdou M, Schafer J, Hu RY, Gil-Diaz T, Garnier C, Brach-Papa C, Chiffoleau JF, Charmasson S, Giner F, Dutruch L, Blanc G (2019). Platinum in sediments and mussels from the northwestern Mediterranean coast: Temporal and spatial aspects. *Chemosphere* **215**, 783–792. doi:10.1016/J.CHEMOSPHERE.2018.10.011

Almecija C, Cobelo-Garcia A, Santos-Echeandia J, Caetano M (2016). Platinum in salt marsh sediments: Behavior and plant uptake. *Marine Chemistry* **185**, 91–103. doi:10.1016/J.MARCHEM.2016.05.009

Cindric AM, Garnier C, Oursel B, Pizeta I, Omanovic D (2015). Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: The Krka River estuary (Adriatic, Croatia). *Marine Pollution Bulletin* **94**, 199–216. doi:10.1016/J.MARPOLBUL.2015.02.029

Cobelo-Garcia A, Neira P, Mil-Homens M, Caetano M (2011). Evaluation of the contamination of platinum in estuarine and coastal sediments (Tagus Estuary and Prodelta, Portugal). *Marine Pollution Bulletin* **62**, 646–650. doi:10.1016/J.MARPOLBUL.2010.12.018

Cobelo-Garcia A, Lopez-Sanchez DE, Almecija C, Santos-Echeandia J (2013). Behavior of platinum during estuarine mixing (Pontevedra Ria, NW Iberian Peninsula). *Marine Chemistry* **150**, 11–18. doi:10.1016/J.MARCHEM.2013.01.005

Cobelo-Garcia A, Lopez-Sanchez DE, Schafer J, Petit JCJ, Blanc G, Turner A (2014*a*). Behavior and fluxes of Pt in the macrotidal Gironde Estuary (SW France). *Marine Chemistry* **167**, 93–101. doi:10.1016/J.MARCHEM.2014.07.006

Cobelo-Garcia A, Santos-Echeandia J, Lopez-Sanchez DE, Almecija C, Omanovic D (2014*b*). Improving the Voltammetric Quantification of Ill-Defined Peaks Using Second Derivative Signal Transformation: Example of the Determination of Platinum in Water and Sediments. *Analytical Chemistry* **86**, 2308–2313. doi:10.1021/AC403558Y

Cobelo-Garcia A, Filella M, Croot P, Frazzoli C, Du Laing G, Ospina-Alvarez N, Rauch S, Salaun P, Schafer J, Zimmermann S (2015). COST action TD1407: network on technology-critical elements (NOTICE)-from environmental processes to human health threats. *Environmental Science and Pollution Research International* **22**, 15188–15194. doi:10.1007/S11356-015-5221-0

Cosden JM, Schijf J, Byrne RH (2003). Fractionation of platinum group elements in aqueous systems: Comparative kinetics of palladium and platinum removal from seawater by *Ulva lactuca* L. *Environmental Science & Technology* **37**, 555–560. doi:10.1021/ES0259234

Fischer L, Smith G, Hann S, Bruland KW (2018). Ultra-trace analysis of silver and platinum in seawater by ICP-SFMS after off-line matrix separation and pre-concentration. *Marine Chemistry* **199**, 44–52. doi:10.1016/J.MARCHEM.2018.01.006

Legović T, Petricioli D, Žutić V (1991). Hypoxia in a Pristine Stratified Estuary (Krka, Adriatic Sea). *Marine Chemistry* **32**, 347–359. doi:10.1016/0304-4203(91)90048-2

Locatelli C (2005). Platinum, rhodium, palladium and lead: Elements linked to vehicle emissions. Their simultaneous voltammetric determination in superficial water. *Electroanalysis* **17**, 140–147. doi:10.1002/ELAN.200403082

Louis Y, Cmuk P, Omanović D, Garnier C, Lenoble V, Mounier S, Pižeta I (2008). Speciation of trace metals in natural waters: The influence of an adsorbed layer of natural organic matter (NOM) on voltammetric behaviour of copper. *Analytica Chimica Acta* **606**, 37–44. doi:10.1016/J.ACA.2007.11.011

Mashio AS, Obata H, Gamo T (2017). Dissolved Platinum Concentrations in Coastal Seawater: Boso to Sanriku Areas, Japan. *Archives of Environmental Contamination and Toxicology* **73**, 240–246. doi:10.1007/S00244-017-0373-1

Matthey J (2018). PGM Marker report. Available at http://www.platinum.matthey.com/services/market-research/pgm-market-reports [verified May 2018]

Monticelli D, Carugati G, Castelletti A, Recchia S, Dossi C (2010). Design and development of a low cost, high performance UV digester prototype: Application to the determination of trace elements by stripping voltammetry. *Microchemical Journal* **95**, 158–163. doi:10.1016/J.MICROC.2009.11.002

Obata H, Yoshida T, Ogawa H (2006). Determination of picomolar levels of platinum in estuarine waters: A comparison of cathodic stripping voltammetry and isotope dilution-inductively coupled plasma mass spectrometry. *Analytica Chimica Acta* **580**, 32–38. doi:10.1016/J.ACA.2006.07.044

Petricioli D, Bakran-Petricioli T, Viličić D, Požar-Domac A (1996). Freshwater phytoplankton bloom in Visovac Lake – A possible cause of benthic mortality in Krka estuary (Adriatic Sea, Croatia). *Marine Ecology* **17**, 373–382. doi:10.1111/J.1439-0485.1996.TB00515.X

Prohic E, Kniewald G (1987). Heavy-Metal Distribution in Recent Sediments of the Krka River Estuary – An Example of Sequential Extraction Analysis. *Marine Chemistry* **22**, 279–297. doi:10.1016/0304-4203(87)90015-6

Rauch S, Hemond HF, Barbante C, Owari M, Morrison GM, Peucker-Ehrenbrink B, Wass U (2005). Importance of automobile exhaust catalyst emissions for the deposition of platinum, palladium, and rhodium in the Northern Hemisphere. *Environmental Science & Technology* **39**, 8156–8162. doi:10.1021/ES050784M

Schafer J, Eckhardt JD, Berner ZA, Stuben D (1999). Time-dependent increase of traffic-emitted platinum-group elements (PGE) in different environmental compartments. *Environmental Science & Technology* **33**, 3166–3170. doi:10.1021/ES990033I

Sen IS, Peucker-Ehrenbrink B (2012). Anthropogenic Disturbance of Element Cycles at the Earth's Surface. *Environmental Science & Technology* **46**, 8601–8609. doi:10.1021/ES301261X

Soyol-Erdene TO, Huh Y (2012). Dissolved platinum in major rivers of East Asia: Implications for the oceanic budget. *Geochemistry Geophysics Geosystems* **13**, Q06009. doi:10.1029/2012GC004102

Soyol-Erdene TO, Huh Y, Hong S, Do Hur S (2011). A 50-Year Record of Platinum, Iridium, and Rhodium in Antarctic Snow: Volcanic and Anthropogenic Sources. *Environmental Science & Technology* **45**, 5929–5935. doi:10.1021/ES2005732

Turetta C, Cozzi G, Varga A, Barbante C, Capodaglio G, Cescon P (2003). Platinum group elements determination in seawater by ICP-SFMS: Initial results. *Journal de Physique. IV* **107**, 1321–1324. doi:10.1051/JP4:20030544

Turner A (2007). Particle-water interactions of platinum group elements under estuarine conditions. *Marine Chemistry* **103**, 103–111. doi:10.1016/J.MARCHEM.2006.08.002

Turner A, Crussell M, Millward GE, Cobelo-Garcia A, Fisher AS (2006). Adsorption kinetics of platinum group elements in river water. *Environmental Science & Technology* **40**, 1524–1531. doi:10.1021/ES0518124

van den Berg CMG, Jacinto GS (1988). The Determination of Platinum in Sea-Water by Adsorptive Cathodic Stripping Voltammetry. *Analytica Chimica Acta* **211**, 129–139. doi:10.1016/S0003-2670(00)83675-2

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Supplementary document to Research paper 2

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Determination of sub-pico-molar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology

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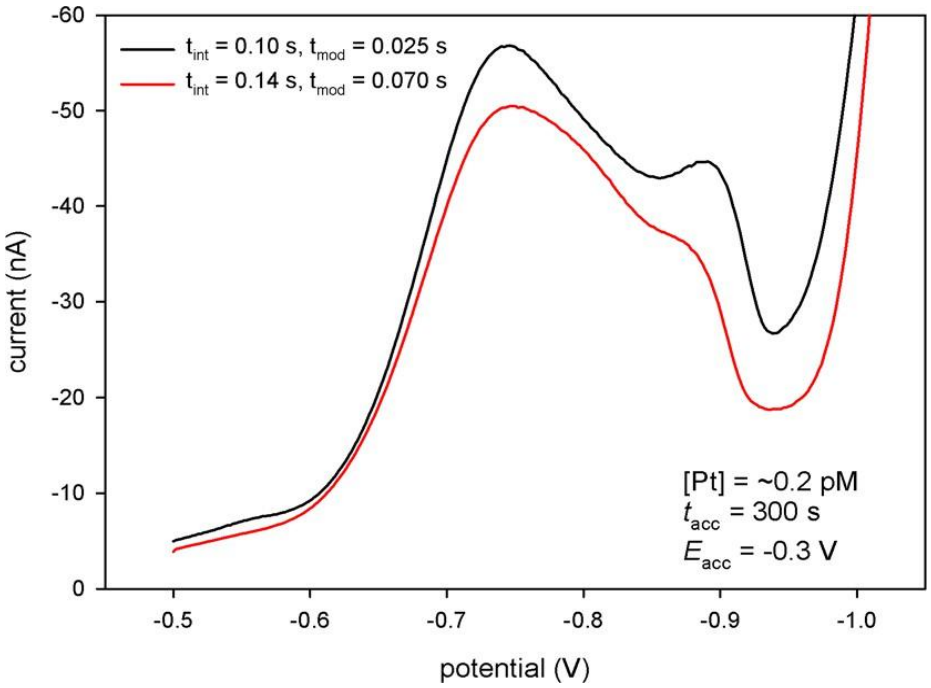

Figure S1. Voltamograms of Pt obtained under two different experimental parameters.

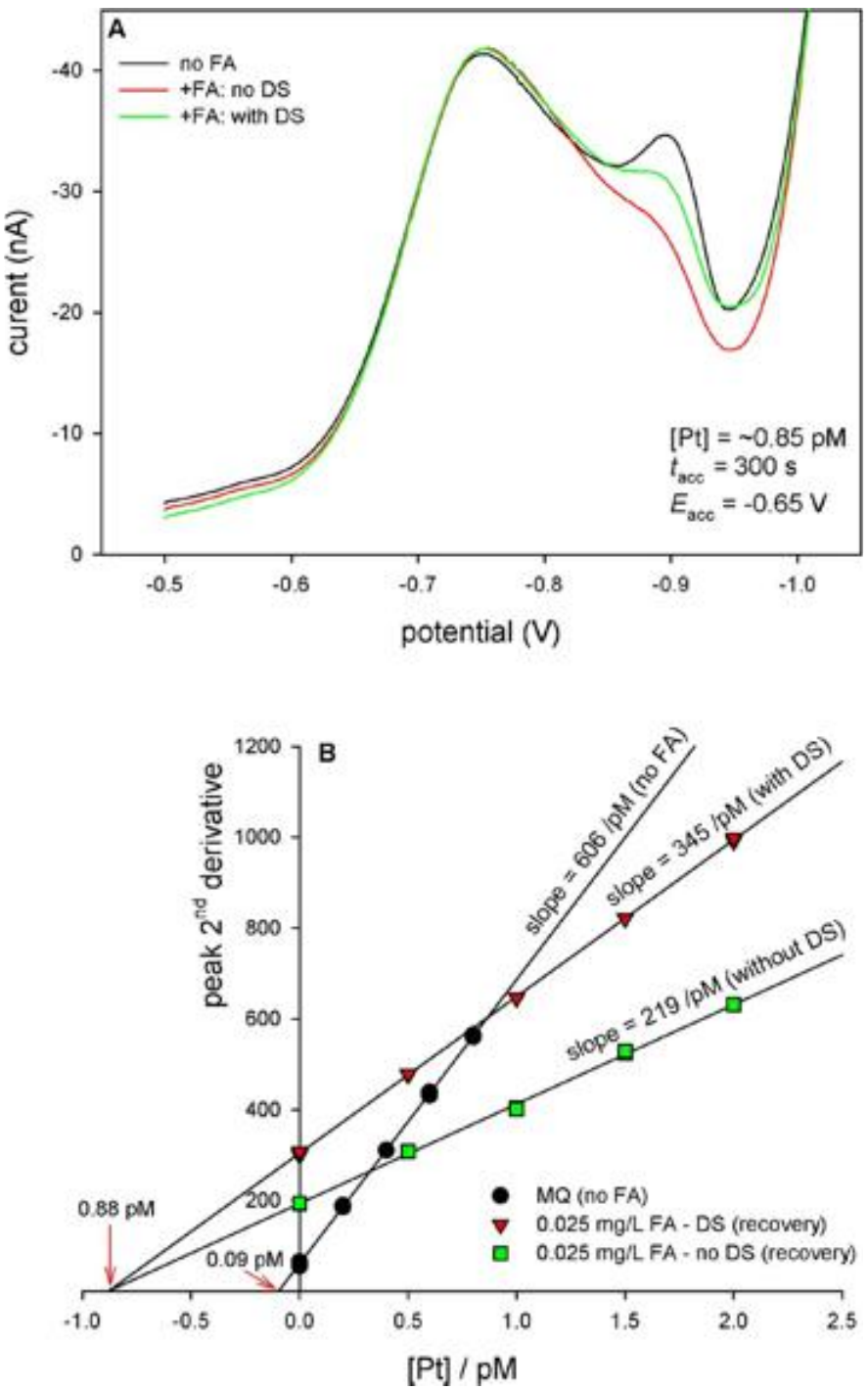

Figure S2. (A) Voltamograms of Pt obtained without added fulvic acid (FA) and with added FA (0.025 mg/L) applying (or not) short desorption step (DS) (*E*ds = -1.35 V, 2 s). (B) Standard addition plots of Pt in MiliQ water without added FA, and recovery plots (with and without DS) of Pt after addition of FA.

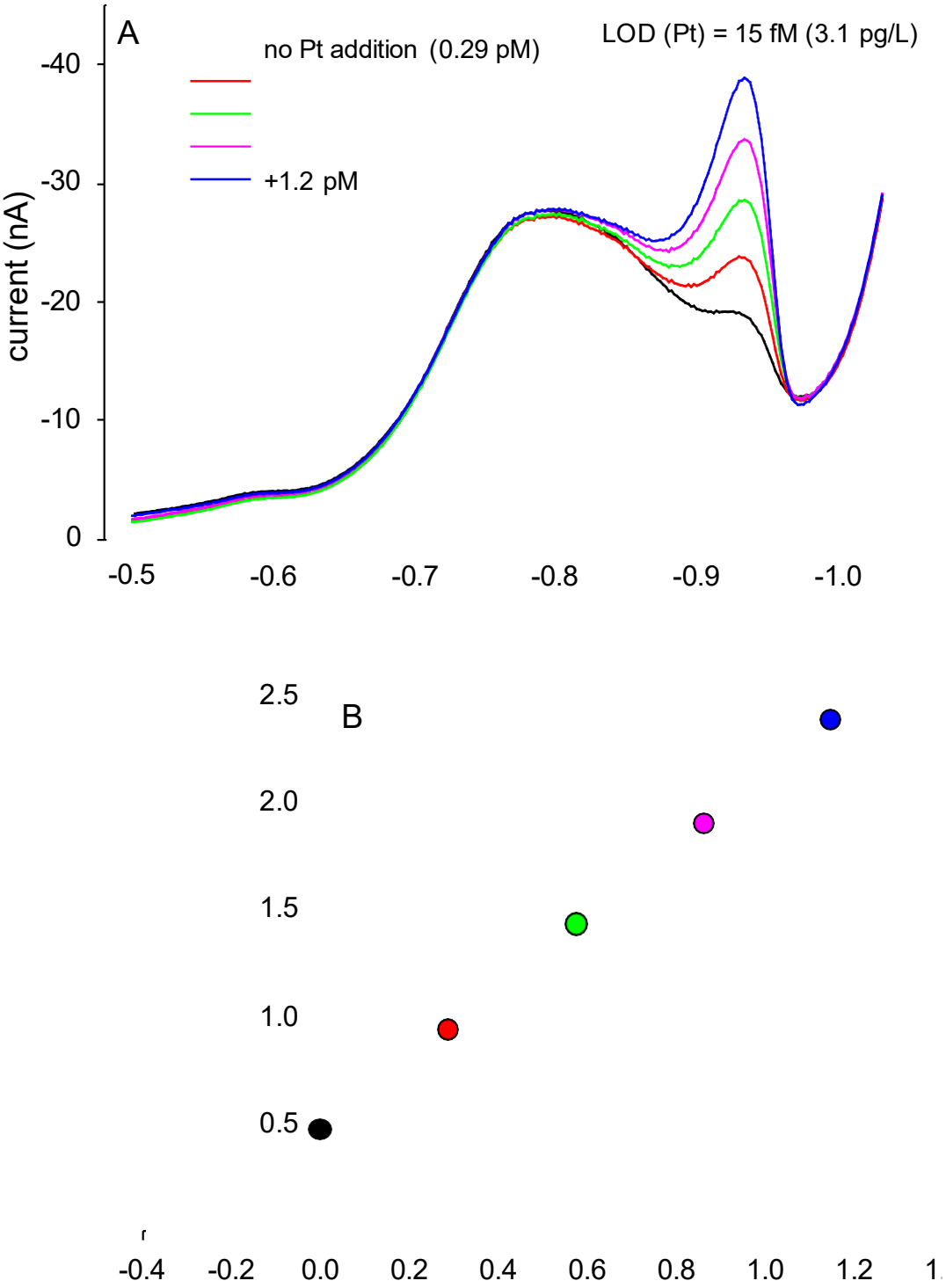

Figure S3. (A) Typical voltamograms of Pt obtained during analysis using standard addition method, along with (B) the corresponding standard addition plot.

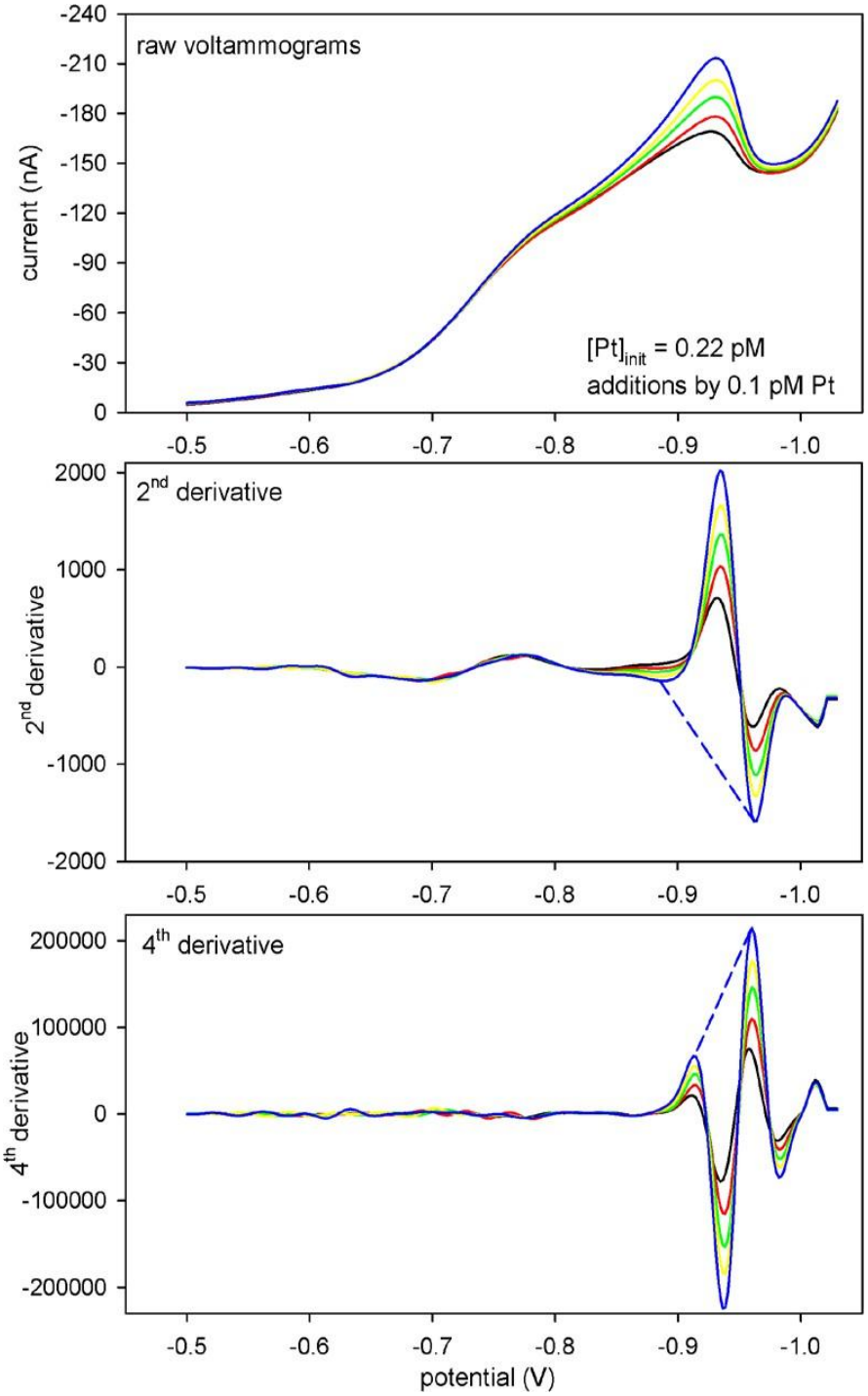

Figure S4. Forward current of DP voltamograms and corresponding 2nd and 4th derivative transformations. Blue dashed line approximates the "tangent fit" line used for peak determination.

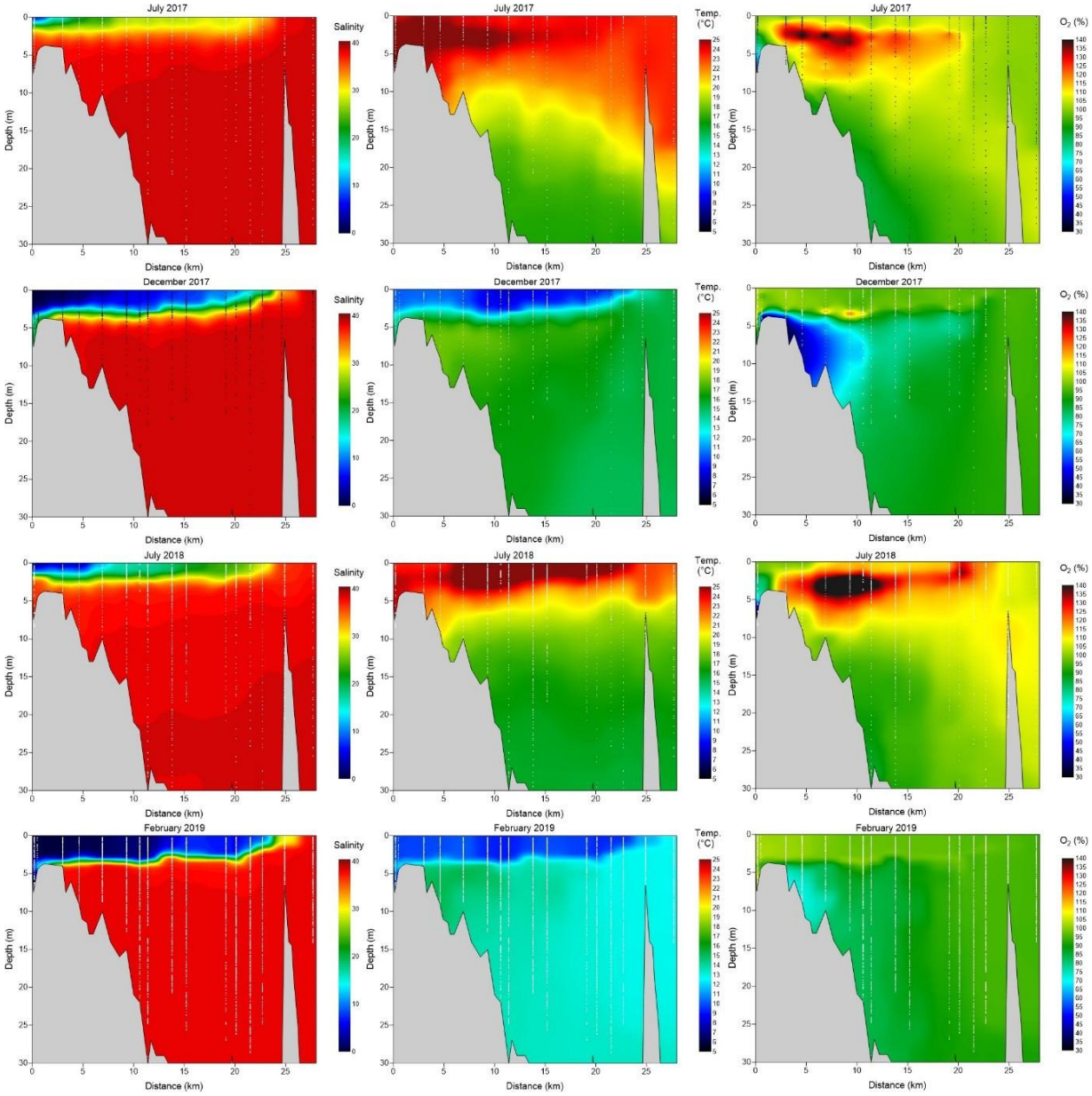

Figure S4. Contour plots of salinity, temperature and oxygen saturation for examined sampling campaigns.

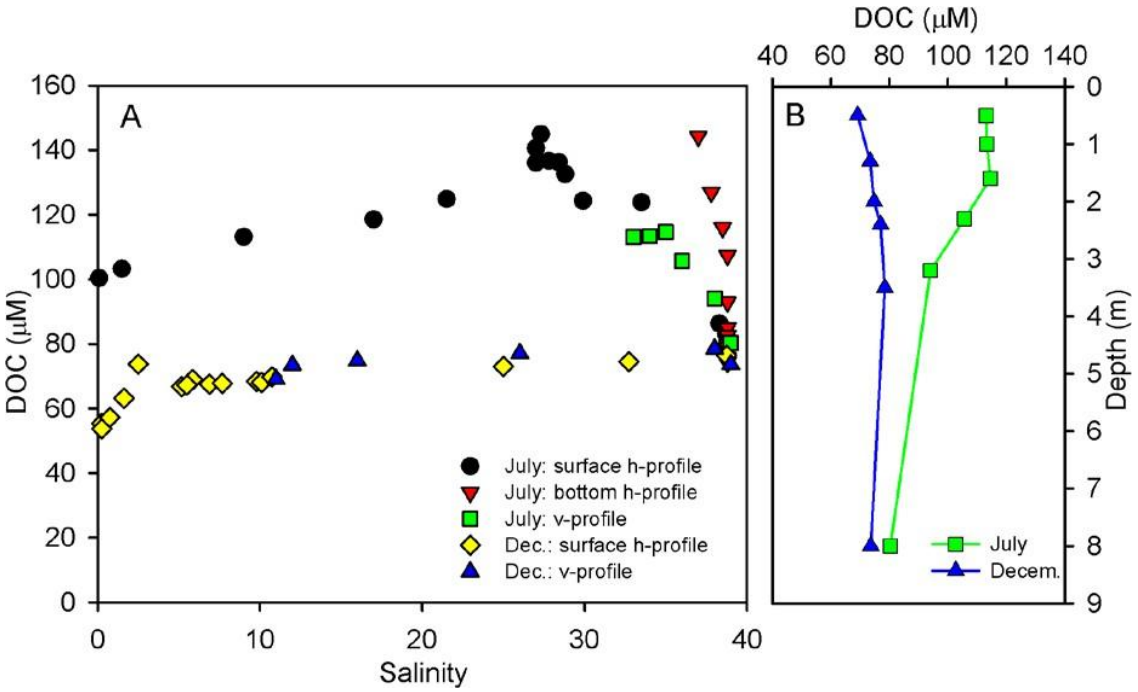

Figure S5. (A) Typical horizontal and (B) vertical profiles of dissolved organic carbon (DOC) for winter and summer period in the Krka River estuary ("h" - horizontal; "v" - vertical)

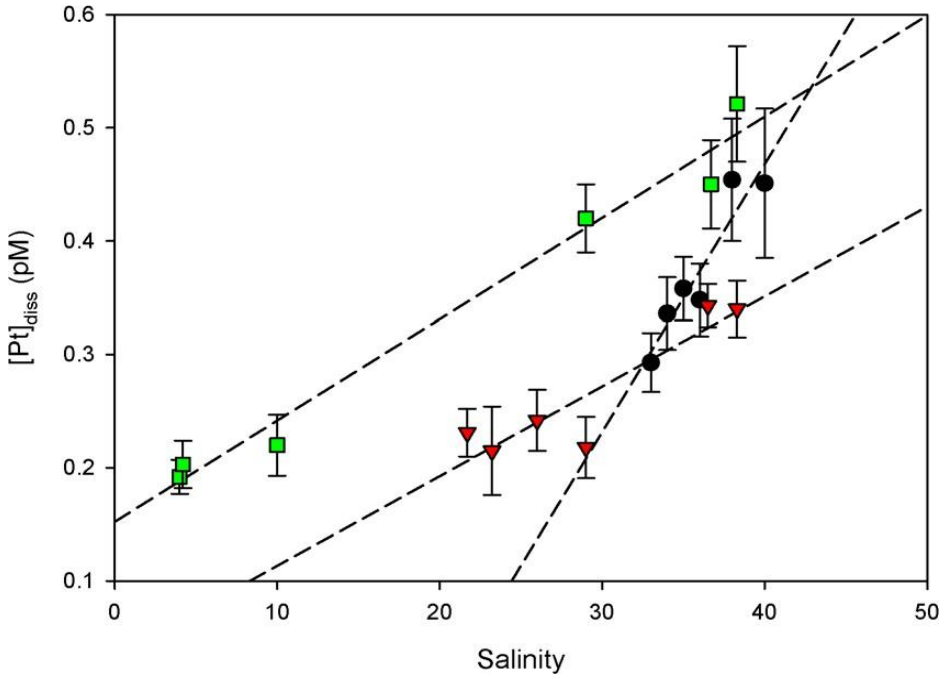

Figure S6. Relationship of dissolved Pt with salinity for examined vertical profiles presented in Fig. S5

Research paper 3

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Organic Copper Speciation by Anodic Stripping Voltammetry in Estuarine Waters With High Dissolved Organic Matter

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The determination of copper (Cu) speciation and its bioavailability in natural waters is an important issue due to its specific role as an essential micronutrient but also a toxic element at elevated concentrations. Here, we report an improved anodic stripping voltammetry (ASV) method for organic Cu speciation, intended to eliminate the important problem of surface-active substances (SAS) interference on the voltammetric signal, hindering measurements in samples with high organic matter concentration. The method relies on the addition of nonionic surfactant Triton-X-100 (T-X-100) at a concentration of 1 mg L^{-1}. T-X-100 competitively inhibits the adsorption of SAS on the Hg electrode, consequently 1) diminishing SAS influence during the deposition step and 2) strongly improving the shape of the stripping Cu peak by eliminating the high background current due to the adsorbed SAS, making the extraction of Cu peak intensities much more convenient. Performed tests revealed that the addition of T-X-100, in the concentration used here, does not have any influence on the determination of Cu complexation parameters and thus is considered "interference-free." The method was tested using fulvic acid as a model of natural organic matter and applied for the determination of Cu speciation in samples collected in the Arno River estuary (Italy) (in spring and summer), characterized by a high dissolved organic carbon (DOC) concentration (up to 5.2 mgC L^{-1}) and anthropogenic Cu input during the tourist season (up to 48 nM of total dissolved Cu). In all the samples, two classes of ligands (denoted as L_1 and L_2) were determined in concentrations ranging from 3.5 ± 2.9 to 63 ± 4 nM eq Cu for L_1 and 17 ± 4 to 104 ± 7 nM eq Cu for L_2, with stability constants $\log K_{Cu,1}$ = 9.6 ± 0.2–10.8 ± 0.6 and $\log K_{Cu,2}$ = 8.2 ± 0.3–9.0 ± 0.3. Different linear relationships between DOC and total ligand concentrations between the two seasons suggest a higher abundance of organic ligands in the DOM pool in spring, which is linked to a higher input of terrestrial humic substances into the estuary. This implies that terrestrial humic substances represent a significant pool of Cu-binding ligands in the Arno River estuary.

Keywords: Arno River estuary, copper, organic ligands concentration, speciation, trace metals, surface active substances, metal complexing capacity

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INTRODUCTION

Copper (Cu) is an essential micronutrient in natural waters, required for the proper functioning of metabolic and respiratory processes for many aquatic species (Peers et al., 2005; Peers and Price, 2006; Annett et al., 2008; Glass and Orphan, 2012; Jacquot et al., 2014). It is also known for its toxic effects when exceeding a critical concentration threshold (Karlsson et al., 2010; Ytreberg et al., 2010) and thus is considered a specific pollutant of great ecotoxicological concern (Corcoll et al., 2019; Lopez et al., 2019). It is not the total copper concentration that is directly related to ecotoxicological effects but the fraction available for biological uptake (free and/or labile concentration) (Campbell et al., 2002; Sanchez-Marin et al., 2016; Zitoun et al., 2019). Therefore, for an accurate assessment of its potential impact on biota, the knowledge of Cu speciation, i.e., predicting the concentration of its bioavailable fraction, is of primary concern. The most important factor influencing Cu speciation in seawater is the concentration and quality (chemical structure) of the dissolved organic matter (DOM) (Sanchez-Marin et al., 2016). The range of copper concentrations between its necessity and toxicity is relatively narrow (Amin et al., 2013; Zitoun et al., 2019). However, the formation of strong complexes with organic ligands can reduce the bioavailable Cu fraction and, in most cases, maintains it in the optimal range (Buck et al., 2012; Whitby and van den Berg, 2015; Whitby et al., 2017). The presence of organic ligands is therefore of main significance in assessing the Cu bioavailability, with respect to both toxicity and necessity. Nevertheless, there is evidence that even organically complex copper is acquired by marine phytoplankton and bacteria (Semeniuk et al., 2015). Coastal areas and estuaries are the most relevant areas for Cu speciation studies because of the high potential for anthropogenic Cu contamination (e.g., Helland and Bakke, 2002; Blake et al., 2004; Louis et al., 2009) including secondary contamination by the release from sediment during estuarine mixing (e.g., Cobelo-Garcia and Prego, 2003). These areas are usually characterized by high concentrations of DOM from both allochthonous and autochthonous sources (Fellman et al., 2011; Retelletti Brogi et al., 2020), including biogenic thiol compounds and terrestrially derived humic and fulvic acids, which all form strong Cu complexes (Tang et al., 2000; Kogut and Voelker, 2001; Laglera and van den Berg, 2003; Shank et al., 2004; Whitby et al., 2017; Dulaquais et al., 2020). Besides regulating its bioavailable concentration, binding with these organic ligands in coastal waters is an important factor in Cu transport to the ocean (Helland and Bakke, 2002).

Due to experimental limitations of separation, extraction, and direct measurement of different metal-organic ligand complexes in seawater, an alternative approach based on complexometric titrations using electrochemical techniques (known as the determination of the metal complexing capacity of the sample) is preferentially used (Buck et al., 2012; Pižeta et al., 2015). As the concentration of metals in seawater is very low, various electrochemical techniques with low detection limits are commonly used, mainly stripping techniques: anodic and competitive ligand exchange adsorptive cathodic stripping voltammetry (ASV and CLE-AdCSV, respectively) (Sanchez-Marin et al., 2010; Buck et al., 2012; Omanović et al., 2015; Pižeta et al., 2015; Whitby et al., 2018). Knowledge about sources and chemical identity of detected ligands is scarce (Vraspir and Butler, 2009) and its acquisition is hindered by very complex chemical composition of natural DOM (Repeta, 2015). Useful qualitative information about the DOM pool in the aquatic environment (e.g., average aromaticity degree, molecular weight, and occurrence of humic-like, fulvic-like, and protein-like substances) can be gained indirectly via UV/Vis and fluorescence spectroscopic studies of its optically active fractions (colored and fluorescent dissolved organic matter, CDOM and FDOM) (Yamashita et al., 2011; Osburn et al., 2012; Lee et al., 2018; Galletti et al., 2019; Marcinek et al., 2020). Including this information in speciation studies could improve our understanding of the sources and composition of metal-binding organic ligands (Sanchez-Marin et al., 2010; Slagter et al., 2017; Watanabe et al., 2018; Wong et al., 2019; Whitby et al., 2020). However, these methods cannot characterize the binding sites or give information about nonfluorescent substances in DOM. This is possible by analyses of the molecular composition of DOM isolated from estuarine water (Minor et al., 2001; Minor et al., 2002; Dalzell et al., 2009; Abdulla et al., 2010; Zhang et al., 2018; Daoud and Tremblay, 2019; Thibault et al., 2019), e.g., using Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) (Abdulla et al., 2010; Zhang et al., 2018) or direct temperature-resolved mass spectrometry (DT-MS) complemented with either size-exclusion chromatography (Minor et al., 2002) or wet chemical techniques (Minor et al., 2001), which provide qualitative and quantitative analyses of major functional groups and information about their distribution.

Voltammetric stripping techniques are particularly sensitive to the composition of the sample solution. The well-known interferences in natural samples are due to the adsorption of surface-active substances (SAS) on the surface of the working electrode (usually Hg-drop) (Sander and Henze, 1996; Batley et al., 2004; Hurst and Bruland, 2005). This is obstructing both deposition/adsorption and stripping steps in ASV and CLE-AdCSV, resulting in lower sensitivity and/or deformation of the resultant voltammetric peak (Boussemart et al., 1993; Scarano and Bramanti, 1993; Plavšić et al., 1994; Louis et al., 2008). The extent to which SAS adsorbs on the electrode surface depends on the sample composition and the type of SAS. The final negative effect of the SAS influence is the inaccuracy of speciation parameters (Louis et al., 2008). Given that SAS is a significant fraction of DOM in seawater (Ciglenečki et al., 2020), the analysis of samples with high DOM content is particularly challenging. At potentials < −1.4 V, desorption of SAS from the electrode surface occurs (Sahlin and Jagner, 1996). Therefore, for removing the interferences during measurement, the "desorption step" (DS) was proposed (Louis et al., 2008; Louis et al., 2009; Gibbon-Walsh et al., 2012), i.e., switching to very negative potentials (e.g., −1.5 V) for a short time (e.g., 1–3 s), at the end of the main deposition period. DS reduces the effects of interferences to a large extent and enables a better electrode

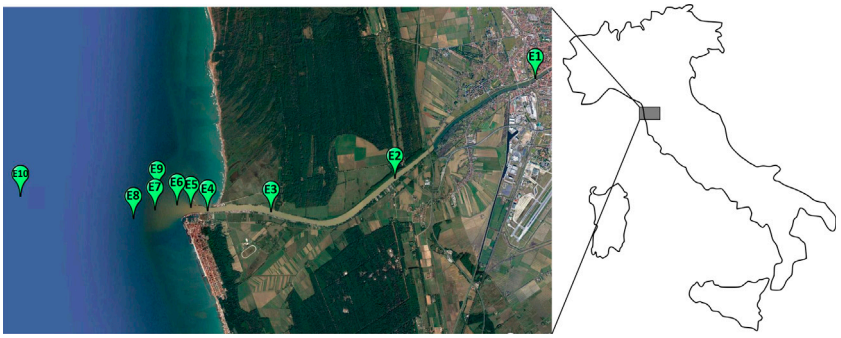

FIGURE 1 | Sampling sites along the Arno River estuary (Pisa, Italy) (image source: Google Earth Pro).

response and the formation of a well-defined peak. However, in samples with high DOM content, adsorption interferences cannot be completely removed, and some effects still exist. Previous studies showed that nonionic surfactant Triton-X-100 (T-X-100) added to the sample has an influence on the redox processes of Cu (Krznarić et al., 1994; Plavšić et al., 1994) and helps in obtaining reliable Cu complexation parameters in model solutions (Omanović et al., 1996). Based on this evidence, we assumed that its adsorption properties could be beneficial also for Cu speciation in natural samples hindered by the adsorbed SAS.

The main objective of this study is to explore the ability of nonionic surfactant T-X-100 to eliminate SAS interferences on the mercury electrode in the presence of high concentrations of DOM, without disturbing the original chemical speciation of Cu in the sample. The method was tested using fulvic acid (obtained from the International Humic Substances Society, IHSS) as a model of natural organic matter. The benefit of the proposed method for copper speciation studies is demonstrated in samples from the Arno River estuary (Italy), characterized by high organic matter content (up to 5.2 mg L^{-1} of dissolved organic carbon, DOC) and significant anthropogenic copper concentration (up to 48 nM of total dissolved copper, DCu). Obtained complexation parameters (i.e., detected Cu-binding ligands) are complemented with UV/Vis and fluorescence measurements (PARAFAC analysis) of DOM present in the estuary.

MATERIALS AND METHODS

Study Area

The Arno River is 242 km long (the 5th largest river in Italy) and its catchment covers an area of 8,228 km^2. The river flows into the Ligurian Sea about 10 km downstream from Pisa. The Arno River is impacted by various anthropogenic sources. Industrial activities like paper-mills, textile, electrochemical plants, and tanneries contribute to high levels of various inorganic and organic contaminants (Dinelli et al., 2005; Cortecci et al., 2009). For example, the Arno River accounts for 7% of the total DOC flux entering the western Mediterranean Sea, highlighting its relevant contribution (Retelletti Brogi et al., 2020).

For this study, the sampling was carried out at the Arno River estuary (**Figure 1**). The estuary region is 12 km long, highly stratified, and characterized by river flow extremes measured from 6 $m^3 s^{-1}$ during summer and up to 2000 $m^3 s^{-1}$ in winter [average discharge of 82.4 $m^3 s^{-1}$ (Retelletti Brogi et al., 2020)].

The lower part of the estuary hosts numerous anchored recreational/sailing boats, whose antifouling paint is a source of copper (Cindrić et al., 2015). This can potentially have unfavorable biological effects on the ecosystem in the estuary and the coastal region. Sampling was carried out in the periods of the year when the most intensive nautical traffic and the highest biological activity was expected (September 27, 2015, and April 5, 2016). Surface samples were collected at 10 sites along the salinity gradient (**Figure 1**).

Samples Collection

Surface samples (depth ~0.5 m) were collected using a van Dorn type 2.2 L horizontal water sampler (Wildco). Bottles for the sampling and sample storage for dissolved Cu measurement and Cu speciation analyses (FEP, fluorinated ethylene propylene, or PFA, perfluoroalkoxy, Nalgene) were previously cleaned with 10% HNO_3 (analytical reagent grade), rinsed several times with ASTM Type I water (labeled hereafter as Milli-Q water, 18.2 MΩ, Millipore, USA), and finally filled with Milli-Q water until use. FEP/PFA bottles were thoroughly rinsed with the sample and 1 L of a sample was then immediately taken for on-board filtration. Samples for dissolved organic carbon (DOC), chromophoric dissolved organic matter (CDOM), and fluorescent dissolved organic matter (FDOM) analyses were collected into 2 L acid-washed polycarbonate bottles (Nalgene) and kept refrigerated and in the dark.

Vertical profiles of the main physicochemical parameters (salinity, temperature, pH, and dissolved oxygen) along the salinity gradient were measured *in situ* at each site using a Hydrolab DS5 multiparameter (CTD) probe. The CTD probe was calibrated before each campaign.

Samples Preparation

For the determination of dissolved Cu concentrations and Cu speciation studies (determination of Cu complexing capacity, CuCC), samples were filtered on-board using precleaned (acid + Milli-Q) 0.22 μm cellulose-acetate syringe filters (Minisart,

Sartorius). For the analysis of total dissolved Cu concentrations, samples were acidified in the Lab with trace metal grade nitric acid (*TraceSelect*, Fluka) to pH < 2 and within the next few days UV-irradiated (150 W medium pressure Hg lamp, Hanau, Germany) directly in the FEP/PFA bottles for at least 24 h in order to decompose natural organic matter (Omanović et al., 2006; Monticelli et al., 2010; Cindrić et al., 2015). Samples for Cu speciation studies were kept at natural pH (pH = 8.2 ± 0.1) and stored in the fridge (+4°C) until analysis which was performed within 2 months.

Samples for DOC, CDOM, and FDOM were filtered in the laboratory (within 4 h of sampling) through a 0.2 µm pore size filter (Whatman Polycap, 6,705–3,602 capsules) and dispensed into 3 × 60 ml acid-washed polycarbonate (Nalgene) bottles, used as analytical replicates. DOC, CDOM, and FDOM were immediately measured after filtration. The filtration system (syringe + both filter types) was selected after several tests with Milli-Q water, since the filtered water showed no contamination with Cu or DOC, CDOM, and FDOM.

Determination of Dissolved Cu

Concentrations of dissolved Cu were determined by differential pulse anodic stripping voltammetry (DPASV). Measurements were carried out on Metrohm-Autolab (EcoChemie) potentiostat (PGSTAT128N) controlled by GPES 4.9 software in a three-electrode cell (663 VA Stand, Metrohm). Ag|AgCl|sat. NaCl electrode was used as the reference electrode, a Pt wire as the auxiliary, and a hanging mercury drop (HMDE) as the working electrode. The DPASV parameters used for the measurement of DCu are presented in **Supplementary Table S1**. Analyses were performed using a fully automated system consisting of the instrument, a home-made autosampler, and five Cavro XL 3000 syringe pumps (Tecan, United States). For the preparation of the project file for the GPES software, as well as for the treatment of the voltammograms and final calculations, the handling software was developed (https://sites.google.com/site/daromasoft/home/voltaa).

Concentrations of trace metals were determined by means of the standard addition method. A certified "Nearshore seawater reference material for trace metals," CASS-5 (NRC CNRC), was used for the validation of the Cu analysis. The obtained value (±standard deviation) was 0.370 ± 0.030 µg L^{-1} (certified value is 0.380 ± 0.028 µg L^{-1}).

Copper Speciation Analysis: Determination of Cu Complexing Capacity (CuCC)

For the determination of CuCC, the DPASV method was used (Louis et al., 2008). The experiments were performed using the same electrochemical system as described in the previous section (excluding the use of the autosampler). The parameters used for the DPASV measurements of electrolabile Cu are presented in **Supplementary Table S1**. In order to avoid the adsorption of Cu into the walls of cell compartments, a quartz cell was used (Cuculić and Branica, 1996). Three automatic burette systems were used to automate Cu titration (XL 3000 syringe pumps).

The titrations of natural samples were performed at a pH of 8.2 buffered with 0.01 M borate/ammonia buffer (the final concentration in the cell). The electrochemical cell was conditioned for ~1/2 h with 15 ml of the sample before a new 10 ml aliquot of the sample was measured. The titrations were performed by measuring the ambient Cu concentration (without Cu addition) and by increasing Cu concentration up to the maximum of 300 nM of total Cu concentration ($[Cu]_T$) (using 10^{-5} M and 10^{-4} M Cu stock solutions prepared by appropriate dilutions of an atomic absorption spectrometry standard solution, TraceCERT, Fluka). Each titration was composed of a total of 15 separate points (measured in triplicate), with the Cu concentrations equally distributed in logarithmic scale, i.e., similar increments in log $[Cu]_T$ (Garnier et al., 2004; Louis et al., 2009). Final points that deviated from the linear part of the titration curve were discarded, and thus some of the final titration curves were composed of less than 15 points (12–14). After each Cu addition, 30 min was estimated as enough time to reach the equilibrium conditions in the titration cell. For each Cu addition, 3 repetitive measurements were performed and peak intensities of all the points were thereafter used for the construction of complexometric curves. Before each titration, a Milli-Q test was performed to check the procedural blank. The concentration of Cu in Milli-Q was always below 0.1 nM. The theoretical background of the complexometric parameter calculation can be found elsewhere (Garnier et al., 2004; Omanović et al., 2015; Pižeta et al., 2015). Obtained complexometric curves were then treated by using the ProMCC software tool (Omanović et al., 2015). The number of ligand classes was estimated based on the shape of the Scatchard plot. For all titrations, the Scatchard plot showed a clear convex shape, indicating the presence of the 2-ligand classes. Complexation parameters were calculated by a nonlinear fitting of the Langmuir-Gerringa isotherm in a "logarithmic mode" (Omanović et al., 2015). The ambient speciation of Cu ($[Cu^{2+}]$ $[CuL_1]$ and $[CuL_2]$) originally present in the water sample was calculated from the concentrations of total dissolved Cu ([DCu]), $[L]_i$, and the conditional stability constants ($\log K_i$) of the Cu-binding organic ligands in the sample.

Model experiments with isolated organic matter (4 mg L^{-1} fulvic acid (FA), IHSS, batch 2S101F) were performed in organic-free, UV-digested seawater (UVSW) at the same pH as samples (pH = 8.2) adjusted with the borate/ammonia buffer. The UVSW was cleaned by MnO_2 slurry following the common procedure (van den Berg, 2006).

Dissolved Organic Carbon (DOC) and Dissolved Organic Matter Optical Properties Measurements

The DOC concentration was determined by high-temperature catalytic oxidation using a Shimadzu TOC-VCSN carbon analyzer (Santinelli et al., 2015). Prior to oxidation, samples were acidified with 2 M high purity HCl and purged for 3 min with pure air to remove inorganic carbon. To achieve satisfying analytical precision (±1%), up to 5 replicate injections were performed. The instrument performance was verified by

comparison of data with the DOC Consensus Reference Material (CRM) (Hansell, 2005) (batch #13/08-13, nominal concentration: 41–44 μM measured concentration: 43.2 ± 1.5 μM, n = 4). A calibration curve was measured with potassium hydrogen phthalate as the organic standard.

UV-Vis absorbance spectra were measured using a Jasco UV-visible spectrophotometer (Mod-7850) equipped with a 10 cm quartz cuvette, following the method reported by (Retelletti Brogi et al., 2015). Scans were performed at excitation wavelengths of between 230 and 700 nm. The spectrum of Milli-Q water, measured in the same conditions, was used as blank and subtracted from each sample. Absorbance spectra were treated by using the ASFit tool (Omanović et al., 2019). Absorbance at 254 nm (A_{254}) was expressed as the absorption coefficient (a_{254}) in m^{-1} (Stedmon and Nelson, 2015). The specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated by dividing the absorption coefficient at 254 nm by the DOC concentration ($m^2\ g^{-1}$ C) and used as an indicator of the percentage of CDOM in the total DOM pool (Marcinek et al., 2020).

Fluorescence excitation-emission matrices (EEMs) were recorded using the Aqualog spectrofluorometer (Horiba-Jobin Ivon) in a 1 × 1 cm quartz cuvette as described in detail by (Retelletti Brogi et al., 2020). Briefly, EEMs were scanned at the excitation wavelength range of 250–450 nm with 5 nm increments and emission wavelengths ranged between 212 and 619 nm with 3 nm increments. Procedural blanks were checked by measuring EEM of Milli-Q water. Fluorescence intensities were normalized to Raman units (R.U.) using daily measured Raman peak of Milli-Q water (λ_{ex} = 350 nm, λ_{em} = 371–428 nm) (Lawaetz and Stedmon, 2009). Parallel factorial analysis (PARAFAC) was applied to identify the different components in the FDOM pool by using the decomposition routines for EEMs drEEM toolbox (version 0.2.0 for MATLAB (R2016a) (Murphy et al., 2013).

RESULTS AND DISCUSSION

Study of SAS Influence on the Voltammetric Signal of Cu

Problem Description

Initial examinations of the samples for the determination of the Cu speciation using the DPASV method with high DOC concentration (>2 mg L^{-1}) revealed that a characteristic Cu oxidation peak at the ambient Cu concentration is not present (even at relatively high Cu concentrations, e.g., 20–50 nM) and that an unusually high background current is obtained. The lack of a Cu oxidation peak was rather expected because the majority of the Cu present in the samples with a high DOM load is predicted to be complexed by strong organic ligands, which are not reducible at the applied deposition potential.

However, the unusually high background current, with one or two wide peaks, obtained in most of the samples, signified a potential problem. This is clearly visible in curve #1 in **Figure 2A**. In this example, the voltammetric procedure consisted specifically of only the deposition step at E_{dep} = −0.5 V, in the sample without any addition, except the borate buffer. The high background current in voltammetric measurements as the result of adsorbed SAS is not unusual, as it was shown for several metals (Scarano and Bramanti, 1993; Louis et al., 2008; Pađan et al., 2019; Pađan et al., 2020). To overcome problems caused by SAS adsorption, a "desorption step" (DS) (the application of a very negative potential, e.g., −1.5 V, for a short period, e.g., 1–3 s), at the end of the deposition period was proposed (Louis et al., 2008). However, in our case, the application of DS did not provide the

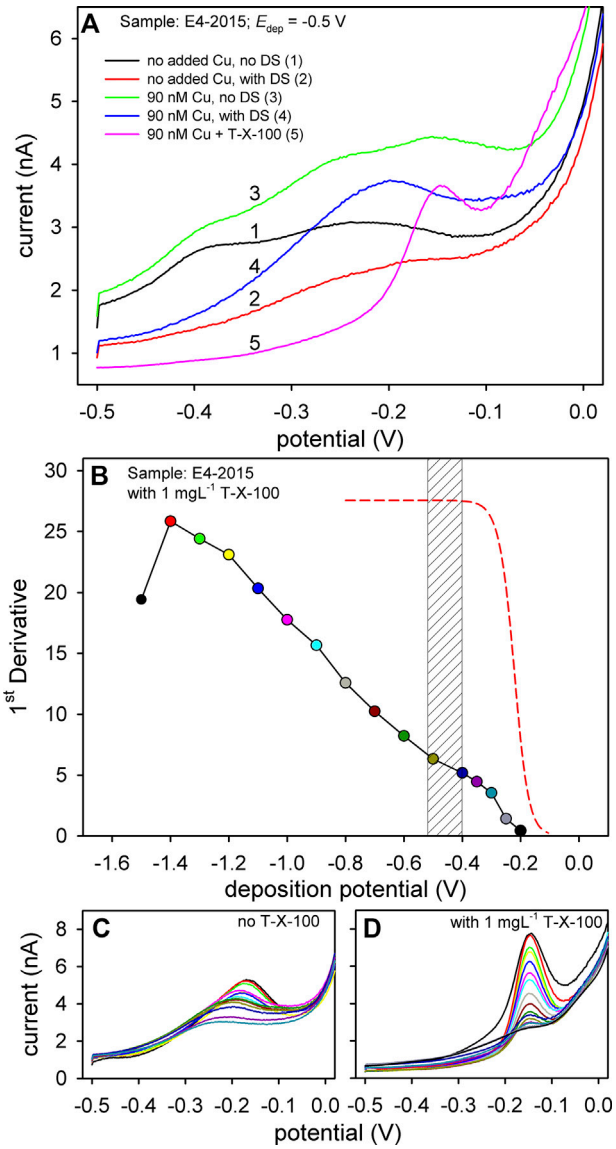

FIGURE 2 | (A) DPASV curves obtained at various scanning conditions, solution compositions, and Cu concentrations as indicated in the figure. **(B)** Pseudopolarogram of Cu obtained in sample taken at the site E4 (2015) with $[Cu]_T$ = 90 nM and with the addition of 1 mg L^{-1} T-X-100. The red dashed line illustrates the Cu pseudopolarogram without organic matter. Bottom plots represent the obtained voltammograms of pseudopolarographic measurements ($[Cu]_T$ = 90 nM) without the addition of T-X-100 but using the desorption step **(C)** and with the addition of 1 mg L^{-1} T-X-100 **(D)**. Different colors represent voltammograms obtained at different deposition potentials.

expected result due to the high content of organic matter, i.e., SAS present in the samples. Although a much better signal was obtained by applying a DS (curve #2, **Figure 2A**), the shape of the voltammogram at the potential range of the anodic Cu peak was still not adequate for the extraction of its intensity. The undefined wide stripping peak at the given potential was present even if the deposition of Cu was performed at potentials more positive than its redox potential; $E_{dep} = > -0.2$ V (either with or without the DS). The problem of the strange background current was even more apparent when the measurements were performed with the addition of Cu. Without the DS, a totally undistinguishable voltammogram was obtained at 90 nM Cu concentration (curve #3, **Figure 2A**). Although considerably better, the scan with the DS still produced a wide and poorly resolved peak (curve #4, **Figure 2A**). Intensities extracted from such voltammograms would be questionable, and consequently, constructed complexation curves and obtained complexation parameters would be entirely unreliable.

To resolve the problem of the adsorbed layer of the SAS on the Cu voltammetric signal, the idea was to introduce a competitive adsorptive process. The used compound should have a competitive effect on SAS adsorption but not on the Cu speciation chemistry in the bulk of the solution. Thus, a nonionic surfactant Triton-X-100 (T-X-100) was chosen for this purpose, since it does not form a complex with Cu (Krznarić et al., 1994; Omanović et al., 1996; Plavšić et al., 2009). The modified procedure employed here consists of adding T-X-100 at a concentration of 1 mg L^{-1} to the sample being analyzed. It has already been shown by several authors that T-X-100 could be beneficial in resolving some of the voltammetric issues. For example, T-X-100 was used for the voltammetric determination of iodine in seawater samples (Luther et al., 1988; Žic et al., 2012), for the separation of poorly resolved redox processes of Cu and Cu-EDTA (Omanović et al., 1996), and for the enhancement of the Cu oxidation by destabilizing formed $CuCl_2^-$ during the anodic scan (Plavšić et al., 1994). The benefit of adding T-X-100 to our samples is illustrated by curve #5 in **Figure 2A**. Compared to all other curves in the graph, a clearly resolved and narrow anodic Cu peak was obtained, from which extraction of Cu peak intensities is much more convenient.

For characterization of the interaction of metals with organic ligands, pseudopolarographic measurements (known also as stripping chronopotentiometry at scanned deposition potential, SSCP, or scanned stripping voltammetry, SSV) are proposed using either Hg (drop or film) or a solid electrode (Lewis et al., 1995; Luther III et al., 2001; Omanović and Branica, 2004; Town and van Leeuwen, 2004; Serrano et al., 2007; Domingos et al., 2008; Gibbon-Walsh et al., 2012; Bi et al., 2013; Town and van Leeuwen, 2019). The methodology has advanced in recent years, enabling the full characterization of the SSCP/pseudopolarographic waves (Serrano et al., 2007; Pinheiro et al., 2020) providing the heterogeneity of labile macromolecular metal-organic complexes. The full characterization of Cu complexes still has to be done using this methodology. As such, it is often used to make a "fingerprint" of the sample (Louis et al., 2008; Nicolau et al., 2008; Domingos et al., 2016). In **Figure 2B**, an example of the Cu pseudopolarogram obtained in an estuarine sample (E4, 2015) is presented, along with the illustrated pseudopolarogram representing the expected shape of labile inorganic Cu in the absence of organic matter (red dashed line). At this point, our goal with pseudopolarographic measurement is only to show the inherent complexity of Cu speciation by using ASV and adequately present the influence of SAS on its voltammetric behavior. The pseudopolarogram presented in **Figure 2B** served as an example of the sample "fingerprint" and to support the estimation of adequate deposition potential for the Cu complexation measurements (shaded region). Based on the basic features of presented waves (the shift of the pseudopolarograms toward negative potentials upon complexation with inorganic/organic ligands or its inclination) (Lewis et al., 1995; Serrano et al., 2007; Bi et al., 2013; Pinheiro et al., 2020), there is clear evidence that the reduction of various Cu-organic complexes occurs along the scanned deposition potentials. The increase of the intensity could be explained by the progressive reduction of various Cu-organic complexes (heterogeneous binding sites). Note that the presented pseudopolarogram was obtained with the addition of T-X-100. The corresponding voltammograms from which the pseudopolarogram was constructed are presented in the right bottom plot, clearly showing the fully resolved Cu peak at the same position. The construction of an analog pseudopolarogram in the sample without T-X-100 was not possible due to inadequate Cu anodic peaks, even with the applied DS step (left bottom plot). A clear difference between voltammograms with and without the added T-X-100 is shown here and is evident during Cu titrations as well (**Supplementary Figure S1**). It is interesting to point out the drop of the peak intensity of the point obtained at $E_{dep} = -1.5$ V. Namely, from this to more negative potentials, the adsorption of T-X-100 strongly decreases (Omanović et al., 1996; Sander and Henze, 1996), and its benefit on the shape of Cu anodic peak weakens (top black voltammogram at right bottom plot).

Although the positive effect of the T-X-100 addition was significant, we kept the desorption step as a procedural parameter because it was found that it does not have a negative effect on the final voltammogram if applied for only 1 s. However, the potential of the DS should not be more negative than −1.5 V and it should be as short as possible to avoid complete desorption of T-X-100.

To verify the proposed method, additional test experiments were performed in "organic-free" seawater (UVSW) with and without added fulvic acid as a model of natural organic matter.

Verification of the Methodology

Organic-Free Seawater

As noted previously, it is very important that added T-X-100 does not have a competitive effect on Cu complexation by natural ligands (especially organic). If that was the case, the effect would be visible on titration curves and pseudopolarograms. According to the basic model experiments, presented in the following sections, this is not the case.

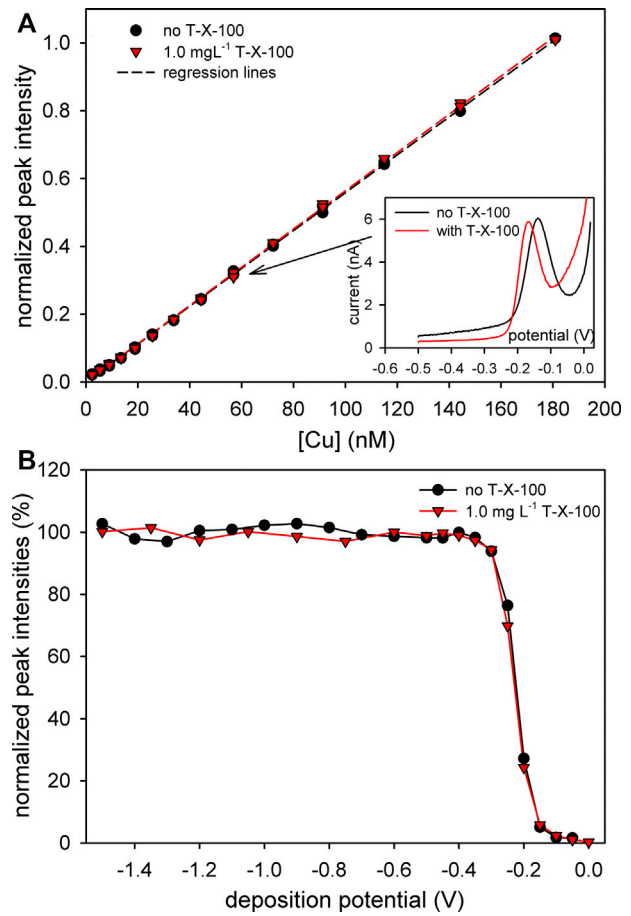

FIGURE 3 | (A) Copper titration curves obtained in UVSW (pH = 8.2) with and without the addition of 1 mg L^{-1} T-X-100 (for better comparison, the peak intensities were normalized on the highest value). Inset: Cu voltammograms with and without T-X-100 corresponding to Cu concentration indicated by the arrow. **(B)** Normalized pseudopolarograms obtained with and without the addition of 1 mg L^{-1} of T-X-100 (intensities normalized to the estimated limiting value).

Titrations with increasing Cu concentrations were first performed in UVSW (i.e., in the absence of organic ligands) with and without added T-X-100. As shown in **Figure 3A**, normalized intensities (with and without added T-X-100) showed the same linear relationship with increasing concentration of Cu. Normalized intensities (the 1st derivative as an analytical signal) were used for better comparison. In both cases, well-resolved Cu anodic voltammograms were obtained (inset in **Figure 3A**) and there is no curvature shape at the foot of the titration curves, characteristic for titration of samples with organic ligands (Pižeta et al., 2015), indicating that there is no complexation of Cu by T-X-100. The small negative shift and a slightly narrower anodic peak obtained with T-X-100 could be explained by the destabilization of formed $CuCl^{2-}$ during the anodic scan (Plavšić et al., 1994).

Furthermore, in cases where Cu forms a complex with T-X-100, a shift and/or a change in the shape of pseudopolarograms at a potential range more negative than the reduction potential of inorganic Cu would be expected (Lewis et al., 1995; Omanović, 2006; Gibbon-Walsh et al., 2012; Pinheiro et al., 2020). However, the pseudopolarograms obtained with and without the addition of T-X-100 showed almost the same shape (**Figure 3B**). This is in slight contrast from the experiment performed by (Sahlin and Jagner, 1996) using striping potentiometry with a mercury film electrode with a fully saturated T-X-100 layer (1,000 mg L^{-1}) in which a slight shift of the pseudopolarographic wave was observed. However, the concentration of T-X-100 used in their experiment was 1,000× higher (1 g L^{-1}) than in our work and as such is not directly comparable. Therefore, based on our experiments presented in **Figure 3**, it can be assumed, with great confidence, that, at the concentration used in our work, T-X-100 did not form a complex with Cu and as such it works as "interference-free" for Cu speciation studies using ASV.

FA as a Model of Natural Organic Matter

Further examination of the proposed methodology using T-X-100 (1 mg L^{-1}) was performed in UVSW with the addition of 4 mg L^{-1} of FA as a model of terrestrial organic matter showing considerable surface activity and 50 nM of added Cu (all experiments performed at pH = 8.2). The selected voltammograms are presented in **Figure 4A** and corresponding pseudopolarograms in **Figure 4B**. For comparison purposes, a voltammogram without FA (but with T-X-100) is presented by curve #1 in **Figure 4A**. In the sample with 4 mg L^{-1} FA and 50 nM of Cu and without the applied DS, a relatively high background current was obtained, with a relatively wide Cu peak (curve #2, **Figure 4A**).

Compared to the poorly shaped Cu signal in the real estuarine sample presented in **Figure 2A**, this peak seems easily resolvable despite the curvature baseline under the peak. However, the voltammogram in the presence of FA without added Cu shows a small peak at a slightly more positive potential (curve #2′, **Figure 4A**), making the manual construction of the curvature baseline more complicated. The above-mentioned problem in the Cu intensity determination is more profound at the low Cu intensities, which correspond to the foot of the titration curve where strong complexing ligands dominate. This in turn may introduce a high uncertainty in the determination of the complexation parameters, making them ultimately unreliable (Omanović et al., 2010). By applying DS, a better resolved and higher Cu peak is obtained (curve #3, **Figure 4A**). It was shown that the main influence of the adsorbed FA occurs during the stripping step, with Cu-FA considered as a labile complex (Town, 1997). Due to the nature of the differential pulse technique, Cu oxidized during the applied pulse is being progressively concentrated in the vicinity of the electrode surface and immediately complexed by the accumulated organic ligands, causing the shift of the oxidation peak to a more negative potential (Town, 1998). This scenario is likely to occur taking into account the fact that the Cu complex with FA is directly reducible (Whitby and van den Berg, 2015). The lability of Cu-FA complexes and problems associated with DPASV measurement of Cu in the case of adsorbable ligands, such as FA, are already documented in the literature (Soares and Vasconcelos, 1994; 1995).

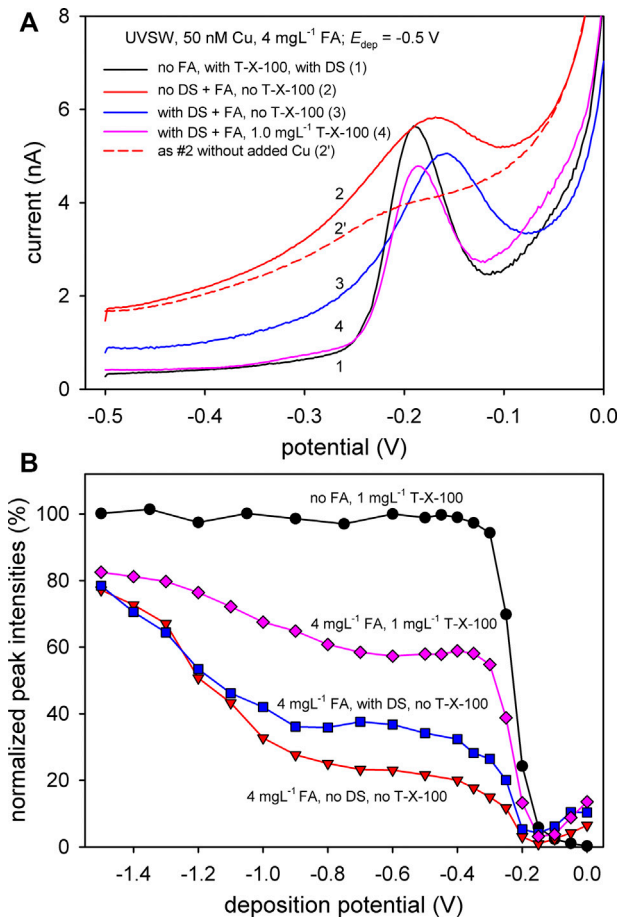

FIGURE 4 | (A) DPASV curves obtained in UVSW at various scanning conditions and solution composition as indicated in figure. **(B)** Pseudopolarogram of Cu obtained in UVSW at various scanning conditions and solution composition as indicated in the figure.

The Cu stripping peak was further improved by the addition of T-X-100 (curve #4, **Figure 4A**). The same shape and the peak potential in presence of FA, after the addition of T-X-100, as the one without added FA (curve #1, **Figure 4A**) indicate that T-X-100 practically eliminated the influence of the SAS adsorbed during the stripping step.

The problems associated with the stripping voltammetry of Cu in the presence of a high concentration of organic matter are reflected in the shape of the pseudopolarograms. As presented in **Figure 4B**, a clear reversible pseudopolarographic wave of Cu was obtained in the absence of FA. Upon the addition of FA, the form of the pseudopolarogram considerably changed. For all pseudopolarograms with added FA, the decrease of the intensity of the initial value without FA was around 20% at a high deposition potential (E_{dep} = −1.5 V). However, in the potential range between −0.8 and −0.4 V, the decrease of the intensity was much higher: ~ 40% in the presence of 1 mg L^{-1} T-X-100 and even ~75% (w/o DS) and ~65% (with DS) in the absence of T-X-100. The decrease of intensities at more positive potentials is already reported in the literature (Town and Filella, 2000; Gibbon-Walsh et al., 2012), indicating the complexation of the Cu with the added FA.

As mentioned earlier, the shift of the pseudopolarographic wave in the case of labile reversible Cu complexes, or those which are irreversibly reduced, could be used to determine the stability constants of formed complexes by using the so-called "chelate scale" (Lewis et al., 1995; Omanović, 2006; Gibbon-Walsh et al., 2012). With this in mind, the steep increase of intensities in pseudopolarograms without T-X-100 at potentials more negative than −0.9 V may indicate the presence of very strong organic complexes with high stability constants which are irreversibly reduced (Lewis et al., 1995; Branica and Lovrić, 1997; Gibbon-Walsh et al., 2012). This increase was not observed in experiments with Cu-FA performed using a gold-vibrating electrode (Gibbon-Walsh et al., 2012), whereas in experiments of (Town and Filella, 2000) or (Chakraborty et al., 2007), this range of deposition potentials was not scanned and could not be compared. If the metal-to-ligand ratio in the bulk of the solution used in the work of (Gibbon-Walsh et al., 2012) is compared to our ratio, the extent of the decrease of intensities at E_{dep} range between −0.8 and −0.4 V in our experiment is larger than expected. This is probably caused by the above-discussed strong influence of accumulated FA on the stripping process, which is much stronger on a Hg electrode than on the vibrating Au-microelectrode. The observed increase of intensities toward more negative potentials could also be partially related to the decreasing FA adsorption. This is supported by the progressive shift of Cu peak potentials (up to ~ Δ20 mV between −1.5 and −0.7 V) in voltammograms used for the construction of pseudopolarograms without DS (data not shown). With the applied DS, the shift was only ~ Δ5 mV, which confirms that DS removes the accumulated FA to a great extent. There was "only" ~ 40% decrease of intensity (of the initial value without FA) at E_{dep} range between −0.8 and −0.4 V after the addition of T-X-100 (with no change in the Cu peak potentials across the scanned range), which is much closer to the values obtained by Gibbon-Walsh et al. (2012). Even after the addition of T-X-100, the small increase of intensities, from E_{dep} = −0.9 V to more negative values, remained. This is also evident in the pseudopolarogram of the natural estuarine sample (**Fig. 2B**). It indicates that 1) there is still a portion of FA adsorbed during the deposition step or 2) a portion of the strong Cu-FA complexes are irreversibly reduced at this range of potentials (Lewis et al., 1995; Gibbon-Walsh et al., 2012). The detailed examination of Cu-FA interactions is beyond the scope of this paper and was not further studied.

Copper Speciation in the Estuarine Samples

Figure 5 presents all the results relevant for the Cu speciation study in samples collected for two different periods, the early spring and the late summer.

Total Dissolved Copper

A strong decrease of dissolved copper (DCu) concentrations with increasing salinity was observed for both campaigns with a slightly different distribution in the salinity gradient (**Figure 5A**). For the late summer campaign (September 2015), which is characterized by a lower river discharge (as evident from

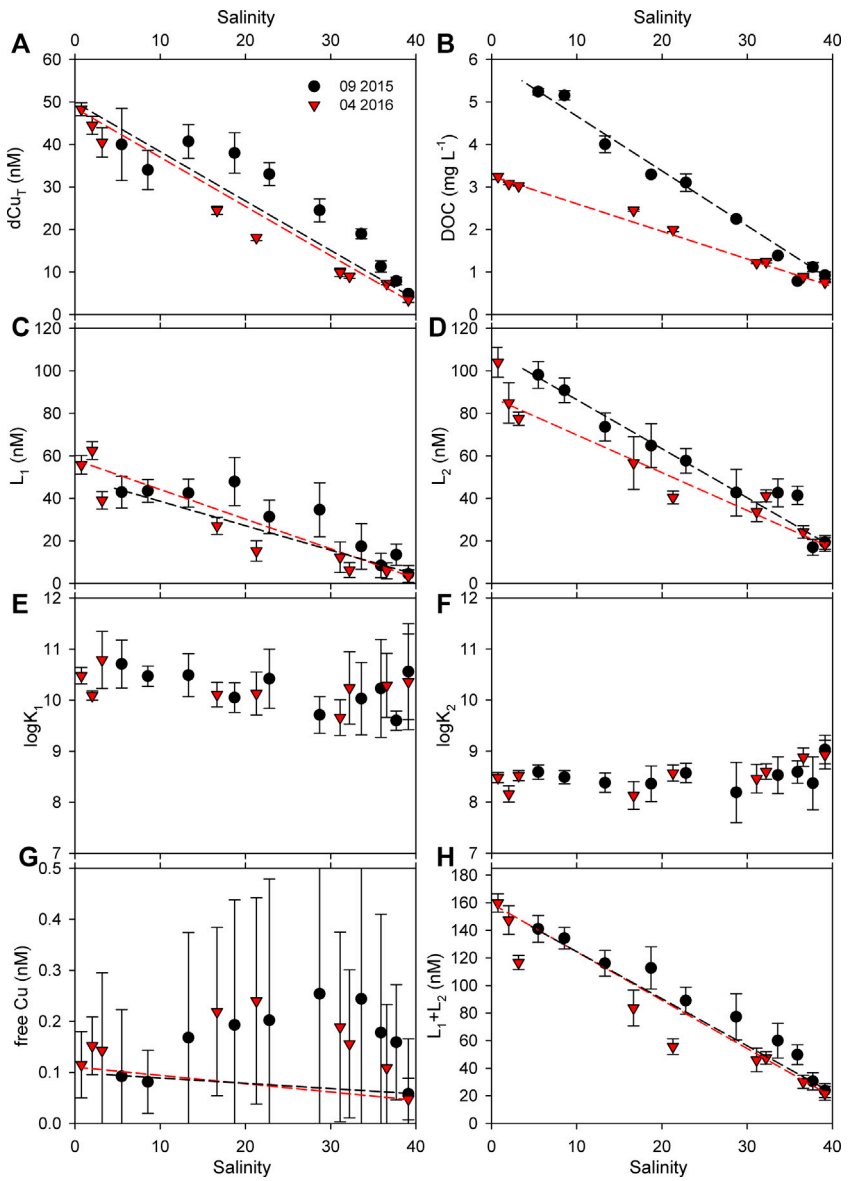

FIGURE 5 | Measured parameters along the estuarine segment for two sampling periods. Uncertainties are expressed as 95% confidence intervals. Dashed lines represent the projected conservative trends.

the salinity at the first site, E1, and the salinity contour plot in **Supplementary Figure S2**) (Retelletti Brogi et al., 2020), there is an increase of Cu concentration at the site E3, most likely caused by an anthropogenic input of Cu. Namely, numerous boat anchorage sites are found in the part of the estuary that begins between sites E2 and E3 (**Supplementary Figure S3**). In the summer period, these locations were fully occupied by boats. Thus, the observed increase of Cu in that region could be ascribed to the leaching of the Cu from the boats' antifouling paints, in which Cu is used as the biocide component (Turner, 2010; Cindrić et al., 2015). In the spring campaign (April 2016), due to the absence of the anchored boats (**Supplementary Figure S3**), Cu input was low. In this period, removal of dissolved Cu along the salinity gradient is implied by its deviation from the conservative line. This is probably the result of the flocculation process, which is common at lower salinities (Sholkovitz, 1976; Waeles et al., 2008). The concentrations of dissolved Cu in the seawater end-member for both periods reached the range characteristic for a clean coastal and open Mediterranean Sea area (Oursel et al., 2013; Migon et al., 2020).

Dissolved Organic Carbon

DOC concentrations show clear conservative behavior along the salinity gradient in both campaigns, with decreasing concentrations toward the sea end-member. Removal and/or production of DOM have a small impact on the DOC

concentration within the estuary, suggesting that the dilution of DOC in riverine water with marine water is the main process affecting DOC dynamics in the estuary. The DOC concentration is almost 2× higher in the late summer than in the spring campaign in the river end-member (5.2 mg L^{-1} in September 2015 in contrast to 3.3 mg L^{-1} in April 2016), whereas the sea end-member shows the same DOC values in both periods (~0.8 mg L^{-1}). In both periods, in the river end-member (at the E1 site), the measured DOC concentrations are in agreement with the DOC seasonal cycle observed in the river (Retelletti Brogi et al., 2020). High concentrations of DOC in the Arno River are the result of the weathering processes, especially during flood events, which primarily bring the terrestrial DOM, as well as the autochthonous production during spring and summer periods (Retelletti Brogi et al., 2020). Anthropogenic input of DOM also plays a role, because of the numerous anthropogenic activities in the upstream riverine region.

Copper-Binding Ligands and Estimation of Copper Bioavailability

Applying the adapted methodology for the determination of Cu speciation, well-shaped complexometric titration curves were obtained (examples are given in **Supplementary Figure S4**). Two classes of ligands were identified in all samples, denoted as L_1 and L_2, corresponding to a stronger and a weaker class of organic ligands, respectively. Concentrations of L_1 ranged from 3.5 ± 2.9 to 63 ± 4 nM eq Cu, whereas L_2 concentrations were higher and ranged from 17 ± 4 to 104 ± 7 nM eq Cu (**Figures 5C,D**). The concentration of both ligand classes decreased with the salinity, as was similarly observed for DCu and DOC. Along the estuary, the concentrations of ligands exceeded the corresponding total dissolved Cu concentrations, which are usually obtained in coastal regions (Bruland et al., 2000; Hurst and Bruland, 2005; Louis et al., 2009; Plavšić et al., 2009; Wong et al., 2018). The apparent stability constants ($\log K_{Cu}$) of the two ligand classes ranged from 9.6 ± 0.2 to 10.8 ± 0.6 for L_1 and from 8.2 ± 0.3 to 9.0 ± 0.3 for L_2, without a noticeable difference between the two sampling periods (**Figures 5E,F**). The ranges of estimated constants are typical for estuarine and coastal regions obtained by the ASV method and reflect the so-called "detection window" (DW) of the method (Apte et al., 1988; Bruland et al., 2000; Pižeta et al., 2015).

One of the purposes of the determination of metal complexing parameters is to estimate the free metal concentration, which is considered to be the most bioavailable/toxic. Having in mind the potential overestimation of free Cu using the ASV method, as will be discussed later, we refer to it as "free" Cu (quoted). In our study, the estimated "free" Cu concentrations ranged from 50 to 250 pM, following the strong positive deviation from the projected conservative line. The obtained trends are the result of the estimated complexation parameters, along with the dissolved Cu concentration. These are fairly high values of "free" Cu, taking into account the fact that the estimated threshold toxicity level is around 10 pM (Sunda et al., 1987; Gledhill et al., 1997), but as already mentioned they are likely overestimated due to the methodological problems.

The measured concentrations of the stronger ligand class (L_1) are similar to dissolved Cu concentrations. Although in some cases L1 was almost saturated with Cu at its ambient concentration, Cu was still mainly controlled by the abundance of strong ligand class (L_1) with high stability constants: between ~55 and 90% of dissolved Cu exists solely in the form of strong complexes.

Optical Properties of DOM

PARAFAC analysis applied to all the samples revealed 5 FDOM components. The components spectra were compared with previous studies by using the Openfluor database (Murphy et al., 2014) and identified as microbial humic-like (C1), terrestrial fulvic-like (C2), protein-like (C3), terrestrial humic-like (C4), and protein + PAH-like (polycyclic aromatic hydrocarbons) (C5). In both periods examined here, the protein-like component dominates the FDOM pool (**Supplementary Table S2**). This is in agreement with the two-year study performed in the Arno River (Retelletti Brogi et al., 2020). The average percentage of microbial humic-like (C1) and protein-like (C3) components is approximately the same in the two seasons (25.1% and 25.1% for C1 and 31.7% and 34.0% for C3 in spring and late summer, respectively), whereas terrestrial components (C2 and C4) represent a higher average percentage of the FDOM pool in spring (21.0% for C2 and 15.8% for C4) than in late summer (14.6% for C2 and 9.6% for C4) (**Supplementary Table S2**), due to the higher river discharge in spring. However, the highest difference is observed for the protein + PAH-like component (C5), whose average percentage in the whole FDOM pool was 6.4% in spring and 16.7% in late summer. This can be the consequence of intense touristic activity in late summer, i.e., extensive automobile and boat traffic in the area, introducing PAH-like DOM from the exhaust. $SUVA_{254}$ supports a change in DOM pool between the two seasons, suggesting a higher percentage of chromophoric DOM in late summer than in spring (**Supplementary Figure S5**).

Linking Copper Organic Ligands with DOM Properties

The stability constants obtained in this study appear to be lower at midsalinity range than at the end-members for both ligand types (**Figures 5E,F**). However, considering the associated uncertainties, there is no clear statistical difference among them. Despite that, the obtained trends could indicate the change in the composition of the organic ligands at different salinities. A change in DOM composition in the salinity gradient is suggested by the decrease of $SUVA_{254}$ and DOC normalized PARAFAC components toward the sea end-member (excluding the C5/DOC in spring) (**Supplementary Figure S5**), signifying the decrease of the chromophoric and fluorescent fraction in the DOM pool (the addition of nonchromophoric DOM and/or removal of chromophoric DOM). While the sum of the ligands (ΣL) correlates with the distribution of DOC, a_{254}, and all PARAFAC components ($r^2 > 0.9$), the obtained trends of stability constants in the salinity gradient do not show a strong link with any of the optical properties of DOM (CDOM/FDOM) (data not shown). Higher stability constants at a high salinity could be a consequence of the addition of organic ligands with

stronger binding constants (Whitby et al., 2017) not visible by UV/Vis and fluorescent measurements. Another possibility is that removal of ASV-labile organic ligands occurs at a high salinity which could lead to a slight increase of conditional stability constants.

A different relationship between the sum of the ligands (ΣL) and the DOC is observed in spring and summer: the estimated slope of their relationship is ~2× higher during spring than during summer (**Figure 6A**). Although the DOC, a_{254}, $SUVA_{254}$ and DOC normalized fluorescence intensities of all PARAFAC components (**Supplementary Figure S5**) are higher in late summer than in spring, the DOM pool in spring is more abundant with Cu-binding ligands. This can be linked to a higher percentage of terrestrial fulvic and humic components (C2 and C4, respectively) in the FDOM pool in spring (**Figures 6C,E**).

This is in agreement with other studies performed in estuarine regions, which suggest that humic and fulvic acids are the major source of organic ligands in these environments (Kogut and Voelker, 2001; Yang and van den Berg, 2009; Whitby and van den Berg, 2015; Wong et al., 2018; Dulaquais et al., 2020). Along with the humic substances, it was found that biogenic thiourea-type thiols are abundant ligands in estuarine environments (Whitby et al., 2017). A strong positive correlation between ΣL and the dissolved Cu concentration is observed in our study, which is on average the same for both periods (**Supplementary Figure S6**), suggesting that Cu-binding ligands could be the driver of DCu estuarine geochemistry. The increase of the organic ligands in the midsalinity range (S = 15–30), in late summer, could be a biological response to Cu release in the boat anchorage area (Zitoun et al., 2019). *In situ* production mainly releases protein-like substances (Osburn et al., 2012) and in this study, the protein component does indeed show an increase in the midsalinity range in late summer, while in the spring this is not the case. However, a similar increase is observed for all other components, showing a positive correlation with ΣL. Even though *in situ* production can also cause the increase of humic-like components (Romera-Castillo et al., 2010; Marcinek et al., 2020), without additional biological information we cannot conclude that the correlation between ΣL and the DCu in late summer is related to any extent to a biological response to Cu-stress.

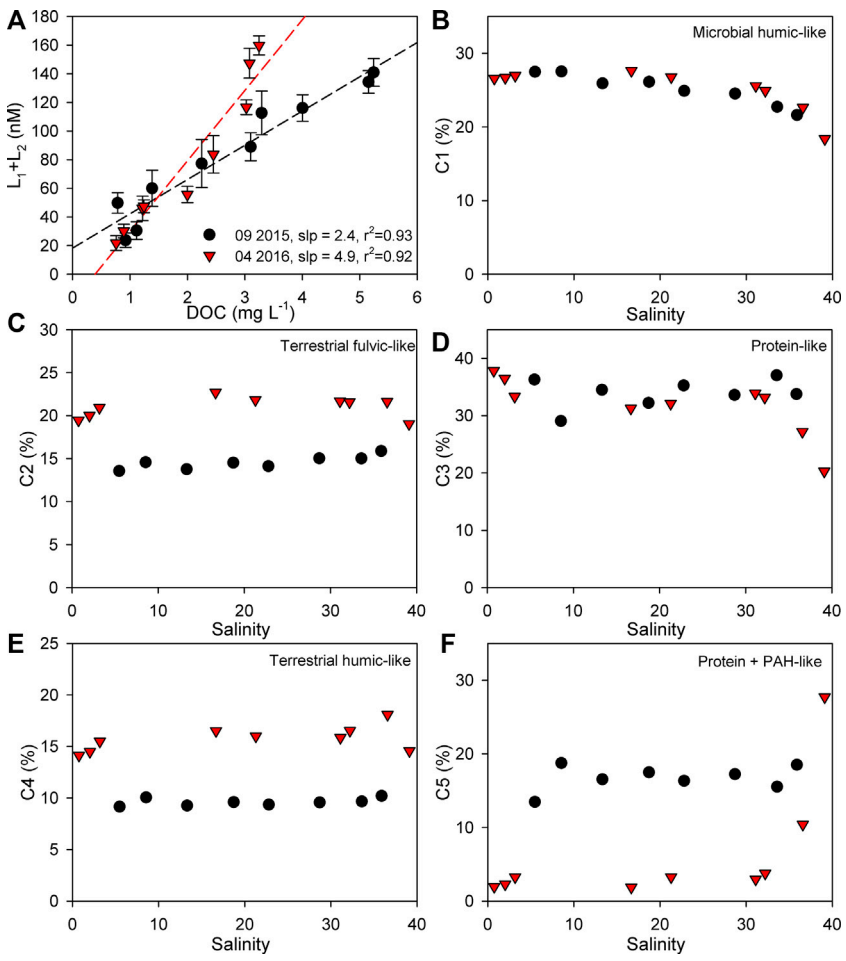

FIGURE 6 | Relationship between the sum of organic ligands (L_1+L_2) and DOC **(A)** and the variation in the percentage of PARAFAC components (C1–C5) along the salinity gradient **(B–F)**. Dashed lines represent the linear regression of the data.

Evaluation of Derived Complexation Parameters and Anodic Stripping Voltammetry Method Limitations

The method of calculation of complexation parameters (free and/or inorganic Cu, ligand concentrations, and conditional stability constants) in natural samples using both ASV and CLE-AdCSV provides results which are "operationally defined" (Bruland et al., 2000). Namely, the derived apparent stability constants depend strongly on the applied DW, which is expressed as the "side reaction coefficient" (α) (Bruland et al., 2000; Buck et al., 2012; Pižeta et al., 2015). For ASV, α is much smaller than it is for the CLE-AdCSV method in which the ligand with known stability constants is used as a competing ligand. In our measurements, the calculated logα is 1.13 (the ratio of the sum of all inorganic Cu species vs. free Cu concentration), whereas for CLE-AdCSV, logα depends on the used competitive ligand type and its concentration, and for Cu, it is usually logα >3 (Bruland et al., 2000; Buck et al., 2012; Whitby et al., 2017; Wong et al., 2018). Thus, if compared to CLE-AdCSV, it might seem like the stronger class of ligands are underestimated (Dulaquais et al., 2020); however, this is the consequence of DW which is not comparable to these two methods. It should also be noted that theoretically the method for calculation of complexation parameters using ASV assumes that the measured signal corresponds purely to the inorganic Cu. However, it is known that, during the deposition time at the appropriate deposition potential, kinetically labile complexes are actually accumulated, which include not only fully labile inorganic Cu species but also a portion of the kinetically labile organic complexes (Town, 1997; Bruland et al., 2000; Town and van Leeuwen, 2004; Chakraborty et al., 2007; Gibbon-Walsh et al., 2012). To prevent the reduction of labile organic complexes as much as possible, the deposition potential should be selected at the most positive value at the range of limiting currents (top of the wave) of the Cu pseudopolarogram without the addition of organic ligands (shaded area in **Figure 2B**). It is generally assumed that stronger complexes are less kinetically labile than the weaker complexes, and as such, it is expected that this problem will more greatly impact the weaker class of ligands.

This problem is partly reflected in the estimated "free/bioavailable" Cu. However, as Cu exists mainly in the form of strong complexes at ambient concentrations, it could be considered that the contribution of kinetically labile complexes to the bioavailable fraction is not high. Despite these potential methodological problems in the estimation of free Cu (which is the basis for the assessment of Cu toxicity), it should be mentioned that some model toxicological experiments showed a very good agreement with the labile Cu estimated by ASV and its bioavailability/toxicity (Tubbing et al., 1994; Lage et al., 1996; Tait et al., 2016; Sánchez-Marín, 2020). Furthermore, the free Cu concentrations obtained by the ASV method in estuarine samples (Jones and Bolam, 2007; Louis et al., 2009) were found to correlate well with those predicted by WHAM VII modeling (Stockdale et al., 2015), highlighting a particular feature of the ASV method.

Overall, it should be noted that each of the currently most-utilized techniques for the estimation of metal speciation (and their bioavailability) in natural waters (diffusive gradients in thin films (DGT), CLE-AdCSV, and ASV) provide results that are essentially "operationally defined"; i.e., they are technique-dependent.

CONCLUSIONS

Here, we present an adapted ASV methodology of copper speciation in estuarine samples with high DOM concentration. The method relies on the addition of the nonionic surfactant T-X-100, which competitively inhibits the adsorption of DOM on the Hg electrode during the deposition and stripping steps. Tests performed with and without the addition of fulvic acid (FA) as a model organic ligand revealed that, at the concentration level used here (1 mg L^{-1}), T-X-100 does not have any detectable influence on the redox process of Cu, labeling it "interference-free" for Cu speciation studies.

The proposed method improvement enabled the Cu speciation analysis in the Arno River estuary (Italy) characterized by high concentrations of both Cu and DOC. Mixing with the clean seawater leads to a decrease in their concentrations, reaching the value characteristic for a clean coastal region. Speciation analyses revealed the existence of the two types of organic ligands responsible for Cu complexation: a strong ligand class (L_1) with a concentration level similar to that of dissolved Cu and a weaker one (L_2) found at higher concentrations. The calculated free Cu concentrations in analyzed samples were above the toxicity threshold level. However, due to the ASV methodological specificities, these values might have been slightly overestimated. The sum of the ligand concentrations (ΣL) had the same linear relationship with dissolved Cu for both sampling periods. By contrast, a different relationship between ΣL and DOC was found in the two sampling periods, implying a change in the contribution of Cu-binding organic ligands to the DOM pool between seasons. The results indicate that the DOM pool comprised a higher percentage of Cu-binding ligands in spring than in summer, due to the higher percentage of terrestrial fulvic and humic components in the FDOM pool. However, as a separate quantification of specific ligand types was not performed, a direct link with detected the Cu-binding ligand classes was not possible to confirm. Assigning the exact type of the organic ligands able to complex Cu in the Arno River estuary demands more detailed studies with additional ligand characterization methods.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTION

JP, CS, SB, OR, CG, and DO performed sampling and sample preparation. JP performed analyses of samples, analyzed the data, and drafted the manuscript. SM conceptualized the manuscript,

organized data processing and interpretation, and wrote the manuscript. A-MC performed analyses and validation of the technique. CS and SB performed analyses of DOM and interpreted the DOM results. OR, CG, CS, and DO organized and conceptualized the study in the Arno River estuary. OR and CG acquired the funding. DO designed the experiments, supervised the method development, and acquired the funding. All authors contributed to results interpretation and manuscript writing/editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2020.628749/full#supplementary-material.

REFERENCES

Abdulla, H. A. N., Minor, E. C., Dias, R. F., and Hatcher, P. G. (2010). Changes in the compound classes of dissolved organic matter along an estuarine transect: a study using FTIR and C-13 NMR. *Geochem. Cosmochim. Acta* 74 (13), 3815–3838. doi:10.1016/j.gca.2010.04.006

Amin, S. A., Moffett, J. W., Martens-Habbena, W., Jacquot, J. E., Han, Y., Devol, A., et al. (2013). Copper requirements of the ammonia-oxidizing archaeon Nitrosopumilus maritimus SCM1 and implications for nitrification in the marine environment. *Limnol. Oceanogr.* 58 (6), 2037–2045. doi:10.4319/lo.2013.58.6.2037

Annett, A. L., Lapi, S., Ruth, T. J., and Maldonado, M. T. (2008). The effects of Cu and Fe availability on the growth and Cu: C ratios of marine diatoms. *Limnol. Oceanogr.* 53 (6), 2451–2461. doi:10.4319/lo.2008.53.6.2451

Apte, S. C., Gardner, M. J., and Ravenscroft, J. E. (1988). An evaluation of voltammetric titration procedures for the determination of trace-metal complexation in natural-waters by use of computer-simulation. *Anal. Chim. Acta* 212 (1–2), 1–21. doi:10.1016/S0003-2670(00)84124-0

Batley, G. E., Apte, S. C., and Stauber, J. L. (2004). Speciation and bioavailability of trace metals in water: progress since 1982. *Aust. J. Chem.* 57 (10), 903–919. doi:10.1071/CH04095

Bi, Z. S., Salaun, P., and van den Berg, C. M. G. (2013). The speciation of lead in seawater by pseudopolarography using a vibrating silver amalgam microwire electrode. *Mar. Chem.* 151, 1–12. doi:10.1016/j.marchem.2013.02.003

Blake, A. C., Chadwick, D. B., Zirino, A., and Rivera-Duarte, I. (2004). Spatial and temporal variations in copper speciation in San Diego Bay. *Estuaries.* 27 (3), 437–447. doi:10.1007/Bf02803536

Boussemart, M., Menargues, L., and Benaim, J. Y. (1993). Anodic-stripping voltammetry of copper in natural-waters-a qualitative approach to the additional peak(S) occurrence. *Electroanalysis.* 5 (2), 125–133. doi:10.1002/elan.1140050206

Branica, G., and Lovrić, M. (1997). Pseudopolarography of totally irreversible redox reactions. *Electrochim. Acta* 42 (8), 1247–1251. doi:10.1016/S0013-4686(96)00297-6

Bruland, K. W., Rue, E. L., Donat, J. R., Skrabal, S. A., and Moffett, J. W. (2000). Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Anal. Chim. Acta* 405 (1–2), 99–113. doi:10.1016/S0003-2670(99)00675-3

Buck, K. N., Moffett, J., Barbeau, K. A., Bundy, R. M., Kondo, Y., and Wu, J. F. (2012). The organic complexation of iron and copper: an intercomparison of competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) techniques. *Limnol. Oceanogr. Methods* 10, 496–515. doi:10.4319/lom.2012.10.496

Campbell, P. G., Errécalde, O., Fortin, C., Hiriart-Baer, V. P., and Vigneault, B. (2002). Metal bioavailability to phytoplankton--applicability of the biotic ligand model. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 133 (1–2), 189–206. doi:10.1016/s1532-0456(02)00104-7

Chakraborty, P., Fasfous, I. I., Murimboh, J., and Chakrabarti, C. L. (2007). Simultaneous determination of speciation parameters of Cu, Pb, Cd and Zn in model solutions of Suwannee River fulvic acid by pseudopolarography. *Anal. Bioanal. Chem.* 388 (2), 463–474. doi:10.1007/s00216-007-1185-3

Ciglenečki, I., Vilibić, I., Dautović, J., Vojvodić, V., Ćosović, B., Zemunik, P., et al. (2020). Dissolved organic carbon and surface active substances in the northern Adriatic Sea: long-term trends, variability and drivers. *Sci. Total Environ.* 730. doi:10.1016/j.scitotenv.2020.139104

Cindrić, A. M., Garnier, C., Oursel, B., Pižeta, I., and Omanović, D. (2015). Evidencing the natural and anthropogenic processes controlling trace metals dynamic in a highly stratified estuary: the Krka River estuary (Adriatic, Croatia). *Mar. Pollut. Bull.* 94 (1–2), 199–216. doi:10.1016/j.marpolbul.2015.02.029

Cobelo-García, A., and Prego, R. (2003). Land inputs, behaviour and contamination levels of copper in a Ria Estuary (NW Spain). *Mar. Environ. Res.* 56 (3), 403–422. doi:10.1016/S0141-1136(03)00002-3

Corcoll, N., Yang, J., Backhaus, T., Zhang, X., and Eriksson, K. M. (2018). Copper affects composition and functioning of microbial communities in marine biofilms at environmentally relevant concentrations. *Front. Microbiol.* 9 (3248). doi:10.3389/fmicb.2018.03248

Cortecci, G., Boschetti, T., Dinelli, E., Cidu, R., Podda, F., and Doveri, M. (2009). Geochemistry of trace elements in surface waters of the Arno River Basin, northern Tuscany, Italy. *Appl. Geochem.* 24 (5), 1005–1022. doi:10.1016/j.apgeochem.2009.03.002

Cuculić, V., and Branica, M. (1996). Adsorption of trace metals from sea-water onto solid surfaces: analysis by anodic stripping voltammetry. *Analyst* 121 (8), 1127–1131. doi:10.1039/An9962101127

Dalzell, B. J., Minor, E. C., and Mopper, K. M. (2009). Photodegradation of estuarine dissolved organic matter: a multi-method assessment of DOM transformation. *Org. Geochem.* 40 (2), 243–257. doi:10.1016/j.orggeochem.2008.10.003

Daoud, A. B., and Tremblay, L. (2019). HPLC-SEC-FTIR characterization of the dissolved organic matter produced by the microbial carbon pump. *Mar. Chem.* 215. doi:10.1016/j.marchem.2019.103668

Dinelli, E., Cortecci, G., Lucchini, F., and Zantedeschi, E. (2005). Sources of major and trace elements in the stream sediments of the Arno river catchment (northern Tuscany, Italy). *Geochem. J.* 39 (6), 531–545. doi:10.2343/geochemj.39.531

Domingos, R. F., Carreira, S., Galceran, J., Salaün, P., and Pinheiro, J. P. (2016). AGNES at vibrated gold microwire electrode for the direct quantification of free

copper concentrations. *Anal. Chim. Acta* 920, 29–36. doi:10.1016/j.aca.2016.03.035

Domingos, R. F., Lopez, R., and Pinheiro, J. P. (2008). Trace metal dynamic speciation studied by scanned stripping chronopotentiometry (SSCP). *Environ. Chem.* 5 (1), 24–32. doi:10.1071/EN07088

Dulaquais, G., Waeles, M., Breitenstein, J., Knoery, J., and Riso, R. (2020). Links between size fractionation, chemical speciation of dissolved copper and chemical speciation of dissolved organic matter in the Loire estuary. *Environ. Chem.* 17 (5), 385–399. doi:10.1071/EN19137

Fellman, J. B., Petrone, K. C., and Grierson, P. F. (2011). Source, biogeochemical cycling, and fluorescence characteristics of dissolved organic matter in an agro-urban estuary. *Limnol. Oceanogr.* 56 (1), 243–256. doi:10.4319/lo.2011.56.1.0243

Galletti, Y., Gonnelli, M., Brogi, S. R., Vestri, S., and Santinelli, C. (2019). DOM dynamics in open waters of the Mediterranean Sea: new insights from optical properties. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* 144, 95–114. doi:10.1016/j.dsr.2019.01.007

Garnier, C., Pižeta, I., Mounier, S., Benaïm, J. Y., and Branica, M. (2004). Influence of the type of titration and of data treatment methods on metal complexing parameters determination of single and multi-ligand systems measured by stripping voltammetry. *Anal. Chim. Acta* 505 (2), 263–275. doi:10.1016/j.aca.2003.10.066

Gibbon-Walsh, K., Salaün, P., and van den Berg, C. M. (2012). Pseudopolarography of copper complexes in seawater using a vibrating gold microwire electrode. *J. Phys. Chem.* 116 (25), 6609–6620. doi:10.1021/jp3019155

Glass, J. B., and Orphan, V. J. (2012). Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. *Front. Microbiol.* 3, 61. doi:10.3389/fmicb.2012.00061

Gledhill, M., Nimmo, M., Hill, S. J., and Brown, M. T. (1997). The toxicity of copper(II) species to marine algae, with particular reference to macroalgae. *J. Phycol.* 33 (1), 2–11. doi:10.1111/j.0022-3646.1997.00002.x

Hansell, D. A. (2005). Dissolved organic carbon reference material program. *Eos. Trans. Am. Geophys. Union.* 86 (35), 318. doi:10.1029/2005eo350003

Helland, A., and Bakke, T. (2002). Transport and sedimentation of Cu in a microtidal estuary, SE Norway. *Mar. Pollut. Bull.* 44 (2), 149–155. doi:10.1016/s0025-326x(01)00195-3

Hurst, M. P., and Bruland, K. W. (2005). The use of nafion-coated thin mercury film electrodes for the determination of the dissolved copper speciation in estuarine water. *Anal. Chim. Acta* 546 (1), 68–78. doi:10.1016/j.aca.2005.05.015

Jacquot, J. E., Horak, R. E. A., Amin, S. A., Devol, A. H., Ingalls, A. E., Armbrust, E. V., et al. (2014). Assessment of the potential for copper limitation of ammonia oxidation by Archaea in a dynamic estuary. *Mar. Chem.* 162, 37–49. doi:10.1016/j.marchem.2014.02.002

Jones, B., and Bolam, T. (2007). Copper speciation survey from UK marinas, harbours and estuaries. *Mar. Pollut. Bull.* 54 (8), 1127–1138. doi:10.1016/j.marpolbul.2007.04.021

Karlsson, J., Ytreberg, E., and Eklund, B. (2010). Toxicity of anti-fouling paints for use on ships and leisure boats to non-target organisms representing three trophic levels. *Environ. Pollut.* 158 (3), 681–687. doi:10.1016/j.envpol.2009.10.024

Kogut, M. B., and Voelker, B. M. (2001). Strong copper-binding behavior of terrestrial humic substances in seawater. *Environ. Sci. Technol.* 35 (6), 1149–1156. doi:10.1021/es0014584

Krznarić, D., Plavšić, M., and Ćosović, B. (1994). Voltametric investigations of the effect of Triton X-100 on copper processes in the presence of oxygen. *Electroanalysis.* 6 (2), 131–137. doi:10.1002/elan.1140060210

Lage, O. M., Soares, H. M. V. M., Vasconcelos, M. T. S. D., Parente, A. M., and Salema, R. (1996). Toxicity effects of copper(II) on the marine dinoflagellate Amphidinium carterae: influence of metal speciation. *Eur. J. Phycol.* 31 (4), 341–348. doi:10.1080/09670269600651571

Laglera, L. M., and van den Berg, C. M. G. (2003). Copper complexation by thiol compounds in estuarine waters. *Mar. Chem.* 82 (1–2), 71–89. doi:10.1016/S0304-4203(03)00053-7

Lawaetz, A. J., and Stedmon, C. A. (2009). Fluorescence intensity calibration using the Raman scatter peak of water. *Appl. Spectrosc.* 63 (8), 936–940. doi:10.1366/000370209788964548

Lee, M. H., Osburn, C. L., Shin, K. H., and Hur, J. (2018). New insight into the applicability of spectroscopic indices for dissolved organic matter (DOM) source discrimination in aquatic systems affected by biogeochemical processes. *Water Res.* 147, 164–176. doi:10.1016/j.watres.2018.09.048

Lewis, B. L., Luther, G. W., Lane, H., and Church, T. M. (1995). Determination of metal-organic complexation in natural-waters by swasv with pseudopolarograms. *Electroanalysis* 7 (2), 166–177. doi:10.1002/elan.1140070213

Lopez, J. S., Lee, L., and Mackey, K. R. M. (2019). The toxicity of copper to crocosphaera watsonii and other marine phytoplankton: a systematic review. *Front. Mar. Sci.* 5 (511). doi:10.3389/fmars.2018.00511

Louis, Y., Cmuk, P., Omanović, D., Garnier, C., Lenoble, V., Mounier, S., et al. (2008). Speciation of trace metals in natural waters: the influence of an adsorbed layer of natural organic matter (NOM) on voltammetric behaviour of copper. *Anal. Chim. Acta* 606 (1), 37–44. doi:10.1016/j.aca.2007.11.011

Louis, Y., Garnier, C., Lenoble, V., Mounier, S., Cukrov, N., Omanović, D., et al. (2009). Kinetic and equilibrium studies of copper-dissolved organic matter complexation in water column of the stratified Krka River estuary (Croatia). *Mar. Chem.* 114 (3–4), 110–119. doi:10.1016/j.marchem.2009.04.006

Luther, G. W., III, Rozan, T. F., Witter, A., and Lewis, B. (2001). Metal-organic complexation in the marine environment. *Geochem. Trans.* 2, 65–74. doi:10.1186/1467-4866-2-65

Luther, G. W., Swartz, C. B., and Ullman, W. J. (1988). Direct determination of iodide in seawater by cathodic stripping square-wave voltammetry. *Anal. Chem.* 60 (17), 1721–1724. doi:10.1021/Ac00168a017

Marcinek, S., Santinelli, C., Cindrić, A. M., Evangelista, V., Gonnelli, M., Layglon, N., et al. (2020). Dissolved organic matter dynamics in the pristine Krka River estuary (Croatia). *Mar. Chem.* 225, 103848. doi:10.1016/j.marchem.2020.103848

Migon, C., Heimburger-Boavida, L. E., Dufour, A., Chiffoleau, J. F., and Cossa, D. (2020). Temporal variability of dissolved trace metals at the DYFAMED time-series station, Northwestern Mediterranean. *Mar. Chem.* 225, 3845. doi:10.1016/j.marchem.2020.103846

Minor, E. C., Boon, J. J., Harvey, H. R., and Mannino, A. (2001). Estuarine organic matter composition as probed by direct temperature-resolved mass spectrometry and traditional geochemical techniques. *Geochem. Cosmochim. Acta* 65 (17), 2819–2834. doi:10.1016/S0016-7037(01)00643-3

Minor, E. C., Simjouw, J. P., Boon, J. J., Kerkhoff, A. E., and van der Horst, J. (2002). Estuarine/marine UDOM as characterized by size-exclusion chromatography and organic mass spectrometry. *Mar. Chem.* 78 (2–3), 75–102. doi:10.1016/S0304-4203(02)00011-7

Monticelli, D., Carugati, G., Castelletti, A., Recchia, S., and Dossi, C. (2010). Design and development of a low cost, high performance UV digester prototype: application to the determination of trace elements by stripping voltammetry. *Microchem. J.* 95 (2), 158–163. doi:10.1016/j.microc.2009.11.002

Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Anal. Methods* 5 (23), 6557–6566. doi:10.1039/c3ay41160e

Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R. (2014). OpenFluor- an online spectral library of auto-fluorescence by organic compounds in the environment. *Anal. Methods* 6 (3), 658–661. doi:10.1039/c3ay41935e

Nicolau, R., Louis, Y., Omanović, D., Garnier, C., Mounier, S., and Pizeta, I. (2008). Study of interactions of concentrated marine dissolved organic matter with copper and zinc by pseudopolarography. *Anal. Chim. Acta* 618 (1), 35–42. doi:10.1016/j.aca.2008.04.038

Omanović, D., Garnier, C., Louis, Y., Lenoble, V., Mounier, S., and Pižeta, I. (2010). Significance of data treatment and experimental setup on the determination of copper complexing parameters by anodic stripping voltammetry. *Anal. Chim. Acta* 664 (2), 136–143. doi:10.1016/j.aca.2010.02.008

Omanović, D., and Branica, M. (2004). Pseudopolarography of trace metals. Part II. The comparison of the reversible, quasireversible and irreversible electrode reactions. *J. Electroanal. Chem.* 565 (1), 37–48. doi:10.1016/j.jelechem.2003.09.026

Omanović, D., Gamier, C., and Pižeta, I. (2015). ProMCC: an all-in-one tool for trace metal complexation studies. *Mar. Chem.* 173, 25–39. doi:10.1016/j.marchem.2014.10.011

Omanović, D., Kwokal, Z., Goodwin, A., Lawrence, A., Banks, C. E., Compton, R. G., et al. (2006). Trace metal detection in Sibenik Bay, Croatia: cadmium, lead

and copper with anodic stripping voltammetry and manganese via sonoelectrochemistry. A case study. *J. Iran. Chem. Soc.* 3 (2), 128–139. doi:10.1007/Bf03245940

Omanović, D., Pižeta, I., Peharec, Z., and Branica, M. (1996). Voltametric determination of the metal complexing capacity in model solutions. *Mar. Chem.* 53 (1–2), 121–129. doi:10.1016/0304-4203(96)00018-7

Omanović, D. (2006). Pseudopolarography of trace metals. Part III. Determination of stability constants of labile metal complexes. *Croat. Chem. Acta* 79 (1), 67–76. doi:10.1021/ja01227a043

Omanović, D., Santinelli, C., Marcinek, S., and Gonnelli, M. (2019). ASFit - an all-inclusive tool for analysis of UV-Vis spectra of colored dissolved organic matter (CDOM). *Comput. Geosci.* 133. doi:10.1016/J.Cageo.2019.104334

Osburn, C. L., Handsel, L. T., Mikan, M. P., Paerl, H. W., and Montgomery, M. T. (2012). Fluorescence tracking of dissolved and particulate organic matter quality in a river-dominated estuary. *Environ. Sci. Technol.* 46 (16), 8628–8636. doi:10.1021/es3007723

Oursel, B., Garnier, C., Durrieu, G., Mounier, S., Omanović, D., and Lucas, Y. (2013). Dynamics and fates of trace metals chronically input in a Mediterranean coastal zone impacted by a large urban area. *Mar. Pollut. Bull.* 69 (1–2), 137–149. doi:10.1016/j.marpolbul.2013.01.023

Pađan, J., Marcinek, S., Cindrić, A. M., Layglon, N., Lenoble, V., Salaün, P., et al. (2019). Improved voltammetric methodology for chromium redox speciation in estuarine waters. *Anal. Chim. Acta* 1089, 40–47. doi:10.1016/j.aca.2019.09.014

Pađan, J., Marcinek, S., Cindrić, A. M., Layglon, N., Garnier, C., Salaun, P., et al. (2020). Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology. *Environ. Chem.* 17 (2), 77–84. doi:10.1071/EN19157

Peers, G., and Price, N. M. (2006). Copper-containing plastocyanin used for electron transport by an oceanic diatom. *Nature.* 441 (7091), 341–344. doi:10.1038/nature04630

Peers, G., Quesnel, S. A., and Price, N. M. (2005). Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. *Limnol. Oceanogr.* 50 (4), 1149–1158. doi:10.4319/lo.2005.50.4.1149

Pinheiro, J. P., Galceran, J., Rotureau, E., Companys, E., and Puy, J. (2020). Full wave analysis of stripping chronopotentiometry at scanned deposition potential (SSCP): obtaining binding curves in labile heterogeneous macromolecular systems for any metal-to-ligand ratio. *J. Electroanal. Chem.* 873, 114436. doi:10.1016/j.jelechem.2020.114436

Pižeta, I., Sander, S. G., Hudson, R. J. M., Omanović, D., Baars, O., Barbeau, K. A., et al. (2015). Interpretation of complexometric titration data: an intercomparison of methods for estimating models of trace metal complexation by natural organic ligands. *Mar. Chem.* 173, 3–24. doi:10.1016/j.marchem.2015.03.006

Plavšić, M., Krznarić, D., and Ćosović, B. (1994). The electrochemical processes of copper in the presence of Triton X-100. *Electroanalysis* 6 (5–6), 469–474. doi:10.1002/elan.1140060518

Plavšić, M., Kwokal, Z., Strmečki, S., Peharec, Z., Omanović, D., and Branica, M. (2009). Determination of the copper complexing ligands in the krka River estuary. *Fresenius Environ. Bull.* 18 (3), 327–334.

Repeta, D. J. (2015). "*Chemical characterization and cycling of dissolved organic matter.*," in *Biogeochemistry of marine dissolved organic matter.* Editors D. A. Hansell and C. A. Carlson (Cambridge: Academic Press), 21–63.

Retelletti Brogi, S., Gonnelli, M., Vestri, S., and Santinelli, C. (2015). Biophysical processes affecting DOM dynamics at the Arno river mouth (Tyrrhenian Sea). *Biophys. Chem.* 197, 1–9. doi:10.1016/j.bpc.2014.10.004

Retelletti Brogi, S., Balestra, C., Casotti, R., Cossarini, G., Galletti, Y., Gonnelli, M., et al. (2020). Time resolved data unveils the complex DOM dynamics in a Mediterranean river. *Sci. Total Environ.* 733. doi:10.1016/j.scitotenv.2020.139212

Romera-Castillo, C., Sarmento, H., Alvarez-Salgado, X. A., Gasol, J. M., and Marrase, C. (2010). Production of chromophoric dissolved organic matter by marine phytoplankton. *Limnol. Oceanogr.* 55 (1), 446–454. doi:10.4319/lo.2010.55.1.0446

Sahlin, E., and Jagner, D. (1996). Influence of Triton X-100 in stripping potentiometry. *Anal. Chim. Acta* 333 (3), 233–240. doi:10.1016/0003-2670(96)00310-8

Sánchez-Marín, P., Aierbe, E., Lorenzo, J. I., Mubiana, V. K., Beiras, R., and Blust, R. (2016). Dynamic modeling of copper bioaccumulation by *Mytilus edulis* in the presence of humic acid aggregates. *Aquat. Toxicol.* 178, 165–170. doi:10.1016/j.aquatox.2016.07.021

Sánchez-Marín, P., Santos-Echeandía, J., Nieto-Cid, M., Alvarez-Salgado, X. A., and Beiras, R. (2010). Effect of dissolved organic matter (DOM) of contrasting origins on Cu and Pb speciation and toxicity to *Paracentrotus lividus* larvae. *Aquat. Toxicol.* 96 (2), 90–102. doi:10.1016/j.aquatox.2009.10.005

Sánchez-Marín, P. (2020). A review of chemical speciation techniques used for predicting dissolved copper bioavailability in seawater. *Environ. Chem.* 17 (7), 469. doi:10.1071/EN19266

Sander, S., and Henze, G. (1996). Alternating current investigations at the mercury drop electrode on the adsorption potential of metal complexes. *Electroanalysis.* 8 (3), 253–262. doi:10.1002/elan.1140080310

Santinelli, C., Follett, C., Brogi, S. R., Xu, L., and Repeta, D. (2015). Carbon isotope measurements reveal unexpected cycling of dissolved organic matter in the deep Mediterranean Sea. *Mar. Chem.* 177, 267–277. doi:10.1016/j.marchem.2015.06.018

Scarano, G., and Bramanti, E. (1993). Voltametric behavior of marine hydrophobic copper-complexes-effect of adsorption processes at a mercury-electrode. *Anal. Chim. Acta* 277 (1), 137–144. doi:10.1016/0003-2670(93)85098-5

Semeniuk, D. M., Bundy, R. M., Payne, C. D., Barbeau, K. A., and Maldonado, M. T. (2015). Acquisition of organically complexed copper by marine phytoplankton and bacteria in the northeast subarctic Pacific Ocean. *Mar. Chem.* 173, 222–233. doi:10.1016/j.marchem.2015.01.005

Serrano, N., Diaz-Cruz, J. M., Arino, C., Esteban, M., Puy, J., Companys, E., et al. (2007). Full-wave analysis of stripping chronopotentiograms at scanned deposition potential (SSCP) as a tool for heavy metal speciation: theoretical development and application to Cd(II)-phthalate and Cd(II)-iodide systems. *J. Electroanal. Chem.* 600 (2), 275–284. doi:10.1016/j.jelechem.2006.10.007

Shank, G. C., Skrabal, S. A., Whitehead, R. F., and Kieber, R. J. (2004). Strong copper complexation in an organic-rich estuary: the importance of allochthonous dissolved organic matter. *Mar. Chem.* 88 (1–2), 21–39. doi:10.1016/j.marchem.2004.03.001

Sholkovitz, E. R. (1976). Flocculation of dissolved organic and inorganic matter during mixing of river water and seawater. *Geochem. Cosmochim. Acta* 40 (7), 831–845. doi:10.1016/0016-7037(76)90035-1

Slagter, H. A., Reader, H. E., Rijkenberg, M. J. A., van der Loeff, M. R., de Baar, H. J. W., and Gerringa, L. J. A. (2017). Organic Fe speciation in the eurasian basins of the arctic ocean and its relation to terrestrial DOM. *Mar. Chem.* 197, 11–25. doi:10.1016/j.marchem.2017.10.005

Soares, H. M., and Vasconcelos, M. T. (1995). Application of potentiometric stripping analysis for speciation of copper complexes with a non-adsorbable ligand on a mercury electrode. *Talanta* 42 (3), 621–626. doi:10.1016/0003-2670(95)00283-6

Soares, H. M. V. M., and Vasconcelos, M. T. S. D. (1994). Study of the lability of copper(ii)-fulvic acid complexes by ion-selective electrodes and potentiometric stripping analysis. *Anal. Chim. Acta* 293 (3), 261–270. doi:10.1016/0003-2670(94)85031-3

Stedmon, C. A., and Nelson, N. B. (2015). "*Chapter 10 - the optical properties of DOM in the ocean.*," in *Biogeochemistry of marine dissolved organic matter.* 2nd Edn, Editors D. A. Hansell and C. A. Carlson (Boston: Academic Press), 481–508.

Stockdale, A., Tipping, E., and Lofts, S. (2015). Dissolved trace metal speciation in estuarine and coastal waters: comparison of wham/model vii predictions with analytical results. *Environ. Toxicol. Chem.* 34 (1), 53–63. doi:10.1002/etc.2789

Sunda, W. G., Tester, P. A., and Huntsman, S. A. (1987). Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. *Mar. Biol.* 94 (2), 203–210. doi:10.1007/Bf00392932

Tait, T. N., Rabson, L. M., Diamond, R. L., Cooper, C. A., McGeer, J. C., and Smith, D. S. (2016). Determination of cupric ion concentrations in marine waters: an improved procedure and comparison with other speciation methods. *Environ. Chem.* 13 (1), 140–148. doi:10.1071/EN14190

Tang, D. G., Hung, C. C., Warnken, K. W., and Santschi, P. H. (2000). The distribution of biogenic thiols in surface waters of Galveston Bay. *Limnol. Oceanogr.* 45 (6), 1289–1297. doi:10.4319/lo.2000.45.6.1289

Thibault, A., Derenne, S., Parlanti, E., Anquetil, C., Sourzac, M., Budzinski, H., et al. (2019). Dynamics of organic matter in the Seine Estuary (France): bulk and structural approaches. *Mar. Chem.* 212, 108–119. doi:10.1016/j.marchem.2019.04.007

Town, R. M. (1998). Chronopotentiometric stripping analysis as a probe for copper(II) and lead(II) complexation by fulvic acid: limitations and potentialities. *Anal. Chim. Acta* 363 (1), 31–43. doi:10.1016/S0003-2670(97)00671-5

Town, R. M., and Filella, M. (2000). Determination of metal ion binding parameters for humic substances - Part 2. Utility of ASV pseudo-polarography. *J. Electroanal. Chem.* 488 (1), 1–16. doi:10.1016/S0022-0728(00)00186-8

Town, R. M. (1997). Potentiometric stripping analysis and anodic stripping voltammetry for measurement of copper(II) and lead(II) complexation by fulvic acid: a comparative study. *Electroanalysis* 9 (5), 407–415. doi:10.1002/elan.1140090511

Town, R. M., and van Leeuwen, H. P. (2004). Dynamic speciation analysis of heterogeneous metal complexes with natural ligands by stripping chronopotentiometry at scanned deposition potential (SSCP). *Aust. J. Chem.* 57 (10), 983–992. doi:10.1071/CH04088

Town, R. M., and van Leeuwen, H. P. (2019). Stripping chronopotentiometry at scanned deposition potential (SSCP): an effective methodology for dynamic speciation analysis of nanoparticulate metal complexes. *J. Electroanal. Chem.* 853. doi:10.1016/j.jelechem.2019.113530

Tubbing, D. M. J., Admiraal, W., Cleven, R. F. M. J., Iqbal, M., Vandemeent, D., and Verweij, W. (1994). The contribution of complexed copper to the metabolic inhibition of algae and bacteria in synthetic media and river water. *Water Res.* 28 (1), 37–44. doi:10.1016/0043-1354(94)90117-1

Turner, A. (2010). Marine pollution from antifouling paint particles. *Mar. Pollut. Bull.* 60 (2), 159–171. doi:10.1016/j.marpolbul.2009.12.004

van den Berg, C. M. G. (2006). Chemical speciation of iron in seawater by cathodic stripping voltammetry with dihydroxynaphthalene. *Anal. Chem.* 78 (1), 156–163. doi:10.1021/ac051441

Vraspir, J. M., and Butler, A. (2009). Chemistry of marine ligands and siderophores. *Ann. Rev. Mar. Sci.* 1, 43–63. doi:10.1146/annurev.marine.010908.163712

Waeles, M., Tanguy, V., Lespes, G., and Riso, R. D. (2008). Behaviour of colloidal trace metals (Cu, Pb and Cd) in estuarine waters: an approach using frontal ultrafiltration (UF) and stripping chronopotentiometric methods (SCP). *Estuar. Coast Shelf Sci.* 80 (4), 538–544. doi:10.1016/j.ecss.2008.09.010

Watanabe, K., Fukuzaki, K., Fukushima, K., Aimoto, M., Yoshioka, T., and Yamashita, Y. (2018). Iron and fluorescent dissolved organic matter in an estuarine and coastal system in Japan. *Limnology* 19 (2), 229–240. doi:10.1007/s10201-017-0536-9

Whitby, H., Planquette, H., Cassar, N., Bucciarelli, E., Osburn, C. L., Janssen, D. J., et al. (2020). A call for refining the role of humic-like substances in the oceanic iron cycle. *Sci. Rep.* 10 (1), 6144. doi:10.1038/S41598-020-62266-7

Whitby, H., Hollibaugh, J. T., and van den Berg, C. M. G. (2017). Chemical speciation of copper in a salt marsh estuary and bioavailability to thaumarchaeota. *Front. Mar. Sci.* 4. doi:10.3389/Fmars.2017.00178

Whitby, H., Posacka, A. M., Maldonado, M. T., and van den Berg, C. M. G. (2018). Copper-binding ligands in the NE pacific. *Mar. Chem.* 204, 36–48. doi:10.1016/j.marchem.2018.05.008

Whitby, H., and van den Berg, C. M. G. (2015). Evidence for copper-binding humic substances in seawater. *Mar. Chem.* 173, 282–290. doi:10.1016/j.marchem.2014.09.011

Wong, K. H., Obata, H., Kim, T., Mashio, A. S., Fukuda, H., and Ogawa, H. (2018). Organic complexation of copper in estuarine waters: an assessment of the multi-detection window approach. *Mar. Chem.* 204, 144–151. doi:10.1016/j.marchem.2018.07.001

Wong, K. H., Obata, H., Kim, T., Wakuta, Y., and Takeda, S. (2019). Distribution and speciation of copper and its relationship with FDOM in the East China Sea. *Mar. Chem.* 212, 96–107. doi:10.1016/j.marchem.2019.04.005

Yamashita, Y., Panton, A., Mahaffey, C., and Jaffe, R. (2011). Assessing the spatial and temporal variability of dissolved organic matter in Liverpool Bay using excitation-emission matrix fluorescence and parallel factor analysis. *Ocean Dyn.* 61 (5), 569–579. doi:10.1007/s10236-010-0365-4

Yang, R., and van den Berg, C. M. (2009). Metal complexation by humic substances in seawater. *Environ. Sci. Technol.* 43 (19), 7192–7197. doi:10.1021/es900173w

Ytreberg, E., Karlsson, J., and Eklund, B. (2010). Comparison of toxicity and release rates of Cu and Zn from anti-fouling paints leached in natural and artificial brackish seawater. *Sci. Total Environ.* 408 (12), 2459–2466. doi:10.1016/j.scitotenv.2010.02.036

Zhang, Y. L., Yang, K. L., Du, J. Z., Zhang, F. F., Dong, Y. P., and Li, W. (2018). Chemical characterization of fractions of dissolved humic substances from a marginal sea-a case from the Southern Yellow Sea. *J. Oceanol. Limnol.* 36 (2), 238–248. doi:10.1007/s00343-017-6202-6

Žic, V., Truesdale, V. W., Garnier, C., and Cukrov, N. (2012). The distribution of iodine in the Croatian marine lake, Mir - the missing iodate. *Estuar. Coast Shelf Sci.* 115, 377–387. doi:10.1016/j.ecss.2012.07.026

Zitoun, R., Clearwater, S. J., Hassler, C., Thompson, K. J., Albert, A., and Sander, S. G. (2019). Copper toxicity to blue mussel embryos (*Mytilus galloprovincialis*): the effect of natural dissolved organic matter on copper toxicity in estuarine waters. *Sci. Total Environ.* 653, 300–314. doi:10.1016/j.scitotenv.2018.10.263

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary document to Research paper 3

Copper speciation by anodic stripping voltammetry (ASV) in estuarine waters with high dissolved organic matter

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Table S1. Parameters for anodic stripping voltammetry used for CuCC measurement.

Parameters	DPASV
Deposition potential (V)	-0.50
Duration (s)	60-180
Desorption potential (V)	-1.5
Duration (s)	1-2
Equilibration time (s)	5
Modulation time (s)	0.05
Interval time (s)	0.1
Initial potential (V)	-0.5
End potential (V)	0.0
Step potential (V)	0.002
Modulation amplitude (V)	0.040

Table S2. Average fluorescence intensity of PARAFAC components (R.U.) and their average contribution (%) to the whole FDOM pool in September 2015 and April 2016.

		C1	**C2**	**C3**	**C4**	**C5**
09/2015	R.U.	0.41	0.22	0.53	0.15	0.26
	%	25.1	14.6	34.0	9.6	16.7
04/2016	R.U.	0.15	0.12	0.19	0.08	0.02
	%	25.1	21.0	31.7	15.8	6.4

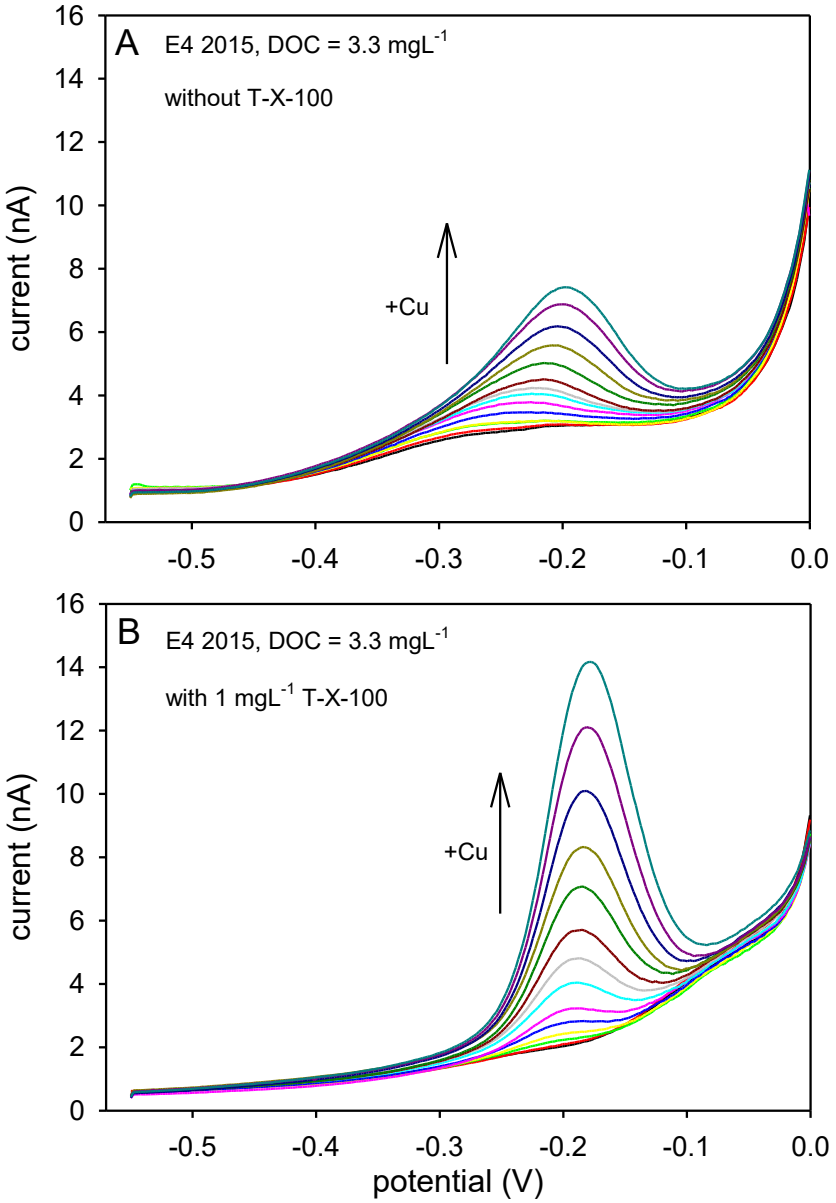

Figure S1. DPASV titration curves obtained by increasing additions of Cu in E4 (2015) sample using (A) desorption step (@E_{DS} = -1.5 V) and without added T-X-100 and (B) with addition of 1 mgL^{-1} T-X-100.

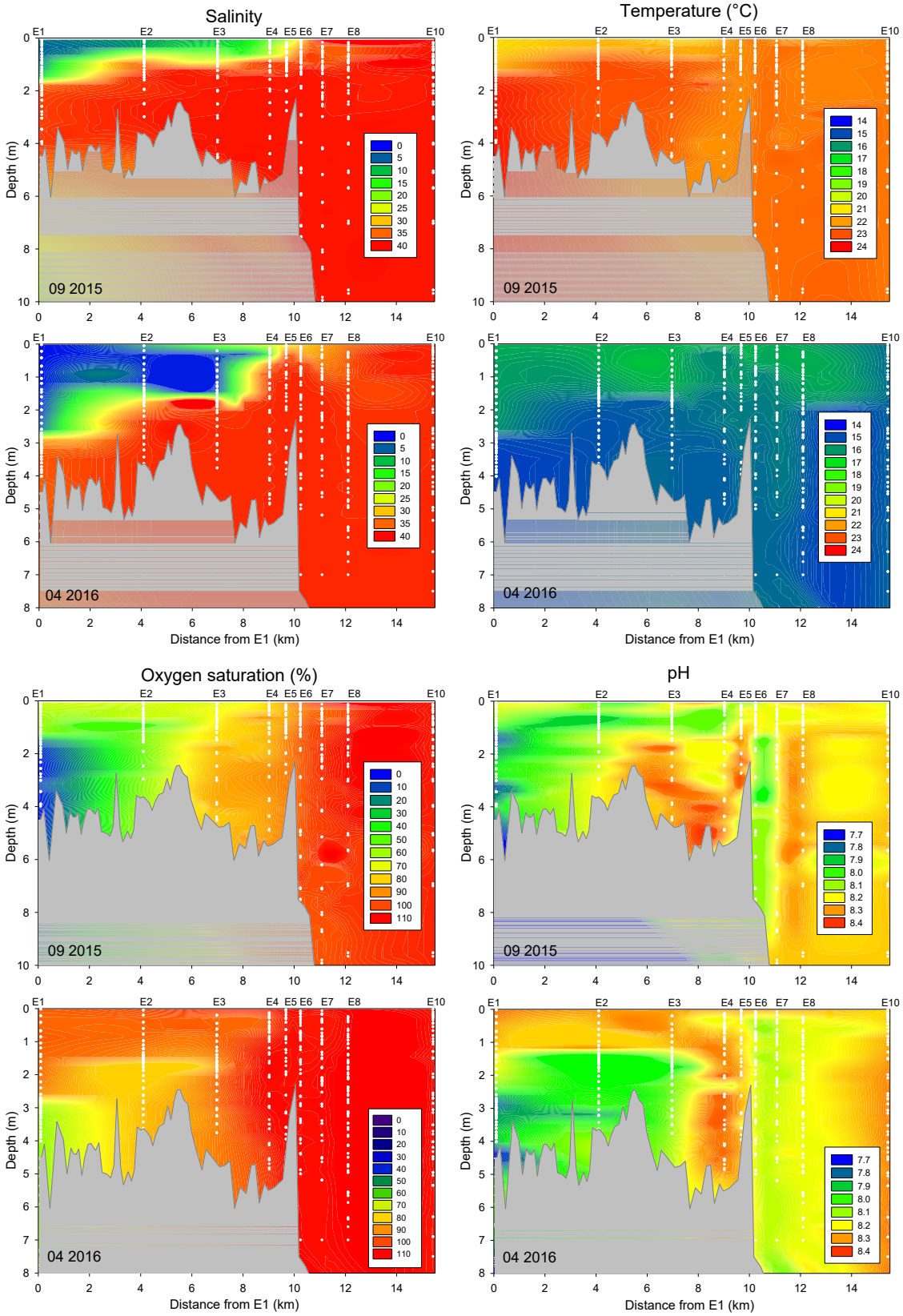

Figure S2. Contour plots of salinity, temperature, dissolved oxygen and pH along the vertical profiles of the Arno River estuary for the two sampling periods.

Figure S3. Images taken at the position of E3 site where during summer period numerous boats are anchored (top image; 28.8.2015.) and winter period where boats are mainly located in dry-marina sites (bottom image, 22.3.2018). Dates for google images were chosen to match our sampling seasons (exact dates were not available).

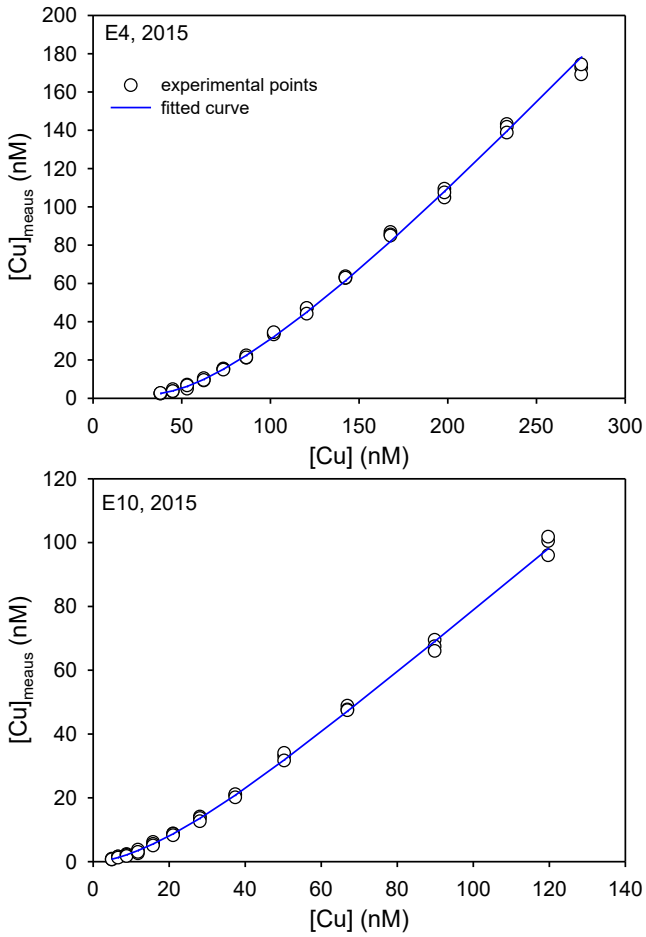

Figure S4. Complexometric titration curves obtained with the addition of 1 mgL^{-1} T-X-100 in E4 and E10 (2015) samples.

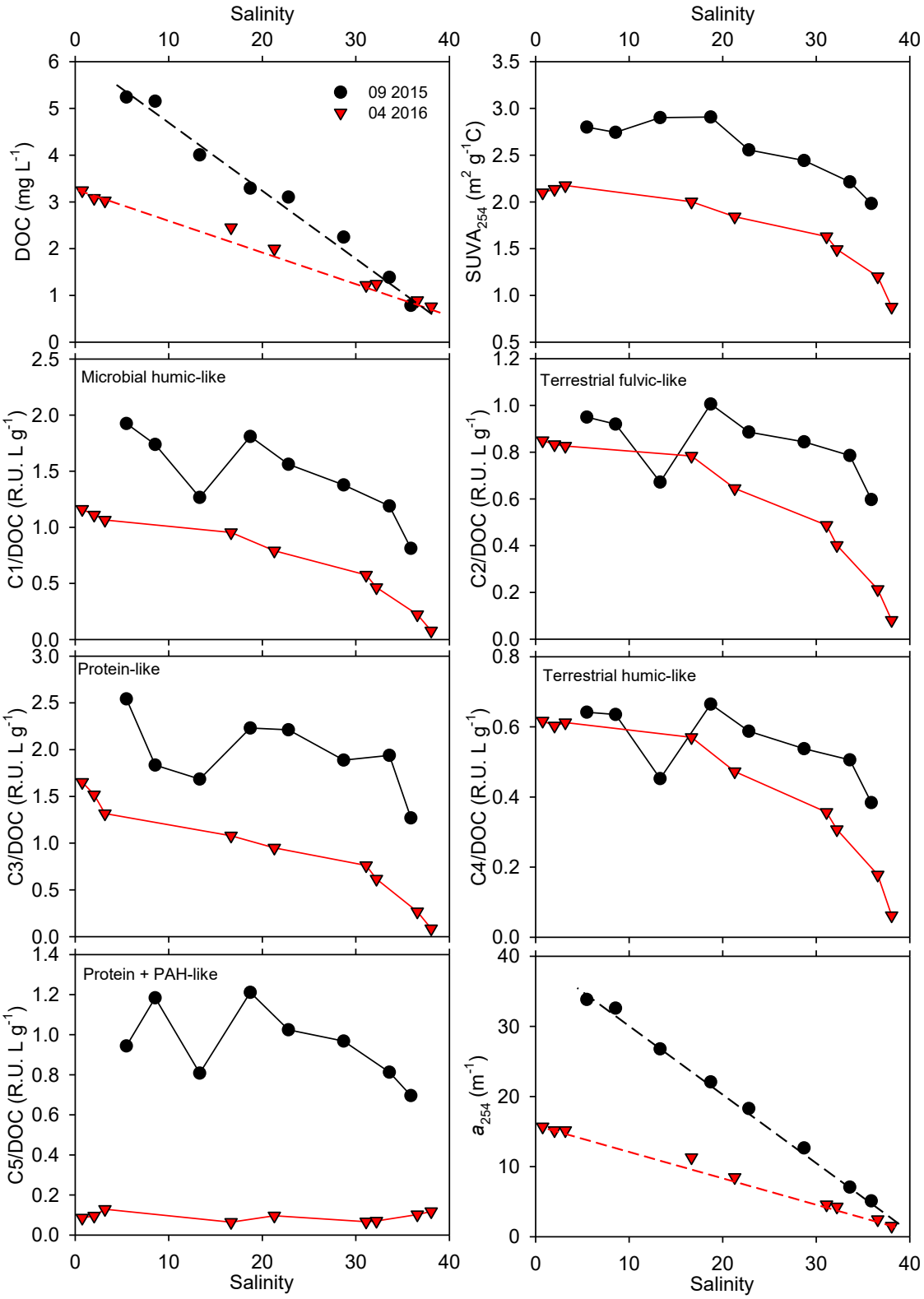

Figure S5. Optical properties of DOM for the two sampling periods. C1-C5 corresponds to components derived by PARAFAC analysis. Dashed lines represent the projected conservative trends.

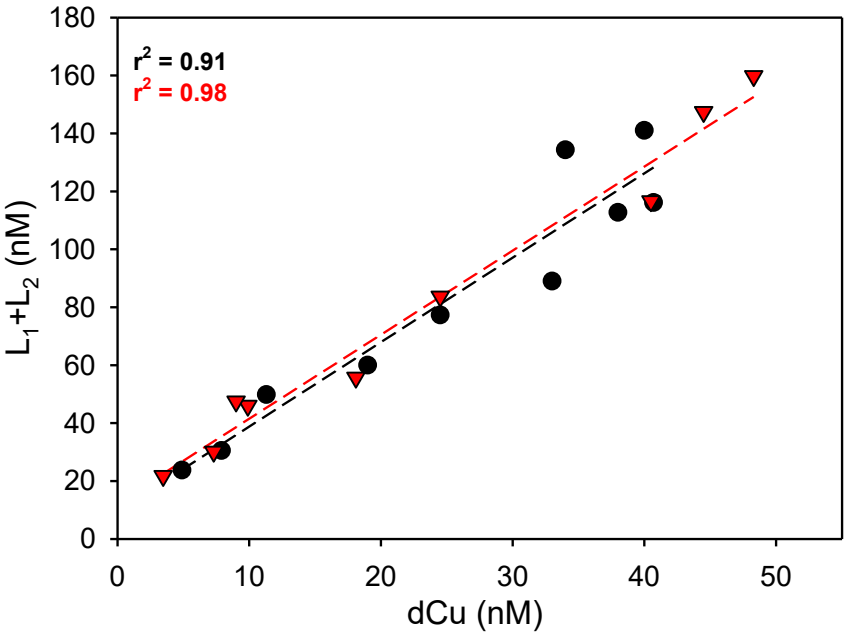

Figure S6. Relationship between the sum of organic ligands (L_1+L_2) and dissolved Cu. Dashed lines represent the linear regression of the data.

CURICULUM VITAE

Jasmin Pađan was born on May 27th, 1988, in Zagreb, Croatia, where he completed his elementary education at Ljubljanica school and later attended the School of natural sciences "Vladimir Prelog". He obtained his Bachelor's degree in 2012 from the Faculty of Chemical Engineering and Technology, University of Zagreb, with a diploma thesis titled "*Procjena koeficijenata prijenosa tvari u mikrokanalu*" under the supervision of Prof. Dr. sc. Đurđa Vasić-Rački and Dr. sc. Martina Sudar. In the same year, he enrolled in the graduate program in Environmental Engineering at the Faculty of Chemical Engineering and Technology, University of Zagreb, where he completed his Master's degree in 2014. His master's thesis, "*Izvedba reaktora za pročišćavanje vode poluvodičkom fotokatalizom*", was conducted under the guidance of Prof. Dr. sc. Sanja Papić and Dr. sc. Ivana Grčić. Since 2014, Jasmin has been employed at the Laboratory for Physical Chemistry of Traces, Division for Marine and Environmental Research, Ruđer Bošković Institute, as a Ph.D. student on the project "New methodological approach to biogeochemical studies of trace metal speciation in coastal aquatic ecosystems – MEBTRACE" funded by the Croatian Science Foundation and led by Dr. sc. Dario Omanović. In 2015, he enrolled in the Interdisciplinary doctoral study in Oceanology at the Department of Geology, Faculty of Science, University of Zagreb, under the mentorship of Dr. sc. Dario Omanović. During his academic career, he actively participated in several conferences and training programs, including the International Summer School GEOTRACES in Brest, France. Since October 2018, Jasmin has been working as a Senior service engineer at Shimadzu, with a specialization in Liquid Chromatography, Mass Spectrometry, Matrix-Assisted Laser Desorption/Ionization (MALDI), and Time-of-Flight Mass Spectrometry (TOF-MS). His responsibilities include the installation, calibration, and validation of high-precision analytical instruments, as well as performing complex troubleshooting and preventive maintenance to ensure optimal system performance. He has gained extensive expertise in method development support, hardware optimization, and integration of chromatography and mass spectrometry systems for advanced analytical workflows. Through this role, Jasmin has developed a deep understanding of the technical aspects of instrument design and operation, contributing to reliable and reproducible analytical results in demanding research and industrial environments.

Peer-review publications

1. Marcinek, S., Cindrić, A. M., **Pađan, J**., Omanović, D., 2022. Trace metal partitioning in the salinity gradient of the highly stratified estuary: A case study in the Krka River estuary (Croatia), *Applied Sciences*, 12 (12): 5816, 13. **doi:** 10.3390/app12125816
2. **Pađan, J.**, Marcinek, S., Cindrić, A.M., Santinelli, C., Retelletti Brogi, S., Radakovitch, O., Garnier, C. and Omanović, D., 2021. Organic copper speciation by anodic stripping voltammetry in estuarine waters with high dissolved organic matter. Frontiers in Chemistry, 8:628749. doi:10.3389/fchem.2020.628749
3. **Pađan, J.**, Marcinek, S., Cindrić, A.M., Layglon, N., Garnier, C., Salaün, P., Cobelo-García, A. and Omanović, D., 2020. Determination of sub-picomolar levels of platinum in the pristine Krka River estuary (Croatia) using improved voltammetric methodology. Environmental Chemistry, 17(2): 77-84. doi:10.1071/EN19157
4. **Pađan, J.**, Marcinek, S., Cindrić, A.M., Layglon, N., Lenoble, V., Salaün, P., Gamier, C. and Omanović, D., 2019. Improved voltammetric methodology for chromium redox speciation in estuarine waters. Analytica Chimica Acta, 1089C: 40-47. doi:10.1016/j.aca.2019.09.014.

Conferences

1. Marcinek, S., Cindrić, A. M., **Pađan, J**., Omanović, D. Diel variability of iron in the estuarine surface microlayer – a preliminary study. *The Iron at the Air-Sea Interface Workshop*, Asheville (USA) 2021. (poster)
2. Marcinek, S., Cindrić, A. M., Garnier, C., **Pađan, J**., Omanović, D. Copper speciation in the Krka River estuary (Croatia). *42nd CIESM Congress*, Cascais, Portugal, 2019. pp. 145-145 (poster)
3. Marcinek, S., Cindrić, A. M., **Pađan, J**., Garnier, C., Omanović, D. Copper speciation in natural waters by CLE-AdCSV - a methodological uncertainties. *Goldschmidt2019 Congress*, Barcelona, Spain, 2019. (poster)
4. Marcinek, S., Layglon, N., **Pađan, J**., Cindrić, A. M., Santinelli, C., Gonnelli, M., Garnier, C., Mounier, S., Omanović, D. Dissolved organic matter (DOM) dynamics in the pristine Krka River estuary (Croatia). *XV International Estuarine Biogeochemistry Symposium (IEBS)*: *Abstract book*. Vigo, Spain, 2019. pp 18-19.
5. Marcinek, S., Layglon, N., **Pađan, J**., Cindrić, A. M., Santinelli, C., Gonnelli, M., Garnier, C., Mounier, S., Omanović, D. Dissolved organic matter (DOM) dynamics in the pristine Krka River estuary (Croatia), *MEBTRACE mini workshop*, Šibenik, Croatia, 2019.
6. **Pađan, J**., Marcinek, S., Cindrić, A. M., Layglon, N., Durrieu, G., Garnier, C., Cobelo-García, A., Omanović, D. Determination of sub-pico-molar levels of platinum in the Krka River estuary (Croatia). *COST ACTION TD 1407 Final Meeting*: *Book of Abstracts*, Filella, M., Omanović D., Dror, I. (Ed.). Zagreb, Croatia, 2019, pp. 70-70. (poster)
7. Marcinek, S., Layglon, N., **Pađan, J**., Cindrić, A. M., Santinelli, C., Gonnelli, M., Garnier, C., Mounier, S., Omanović, D. UV/Vis spektrofotometrijska karakterizacija kromoforne otopljene organske tvari (CDOM) u estuariju rijeke Krke. *Treći Simpozij studenata doktorskih studija PMF-a*, Kumerički K. (Ed.). Zagreb, Croatia, 2019. pp. 24-24
8. Marcinek, S., Layglon, N., **Pađan, J**., Cindrić, A. M., Santinelli, C., Gonnelli, M., Garnier, C., Mounier, S., Omanović, D. UV-Vis spectroscopic study of colored dissolved organic matter (CDOM) in the pristine Krka River estuary (Croatia). *International Workshop on Organic Matter Spectroscopy 2018 (WOMS 2018)*, La Garde City, France, 2018.
9. **Pađan, J**., Marcinek, S., Cindrić, A.M., Layglon, N., Garnier, C., Omanović D. Determination of sub-pico-molar levels of platinum in the Krka River estuary (Croatia). *40th International Conference on Environmental & Food Monitoring*, Santiago de Compostela, Spain, 2018.

10. Marcinek, S., Garnier, C., **Pađan,** J., Pižeta, I., Omanović, D. Segmented multi–detection window approach for organic speciation of trace metals – a model and experimental study in estuarine system. *40th International Conference on Environmental & Food Monitoring*, Santiago de Compostela, Spain, 2018.
11. **Pađan, J**., Marcinek, S., Cindrić, A. M., Layglon, N., Garnier, C., Omanović, D. Determination of sub-pico-molar levels of platinum in the Krka River estuary. *5. Dan elektrokemije & 8th ISE Satellite Student Regional Symposium on Electrochemistry – Book of abstracts*, Zagreb, Croatia, 2018. pp. 22
12. Marcinek, S., Garnier, C., **Pađan, J**., Pižeta, I., Omanović, D. Teoretska i eksperimentalna razrada metodologije "multi–detection window" za specijaciju metala u tragovima u estuarijskom sustavu, *5. Dan elektrokemije & 8th ISE Satellite Student Regional Symposium on Electrochemistry – Book of abstracts*, Zagreb, Croatia, 2018. pp. 23

Trainings

2017 International Summer School GEOTRACES; Brest (France), 19-27. August 2017