Doctoral dissertation submitted to the Department of Biology, Chemistry, and
Pharmacy of the Freie Universität Berlin

Coastal pollution of aquatic systems

Literature review and experiments focusing on metal fate on estuaries

Anderson Abel de Souza Machado

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English title

Coastal pollution of aquatic systems:

Literature review and experiments focusing on metal fate on estuaries

German title

Küstenverschmutzung in aquatischen Systemen:

Literaturrecherche und Experimente zum Umweltverhalten von Metallen in Ästuaren

A Dissertation

Submitted in Partial Fulfilment of the Requirements for the Degree of Ph.D. in River Science to the Department of Biology, Chemistry, and Pharmacy of the Freie Universität Berlin

By

Anderson Abel de Souza Machado

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SMART

Science for MAnagement of Rivers and their Tidal systems

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Primary Supervisor: Jun. Prof. Dr. Christiane Zarfl, Universität Tübingen
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“We see a surprising speed in the growth of mankind’s geochemical work.
We see a more and more pronounced influence of consciousness and collective human reason upon geochemical processes.

Man has introduced into the planet’s structure a new form of effect upon the exchange of atoms between living matter and inert matter.

Formerly, organisms affected the history only of those atoms that were necessary for their respiration, nutrition and proliferation. Man has widened this circle, exerting influence upon elements necessary for technology and for the creation of civilized forms of life. Man acts here not as Homo sapiens, but as Homo sapiens faber”.

Vernadsky, V. 1924 La géochimie. Paris, France: Librairie Félix Alcan

Acknowledgements

Several times humans felt the urge to unberth their vessels and to explore the oceans and their hidden worlds. That was the chance to touch horizons, to encounter new lands before painted solely by third-party narratives and blurred images. Complex and multifaceted reasons were behind this impetuous of taking risk in adventures for unguaranteed awards. Sometimes pure curiosity and romantic spirit of discovery, other moments ambition and desire, perhaps even a sense of duty for a major cause. Whatever controversial fuels and engines powered our crossing of the seven seas it always brought marked changes of paradigms for those in land who saw the crew in the departure, those who sailed with the squad, and those who were with them at the arrival.

As an oceanographer, I can see plenty of similarities between these last three years of academic life and every aspect of the navigations and discoveries above mentioned. I feel an immense sense of gratitude and accomplishment. Which is in part explained by the many horizons that were broadened, by the numerous narratives that I could appropriate, and by the countless images that are now sharper. It is the human part of my personal great navigation that makes the whole experience so noticeable to me however. Every person who helped me to undock, to sail, and eventually to arrive to the final destination deserve a place in this acknowledgement. Nevertheless, there are some people that I must mention.

Among those who were with me during the departure, my family was the safe harbour in which I could dock any time of bad weather. Mom, Dad, brothers and sisters: A huge thanks! I also would like to thank my fellow oceanographers from Brazil that were good memory and delightful presence here many times. In this sense, thank to Rafael, Ingrid, Lumi, Tati, Marina, and Adriano. Johannes was also an essential friend that play important role in helping me to find a place to call home abroad. Also, I would like to sincerely thank Corrie, who became my love, one of the most important people in my life and an essential support during this journey.

The group of those who sailed along the way with me is enormous, with members at IGB and Freie Universität Berlin (Germany), Queen Mary, University of London (UK), University of Trento (Italy), and Deltares (The Netherlands). Many of the staff and students of each one of these institutions were my physically present friends and family during most of the Ph.D. I was especially fortunate to have at my side people as Maja and Navid, Sasha and Saskia, Tesfaye and Amra, Aditya and Alex, Roshni and India, and Alessandro. I am grateful because your friendship made my days even better. I also thank Laura and Simone by the many pleasant philosophical and practical discussions in our office at IGB.

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I additionally thank the financial support provided by EACEA (Education, Audiovisual and Culture Executive Agency) of the European Union.

Responsibility Claim

I, Anderson Abel de Souza Machado, confirm that the research presented within this thesis is result of my own individual and collaborative work. As some studies were carried out in collaboration with, or supported by others, the detailed information on the support is duly acknowledged in the collaborations statement, and in the respective chapters as of co-authorship of the manuscripts and articles, or on the acknowledgements session accordingly to the relevance of the contribution given. Previously published material is also clearly indicated.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, or European and German regulations. Additionally, this work does not infringe third party's copyright or other Intellectual Property Right, nor contains any confidential material.

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Anderson Abel de Souza Machado
September 2016, Berlin.

Collaboration Statement

The multidisciplinary work reported in the present document would not be possible without the many collaborations stablished. In the following paragraphs it is described in detail the contribution of every co-author to the development of the current document.

Chapter 1: Coastal and marine pollution in the Anthropocene: Identifying contaminants and processes
The first chapter is an introductory review published in the Annals of YOUMARES 7 (Hamburg, 2016), for which the search for scientific literature, interpretation and discussion of findings were completedly performed by the solely author.

Chapter 2: Assessment of pollution and environmental restoration in coastal areas: challenges and solutions
The second chapter is an opinion paper published in the Annals of YOUMARES 6 (Bremen, 2015). This opinion paper was an outcome of equal contribution of the two authors: Anderson Abel de Souza Machado and Tatiane Combi.

Chapter 3: Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity
The third chapter is a review and conceptual model published at Science of the Total Environment. It resulted of independent and broad literature search and writing mostly done by the main author Anderson Abel de Souza Machado. All other co-authors (Kate Spencer; Werner Kloas; Marco Toffolon; Christiane Zarfl) significantly contributed to the article throughout the discussion of ideas proposed there, as well as improving clarity and structure of the manunscript.

Chapter 4: Unravelling metal mobility under complex contaminant signatures
The fourth chapter reports experimental results and empirical models for the mobilisation of metals in intertidal areas to be submitted to Estuarine and Coastal Shelf Science. All the experimental results were collected by the main author, Anderson Abel de Souza Machado, who also designed research and analysed data. Francis O’Shea provided important data set on metal spatial distribution in situ, results which were obtained within the scope of his doctoral thesis. Kate Spencer contributed with material and methods. Christiane Zarfl and all the authors discussed the results. At the current form, only the main author has written the manuscript.

Preface

Coastal systems provide a multitude of environmental services that have supported intense human development. Important examples of coastal systems are estuaries, environments in which diverse and abundant freshwater and marine natural resources can be easily assessible. Indeed, it is estimated that about 70% of the global population live in coastal areas, with 11 out of the 15 largest cities in the world being estuarine or coastal. Moreover, coastal and estuarine cities in U.S. growth approximately 3 times faster than inland urban centres. Such intense habitation and urbanization has direct impacts on the environmental health of coastal systems. Therefore, a broad understanding of coastal pollution and its effects is essential for the sustainability of these areas. In this context, the research presented here was developed within the SMART research area 'C', an effort to better understand the potential to rehabilitate compromised functions in impacted riverine and tidal systems.

Purpose

This document was submitted to the Erasmus Mundus Joint Doctorate Committee of the Science for Management of Rivers and their Tidal Systems program (SMART) as partial fulfilment of the requirements of the joint PhD degree in River Science (corresponding to PhD degree in Physical Geography by Queen Mary, University of London and to the degree of Doc Rer Nat by Freie Universität Berlin).

Relevance

Coastal and aquatic pollution are here broadly addressed from a perspective of providing multidisciplinary scientific background for the management of coastal and aquatic systems. An overview of the literature is provided, while additional advances in our current understanding of coastal pollution is also delivered by reporting results from numerical modelling and laboratory experiments.

Structure

This Ph.D. dissertation was submitted in the form of a cumulative thesis, i.e. papers published by the author are organized in a logical sequence resembling to chapters. Only general conclusions and future directions were drawn. It might be worth to mention that as some of the findings reported here were already published elsewhere, eventual inconsistency on chapter format, citation, and language (i.e. British or American English) might occur.

The first part of this document introduces the problematic of current and legacy pollution in coastal areas. Chapter 1 presents the contemporary coastal (fresh and brackish waters) and marine pollution by identifying the various types of stressors and the processes affecting the contaminant distributions and effects. Chapter 2 addresses the scientific and methodological challenges regarding monitoring environmental health in coastal areas.

The second part of this document pays special attention to the current and historically global problem of metal pollution in estuaries. It involved two main goals. The first was an in-depth conceptualization of the problem (chapter 3). Secondly, the chapter 4 discusses the biogeochemical mechanisms of metal mobility are discussed in light of an extensive set of laboratory and field measurements performed in a coastal tidal flat on the Thames Estuary under complex contamination sources.

Additionally, the afterword chapter (after the general conclusions of the thesis) reports further research developed during the PhD period. This independent part focuses on currently unsolved ecotoxicological concerns, such as exposure to contaminant mixtures, non-monotonic dose-responses and toxicity of biopesticides. All research outputs reported here were funded with the support of EACEA (Education, Audiovisual and Culture Executive Agency) of the European Union.

Contents

Abstract

Anthropocene is the current era in which human activities modify various environmental properties, which have implications for many coastal processes. Anthropogenic stressors increasingly affect coasts and push these environments to a new altered equilibrium state. However, monitoring such pollution is a challenging task because coastal systems are highly dynamic and integrate the physicochemical forces at work on freshwater bodies, estuaries and lagoons with the oceanographic characteristics of adjacent seas. The current thesis addresses pollution of coastal environments in a broad way, with special attention to the current and historic problematic of estuarine contamination by metals. Firstly, it introduces the chemical (e.g. metals, persistent organic pollutants, and emerging contaminants), physical (e.g. microplastics, sediment loads, temperature), and biological (e.g. microbiological contamination, invasive species) pervasive anthropogenic influence in coastal areas. This introductory chapter is followed by a discussion on the limitations towards holistic environmental health assessments that are imposed by the scarcity of tools and multidisciplinary approaches. At that juncture, we perform a deep investigation of metal fate and its effects in estuaries. The review of the scientific literature in the third chapter provides a transdisciplinary conceptual framework for the estuarine behaviour of metals and its impacts on fauna and flora. This comprehensive overview and conceptual model are further accompanied by an elaboration on empirical models, as well as discussion of data on metal behaviour under laboratory and field conditions. While our review postulates that most studies had observed a non-conservative behaviour of metals in estuaries, our data suggests that at local scale such phenomenon is greatly explained by a high metal mobilisation driven by biogeochemical gradients. In fact, our results demonstrate that iron mobilisation regulates the pollution levels of iron and potentially other metals in an intertidal area under strong anthropogenic influence. In summary, estuarine physicochemical gradients, biogeochemical processes, and organism physiology are jointly coordinating the fate and potential effects of metals in estuaries, and both realistic model approaches and attempts to postulate site-specific water quality criteria or water/sediment standards must consider such interactions.

Zusammenfassung

Das Anthropocen ist das jetzige Zeitalter, in dem menschliche Aktivitäten zu Umweltveränderungen führen, die sich auch auf viele Küstenprozesse auswirken. Anthropogene Stressoren beeinflussen zunehmend Küsten und zwingen diese Ökosysteme in einen neuen veränderten Gleichgewichtszustand. Eine Überwachung dieser Verschmutzung ist eine anspruchsvolle Aufgabe, da Küstensysteme sehr dynamisch sind und die physikalisch-chemischen Kräfte von Binnengewässern, Flussmündungen und Lagunen mit den ozeanografischen Prozessen der angrenzenden Meere verbinden. Die vorliegende Doktorarbeit befasst sich mit der Verschmutzung der Küstengebiete in einem breitgefächerten Ansatz. Sie beleuchtet hauptsächlich das noch ungelöste Problem der Ästuarverseuchung mit Metallen. Zum einen werden die chemischen (z.B. Metalle, persistente organische Schadstoffe, und “Emerging Contaminants“), physikalische (zum Beispiel Mikroplastik, Sedimentfrachten, Temperatur) und biologische (zum Beispiel mikrobiologische Kontamination, invasive Arten) anthropogenen durchdringen Einfluss in den Küstengebieten eingeführt. Diesem einleitenden Kapitel schliesst sich eine Diskussion an über die Grenzen für eine ganzheitliche Umweltgesundheitsuntersuchung, die durch den Mangel an Werkzeugen und multidisziplinäre Ansätze bedingt sind. In diesen Zusammenhang wird eine eingehende Untersuchung über das Schicksal von Metallen und ihre Auswirkungen in Ästuaren durchgeführt. Wir haben anhand der wissenschaftlichen Literatur einen transdisziplinären konzeptionellen Rahmen entwickelt für das Verhalten von Metallen in Mündungsgebieten und deren Auswirkungen auf die Fauna und Flora. Diese umfassende Übersicht und unser konzeptionelles Modell ergänzeten wir durch eine Abhandlung empirischer Modelle, sowie die Diskussion vorhandener Daten über das Verhalten von Metallen unter Labor- und Feldbedingungen. Während beispielsweise die meisten Veröffentlichungen ein nicht-konservatives Verhalten von Metallen in den Mündungen berichteten, legen unsere Daten nahe, dass ein solches Phänomen auf lokaler Skala als einer hohen Metall-Mobilisierung durch biogeochemische Gradienten erklärt werden kann. Wir konnten tatsächlich zeigen, dass die Eisen-Mobilisierung die Schadstoffbelastung reguliert von diesem und wahrscheinlich auch andere Metalle in einer stark antropogen beeinflußten Gezeitenzone.

Insgesamt können wir also zeigen, dass physikalisch-chemische Gradienten, biogeochemische Prozesse und die Physiologie von Lebewesen gemeinsam das Schicksal und die möglichen Auswirkungen von Metallen in den Mündungen beeinflussen. Realistische Modellansätze und Festsetzungen ortsspezifischer Wasser-Qualität-Kriterien oder Wasser / Sediment-Standards müssen solche Interaktionen berücksichtigen.

PART 1

Introduction to coastal and marine pollution

CHAPTER I

Annals of YOUMARES 7 (2016)

It is now widely accepted that anthropogenic influence in the Earth System has achieved a level compared to that of the natural geophysical processes. Humans have become a major forcing that shifts biogeochemical cycles on lands, atmosphere and oceans. Therefore, human-related activities have great consequences to the interface of these three systems: The coastal area. In this chapter I introduce the pervasive impact of human activities on the function of coastal and marine systems.

Coastal and marine pollution in the Anthropocene: Identifying contaminants and processes

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Abstract:
The emerging concept of the Anthropocene era in which human activities modify various environmental properties has direct implications for coastal and marine research. Anthropogenic chemical (e.g. metals, persistent organic pollutants, and emerging contaminants), physical (e.g. microplastics, sediment loads, temperature), and biological (e.g. invasive species) stressors increasingly affect marine and coastal aquatic systems, pushing these environments to a new equilibrium state. This article addresses coastal and marine pollution in a broad context. Examples of representative baseline changes related to human activity and with deleterious environmental effects are reviewed here. The main goal is to highlight that human influence is pervasive in coastal aquatic and marine systems, and therefore increase awareness of marine researchers that the understanding of the difference between natural and anthropogenic controls is essential to better quantify impacts and elaborate mitigation actions.

Keywords: Biodiversity loss, contaminant fate, ecotoxicology, emerging contaminants, light pollution, microplastics, shifting baselines.

1. Introduction

The Anthropocene describes the current epoch in which humans reached development and numbers to rival and modify the natural geophysical function of our planet (Steffen et al. 2011). This concept is established in environmental sciences (Meybeck 2004) despite the fact that it is still to be proven as a stratigraphic unit on geological history (Quaternary Stratigraphy Working Group, 2016). In this sense, the Anthropocene began ~200 years ago with the onset of industrialization and the enormous expansion in the use of fossil fuels (Steffen et al. 2007). The industrial revolution shattered the energy bottleneck that humans had faced throughout the history, with the consequently remarkable explosion in human enterprises modified the baselines for several processes on Earth System (Steffen et al. 2011).

From urban coastlines with long historic industrialization such as the Thames Estuary up to environments as remote as the Arctic Ocean, the imprint of human activities alters natural physics, chemistry, and biology (Baugh et al. 2013; National Academy of Science 2014). Shifts on processes of the Earth System are clear for many (if not most) processes taking place in coastal areas. Sea level rise, ocean acidification, and coral reef bleaching are some examples of the abiotic and biotic anthropogenic disruptions (IPCC 2014). In fact, the chemical composition of the atmosphere and terrestrial, marine and aquatic continental ecosystems display significant signs of anthropogenic perturbations (Meybeck 2003). Baseline changes are especially noticeable for the natural cycles of chemical elements such as carbon, nitrogen, phosphorus, and various metals (Steffen et al. 2011; IPCC 2014; Machado et al. 2016). Additionally, a complex cocktail of various synthetic and potentially toxic xenobiotics have been released into the environment in the form of persistent organic pollutants (POPs) and emerging contaminants (Box 1).

Thus, the present study introduces selected anthropogenic impacts on the biosphere. The main focus lies on changes that have consequences on the physical, chemical, and biological natural function of the coastal and marine components of the Earth System. I identify potential contaminants and processes to which human influence is already well shown global and distinguishable.

2. Identifying contaminants

After the industrial revolution, human societies have overcome most of energy and natural resources limitations that had previously constrained our species, which allowed remarkable population growth (Fig. 1A) (Steffen et al. 2011). While industrialization happened at early stages of Anthropocene, some of the technological developments were perceived only lately, after the Great Acceleration starting ~1940 (Fig. 1B) (Steffen et al. 2007). Thus, timely diverse contaminants have been released into the environment since the beginning of the Anthropocene. Currently, the American Chemical Society reports >117 million of organic and inorganic chemical compounds, that have been manipulated by humans (CAS Registry, 2016). Indeed, the CAS Registry updates daily ~15,000 new compounds, with 1-2% of them entering the market and eventually the environment.

Box 1. Trace contaminants

Trace contaminants form a chemically diverse group with toxicity or environmental concentrations at range of µg L^{-1} or below. They might be essential micronutrients (e.g. Cu), non-essential elements (e.g. Ag) or synthetic xenobiotics (e.g. polychlorinated biphenyls). This latter group is of particular concern because organisms have been exposed to them only during the Anthropocene, and have not completely evolved specific detoxification mechanisms.

- **Metals:** The most studied and still problematic group of contaminants including several chemically stable elements with high toxicity and extremely long residence times in aquatic systems (Machado et al. 2016). Al and Zn are most threatening contaminants for the aquatic wildlife in the UK.
- **Persistent organic pollutants (POPs):** Toxic chemicals with relatively long environmental persistence and high mobility. Their extremely high affinity to biomolecules and difficulties of metabolic detoxification allow most of POPs to bioaccumulate and biomagnify (EPA US, 2016). POPs are transported long distances in water and atmosphere (Jiménez et al. 2015). Thus, high concentrations are found in top predators like large mammals even in remote areas of Arctic or Antarctic oceans.
- **Emerging contaminants & endocrine disruptors:** Emerging contaminants are modern or long use chemicals, including pharmaceuticals, that have environmental toxicity (mostly sublethal, e.g. carcinogenesis, genotoxicity, mutagenicity) only recently discovered. Endocrine disruptors are often emerging contaminants that interfere with hormonal responses (metals and POPs might also cause endocrine disruption). It is difficult to estimate impacts of endocrine disruptors because they may cause non-monotonic effects at low levels (ng - fg L^{-1}) (Vanderberg et al. 2012).

As single contaminants present significantly different usage and environmental behavior, it is currently impossible to estimate the exact extent of human impacts on environmental chemistry. Some pollutants occur at very low concentrations, often below quantification limits. Such trace concentrations do not imply the absence of effects, however (Box 1).

Other chemicals provide insights on the dimension of chemical pollution. Carbon dioxide (CO_2; Fig. 1C) is perhaps the most notorious sign of anthropogenic influence on geophysical and geochemical forces (Steffen et al. 2007). The atmospheric concentration of this greenhouse gas increased since the beginning of Anthropocene causing climate change, ocean acidification, and sea-level rise, among other effects (IPCC, 2014). Moreover, CO_2 has an estimated residence time between 3 and 40 years for the atmosphere and ~350 years for the oceans. This means that most of anthropogenic CO_2 has been stored in the oceans and will continue impacting the Earth System for the next several centuries (IPCC, 2014).

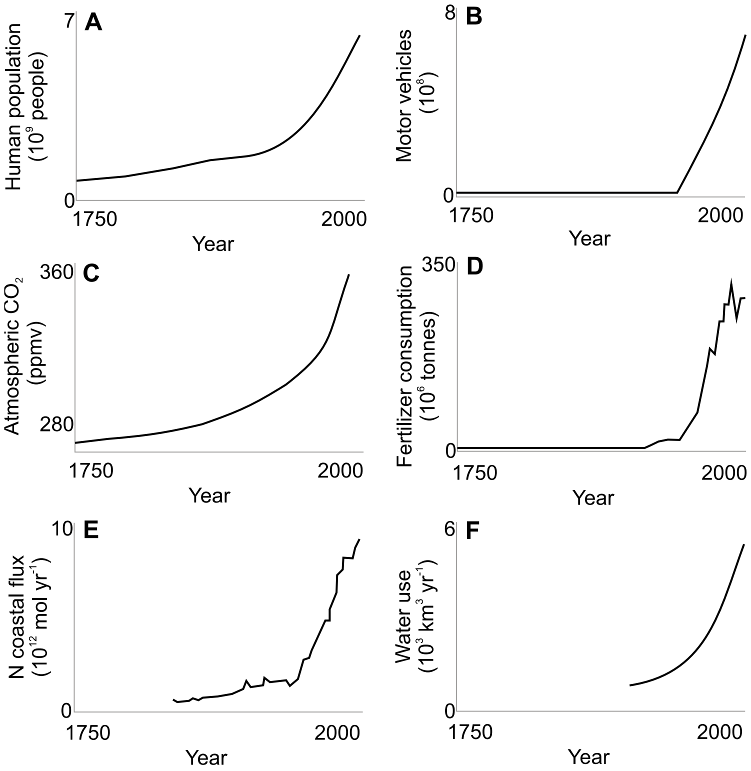


Figure 1: Characteristic markers of the Anthropocene*. Human population (A), popularization and consumption of technology (B), carbon dioxide atmospheric contamination (C), fertilizer consumption (D), coastal nitrogen input (E), and water usage (F). *Figures are adapted from Steffen et al. 2011.

Fertilizers are also undoubtedly chemical contaminants causing shifts in coastal and marine systems worldwide (Fig. 1D). Nutrient contamination on coastal aquatic systems has widespread sources such as agriculture, house waste, aquaculture, industrial effluents, and erosion (Cloern 2001). Thus, coastal and marine systems are receiving enhanced influx of nutrients such as nitrogen (Fig. 1E) and phosphorus (Steffen et al. 2011). Indeed, nutrient enrichment has been observed globally with impacts on trophic structures and coastal eutrophication (Cloern 2001). Such nutrient effects are also associated with the increase in water use (Fig. 1F), mostly for irrigation, that compromises quality and quantity of water flowing to coastal areas (Meybeck 2003, 2004) (see chapter “Fighting eutrophication in coastal waters”).

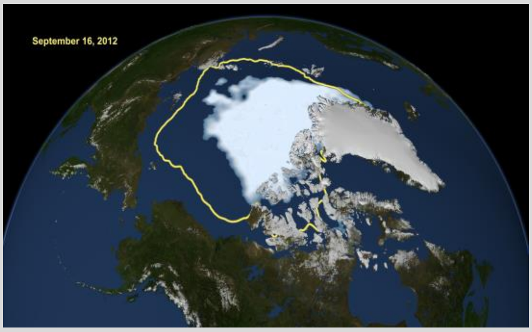
Chemical contamination is not the exclusive type of coastal pollution. Other important anthropogenic stressors are physical. For instance, shifts in water temperature can trigger significant changes on the planktonic community of the Thames Estuary (Lázár et al. 2012). More recently, the potential effects of night-time lightning in harbors and coastal areas have been highlighted as pollutant able to affect epifaunal benthonic communities (Davies et al. 2015). Another relevant physical stressor is the pollution by sediments. In watersheds not properly managed, high sediment input can increase turbidity in estuaries and coastal waters (Baugh et al. 2013). This affects aquatic photosynthesis, fish respiration, predator behavior, and benthonic communities. Conversely, intense water abstraction can decrease sediment input, often associated with coastal erosion (Meybeck 2004).

Plastic debris, such as microplastics, is another example of the physical stressor. It has been claimed that plastic pollution might be the most ubiquitous and long-lasting anthropogenic changes to the surface of our planet (Barnes et al. 2009). Environmental abrasion of large plastic fragments generates microplastics (Zubris & Richards 2005), generally defined as plastic particles smaller than 5 mm (Barnes et al. 2009). The abundance of small plastic particles is further increased by the use of plastic particles in scrubbers and abrasives, spillage of pre-production plastic pellets and powders (Barnes et al. 2009). While large plastic debris can cause ecological effects e.g. by organism entangling and decreasing feeding efficiency, microplastics can additionally cause abrasion of sensitive tissues such as gills, and if internalized they can physically disrupt physiological functions. Furthermore, there is a controversial debate on the potential of microplastics to adsorb and release chemicals under certain conditions, which would have implications on the facilitation of organism interaction with other contaminants (Koelmans et al. 2016). It is unquestionable that plastic pollution is a widespread problem, with plastic fragments present in most of aquatic continental and most oceanic environments (Zubris & Richards 2005, Barnes et al. 2009), thus representing a clear signature of the later phase of Anthropocene. The dimension of its impacts still requires research.

There are also anthropogenic biological stressors. Aquaculture, for instance, enhances the risk of pathology in the aquatic fauna and flora by introducing and increasing the density of certain microbes and their host organisms. In fact, microbial contamination is recently acknowledged as a threat to the global biodiversity (Schwartz et al. 2006). Changes on distribution of diverse species (see chapter "Going global: invasive and range-expanding species") implies anthropogenic disruptions of natural ecological niches (Dirzo et al. 2014). Moreover, the use of the entomopathogenic *Bacillus thuringiensis* as bioinsecticide to control mosquito nuisance in French wetland reserves altered predatory pressure on insects and trophic structure with reduced bird fecundity (Poulin et al. 2010). The community composition and ecological interactions that we observe in the contemporary coastal systems are under strong influence of microbial contamination. Thus, research is required to elucidate which processes are natural and which result from biological

Box 2. Climate change
Climate change refers to the unequivocal and unprecedented warming of the climate system. The main causes are anthropogenic emissions of greenhouse gases (the highest in the history of the planet), which have widespread effects on atmosphere, oceans, cryosphere, etc. (PCC, 2014). For instance, Arctic environments experience changes in climate, biology, and society on a rapid, complex, and interactive non-linear fashion, with regional and global consequences (National Academy of Science, 2015).
Nearly all natural and human systems will be affected by climate change. There is uncertainty on the magnitude of:

- **Temperature changes**
- **Sea level rise**
- **Ocean acidification**
- **Extreme events**
- **Coastal squeeze**
- **Climate forcings and feedbacks**



Sea ice minimum in the Arctic Ocean on September 2012. Source: NASA (http://www.nasa.gov/topics/earth/features/2012-seaicemin.html)

3. Identifying processes

a. Global changes

Global change refers to the conjunction of processes which anthropogenic forcing drives the Earth System away from its natural function. Thus, global change includes a wide range of global-scale phenomena: land use and land cover, urbanization, globalization, coastal ecosystems, atmospheric composition, riverine flow, nitrogen cycle, carbon cycle, physical climate, marine food web chains, biological diversity, population, economy, resource use, energy, transport, communication, and so on (Steffen et al. 2011). Climate change is the most broadly recognized of global changes and it remains a challenge from a scientific and environmental management point of view (IPCC, 2014; Box 2).

Another example of global change is the formation of the ozone hole (Fig. 2A) caused by Chlorofluorocarbons (CFCs) emissions and other halogenated ozone depleting substances (ODS) (Steffen et al. 2011). It had implication on increasing global UV radiation, and therefore potentially increasing mutation rates on various biomes since 1970 (NASA, 2016). The ozone hole is currently decreasing and represents an example of successful control of environmental damage that took place after 1990 (Steffen et al. 2011).

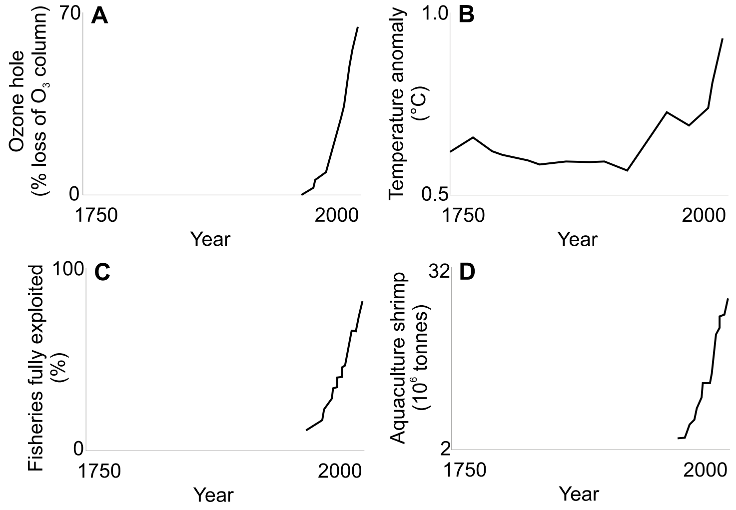


Figure 2: Anthropocene processes affecting life on coastal and marine environments*. Stratospheric ozone depletion (A), global warming (B), and ecosystem impacts: fisheries (C) and aquaculture (D). *Figures are adapted from Steffen et al. 2011.

Coastal structures and coastal engineered morphology also alter the hydrodynamics, and consequently the geochemistry and ecology of coastal systems (Machado et al. 2016). The most iconic example is perhaps “coastal squeeze”, the loss of tidal areas due to a combination of sea level rise and construction of hard defenses to maintain historic coastlines (Pethick 2002). Coastal squeeze affects tidal propagation and compromises ecosystem services provided by tidal areas (Pethick 2002).Additional relevant examples of global change that affect directly biota of coastal and marine ecosystems are global warming (Fig. 2B), and the ecosystem pressures fisheries overexploitation (Fig. 2C) and aquaculture (Fig. 2D).

The impact of global changes on water cycle is of particular concern for estuaries (Meybeck 2004). Estuaries are amongst the most biologically productive and biodiverse ecosystems on the planet, which support a significant part of global fisheries (Cloern 2001). Being areas of traditionally intense human habitation, estuaries experience various current and historical stressors (Pethick 2002). Loss of vegetal cover and extreme weather (Fig. 3A-B) are causing erosion and increased suspended particle transport by rivers, resulting in sediment contamination and its effects (see above). Because of its transitional position between land and sea, at the lowest end of rivers, estuaries are also affected by flooding (Pethick 2002). High flow velocities during floods interfere with the sediment balance, i.e. the net outcome of sedimentation and erosion. Alterations on the water level and flooding frequency can change the chemistry of sedimentary layers and can affect estuarine contaminant remobilization (Machado et al. 2016). Moreover, the freshwater input is a determining the position of the salinity fronts (Meybeck 2004). In turn, the zones of equal salinity, i.e. the isohalines, are determining the communities of estuarine organisms (Jassby et al. 1995). Therefore, it is sensible that the current global boom in construction of river dams (Zarfl et al. 2015) (Fig. 3C) affects the ecology of estuarine systems.

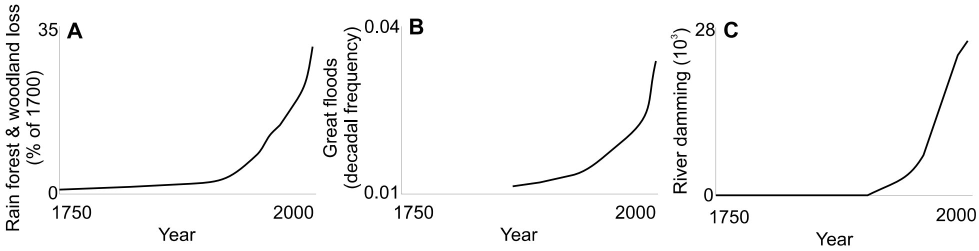


Figure 3: Anthropocene processes that directly impact estuarine systems*. Deforestation (A), extreme events as great floods (B), and river dam construction (C). *Figures are adapted from Steffen et al. 2011.

b. Atmospheric circulation

The absence of human settlements does not assure nonexistence of anthropogenic impacts. Contaminants that present a gas-phase or that are adsorbed by aerosols can be transported in the atmosphere over long distances, which can be up to thousands of kilometers depending on the contaminants physico-chemical properties (Li et al., 2010). Given the global scale of atmospheric circulation and aerosols transport patterns (Fig. 4, NASA 2016), such contamination source is not trivial.

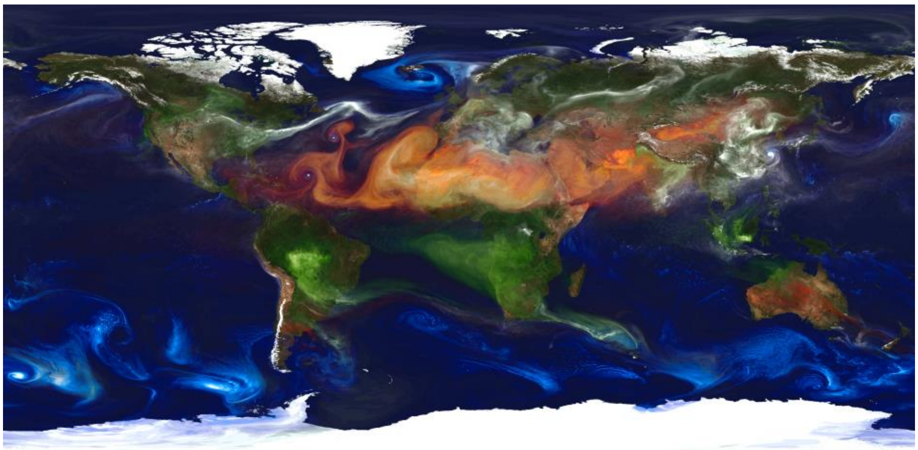


Figure 4: Global atmospheric aerosols. Source: NASA (http://www.nasa.gov/multimedia/imagegallery/image_feature_2393.html).

The three main processes controlling atmospheric deposition of contaminants into aquatic environments are dry deposition, wet deposition, and air–water diffusive fluxes (Jiménez et al 2015). The importance of each process depends on the physicochemical properties of pollutants (e.g., solubility, vapor pressure, and octanol–water partition coefficient) and the environmental conditions (e.g., temperature, wind speed, precipitation rates, etc.) (Jiménez et al 2015). In this context, wet deposition is more relevant in regions with higher precipitation rates, and it affects both gas-phase and aerosol-bound contaminants. In turn, dry deposition drives aquatic contamination for pollutants associated with the atmospheric particle phase (e.g., metals, higher molecular weight polychlorinated dibenzo-p-dioxins, and dibenzofurans and polycyclic aromatic hydrocarbons-PAHs), while diffusive fluxes dominate for contaminants with a large fraction in the atmospheric gas phase (e.g., polychlorinated biphenyls and the low-molecular-weight PAHs) (Jiménez et al 2015).

Atmospheric deposition is suggested as the main source of POPs to open marine systems and large inland lakes. The importance of the atmospheric pathway for the major POPs families has been confirmed in the oceans and seas worldwide far from coasts, and such pathway likely supports POPs bioaccumulation and biomagnification on planktonic food webs throughout the globe (Jiménez et al. 2015).

Atmospheric deposition of metals can also account for metal input on coastal and marine systems (Machado et al. 2016). Depositions of metals to the English Channel and the southern bight of the North Sea are not negligible, representing 20 to 70% of the total input (riverine, direct, and atmospheric inputs) (Deboudt et al. 2003). It has been demonstrated that the atmospheric deposition of toxic metals has changed since the industrial revolution and potentially alters planktonic communities and impairs primary production at the global scale (Paytan et al. 2009). Therefore, it is sensible that coastal and marine systems distant from urban centers and human influence may have their function modified due to atmospheric deposition of contaminants.

c. Biological processes and effects

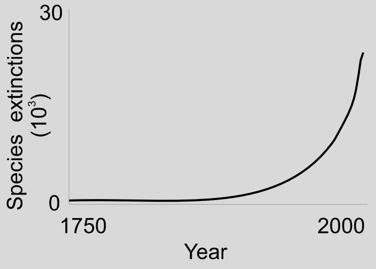
The Anthropocene impacts discussed above may underlay a physiological and ecologically altered state of wild populations. It is accepted that aquatic organisms as microalgae, mussels, fish and others inhabiting polluted sites present altered morphology and physiology (Monserrat et al. 2007). Biological responses (from molecular shifts up to behavioral changes) to exposure to contaminants have been extensively employed as biomarkers of aquatic contamination (Monserrat et al. 2007). Biomarker studies normally compare the physiology of organisms from polluted site to the physiology of control organisms from more pristine environments (Monserrat et al. 2007, Machado et al. 2014). This approach has allowed improving the understanding of the physiological basis of pollution effects (Machado et al. 2014). However, given the widely spread anthropogenic influences, natural baselines for many physiological pathways of most of the species can no longer be determined with this approach.

Box 3. Anthropocene biodiversity loss

It is been increasingly argued that the Earth System is facing it 6th great extinction. The Anthropocene defaunation is a pervasive phenomenon caused by human activity (Dirzo et al. 2014). While species extinctions represent concerning losses in terms of genetic biodiversity, many changes already observed in terms of species compositions might have even more drastic impact on the functional biodiversity.

Causes of biodiversity loss:

- **Habitat destruction**
- **Chemical pollution**
- **Climate changes**
- **Predation**



Anthropocene loss of biodiversity. Adapted from Steffen et al. 2011.

In fact, several studies detected rapid evolutionary changes in morphology or life history on wild populations of short-lived organisms or human-exploited species (Dirzo et al. 2014 and references therein). Such biological shifts are partially explained by extinction and ecological pressure on wild populations (Box 3), and by sublethal toxicity leading to altered metabolism. The latter is of special concern in the case of endocrine disruption because very low concentrations of contaminants might activate and deactivate many physiological responses (Fig. 5) causing, for instance, hormonal unbalance and increasing mutation rates (Vanderberg et al. 2012). Additionally, we lack scientific knowledge to properly assign causality on many of these effects (Vanderberg et al. 2012), because such pollutants may trigger non-monotonic dose-responses (Box 1). Thus, it is impossible to predict the physiological outcome of the contaminants cocktails currently present in coastal and aquatic systems during the Anthropocene.

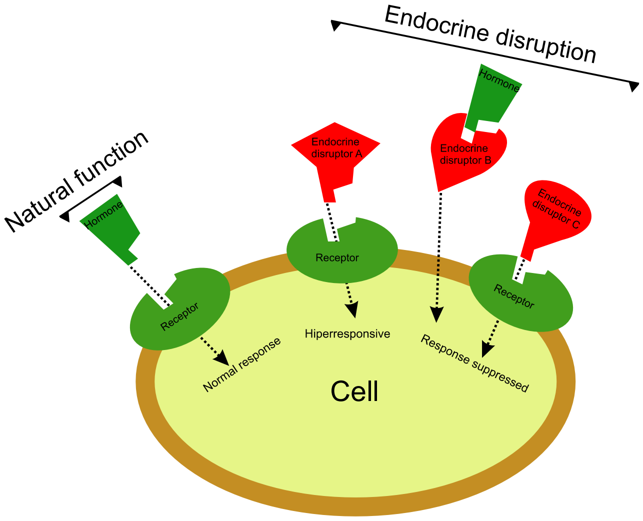


Figure 5: Main mechanisms of endocrine disruption. This process might be pervasive throughout organisms and marine environments.

4. Final Remarks

The Anthropocene describes the epoch in which human activities rival with the natural biogeochemical forces on the Earth System. Anthropogenic activities strongly modify the natural function of the planet, therefore setting some of the greatest research and policy challenges that ever confronted humanity.

The present review addresses how the Anthropocene influences chemistry, physics, and biology of coastal and marine systems, and the related changes in the natural function of aquatic coastal and marine systems towards a new ‘equilibrium’ state. The reality of coastal systems in the Anthropocene is as systems undergoing shifts on baselines. This awareness from general marine researchers will allow better quantification of the impacts and elaboration of management actions to ensure sustainability for the future generations.

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References

Barnes D.K.A., Galgani F., Thompson R.C., Barlaz M. 2009. Accumulation and fragmentation of plastic debris in global environments. Phil. Trans. R. Soc. B 364: 1985-1998.

Baugh J., Feates N., Littlewood M., Spearman J. 2013. The fine sediment regime of the Thames Estuary - A clearer understanding. Ocean & Coastal Management. 79:10-19

Cloern J.J. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series. 210: 223-253.

Davies T.W., Coleman M., Griffith K.M., Jenkins S.R. 2015. Night-time lighting alters the composition of marine epifaunal communities. Biology Letters. 11: 20150080. doi: 10.1098/rsbl.2015.0080

Deboudt K., Flament P., Bertho M-L. 2004. Cd, Cu, Pb and Zn concentrations in atmospheric wet deposition at a coastal station in Western Europe. Water, Air & Soil Pollution. 151:335-359.

Dirzo R., Young H.S., Galetti M., Ceballos G., Isaac N.J., Collen B. 2014. Defaunation in the Anthropocene. Science. 345 (6195):401-406.

Lázár A.N., Wade A.J., Whitehead P.G., Neal C., Loewenthal M. 2012. Reconciling observed and modelled phytoplankton dynamics in a major lowland UK river, the Thames. Hydrology Research 43: 576-588.

Li, Y.F., Harner, T., Liu, L., Zhang, Z., Ren, N.Q., Jia, H., Ma, J., Sverko, E., 2010. Polychlorinated biphenyls in global air and surface soil: Distributions, air-soil exchange, and fractionation effect. Environmental Science & Technology. 44, 2784–2790.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jassby A.D., Kimmerer W.J., Monismith S.G., Armor C., Cloern J.E., Powel T.M., Schubel J.R. Vendlinski T.J. 1995. Isohaline position as a habitat indicator for estuarine populations. Ecological Applications. 5:272-289.

Jiménez J.C., Dachs J., Eisenreich S.J. 2015. Atmospheric Deposition of POPs: Implications for the Chemical Pollution of Aquatic Environments. *In: Eddy, Y.Z. (Ed.), Comprehensive Analytical Chemistry. Elsevier, pp. 295-322*.

Koelmans A.A., Bakir A., Burton A., Janssen C.R. 2016. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. Environmental Science & Technology. 50: 3315-3326.

Machado A.A.S., Wood C.M., Bianchini A., Gillis P.A. 2014. Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. Ecotoxicology. 23:1345-1358.

Machado A.A.S., Spencer K., Kloas W., Toffolon M., Zarfl C. 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. Science of The Total Environment 541:268-281

Meybeck M. 2003. Global analysis of river systems: from Earth system controls to Anthropocene Syndromes Philosophical Transactions of the Royal Society of London B Biological Sciences 29; 358(1440): 1935–1955

Meybeck M. 2004. The global change of continental aquatic systems: dominant impacts of human activities. Water Science and Technology. 49:73-83.

Monserrat J.M., Martinéz P.B., Geracitano L.A., Amado L.L., Martins C.M.G., Pinho G.L.L., Chaves I.S., Ferreira-Cravo M., Ventura-Lima J., Bianchini A. 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology C. 146:221-234.

Committee on Emerging Research Questions in the Arctic. 2014: The Arctic in the Anthropocene: Emerging Research Questions. The National Academies Press, Washington DC.

Paytan A., Macey K.R.M., Chen Y., Lima I.D., Doney S.C., Mahowald N. Labiosa R., Post A.F. 2009. Toxicity of atmospheric aerosols on marine phytoplankton. Proceedings of the National Academy of Science. 106:4601-4605.

Pethick, J. 2002. Estuarine and tidal wetland restoration in the United Kingdom: Policy versus practice. Restoration Ecology. 10, 431–437.

Poulin B., Lefebvre G., Paz L. 2010. Red flag for green spray: adverse trophic effects of Bti on breeding birds. Journal of Applied Ecology. 47:884-889

Schwartz, M.W., Hoeksema, J.D., Gehring, C.A., Johnson, N.C., Klironomos, J.N., Abbott, L.K., Pringle, A. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecology Letters. 9:501-515

Steffen W., Crutzen P.J., McNeill J.R. 2007. The Anthropocene: Are Humans Now Overwhelming the Great Forces of Nature. AMBIO: A Journal of the Human Environment, 36(8): 614-621.

Steffen W., Grinevald J., Crutzen P., McNeill J. 2011. The Anthropocene: conceptual and historical perspectives. Philophical Transactions of Royal Society. A. 369: 842-867.

Vandenberg L. N., Colborn T., Hayes T. B., Heindel J. J., Jacobs D. R. Jr., Lee D. H., Shioda T., Soto A.M., vom Saal F., Welshons W.V., Zoeller T. Myers J.P. 2012. Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. Endocrine Reviews. 33 (3):378-455.

Zarfl C., Lumsdon A.E., Berlekamp J., Tydecks L., Tockner K. 2015. A global boom in hydropower dam construction. Aquatic Sciences. 77:161-170.

Zubris K.A.V., Richards B.K. 2005. Synthetic fibers as an indicator of land application of sludge. Environmental Pollution. 138: 201-211.

http://quaternary.stratigraphy.org/workinggroups/anthropocene/Accessed in 04.08.2016

https://www.epa.gov/international-cooperation/persistent-organic-pollutants-global-issue-global-response Accessed in 04.08.2016

https://www.cas.org/content/chemical-substances Accessed in 10.08.2016

http://earthobservatory.nasa.gov/Features/UVB/ Accessed in 23.08.2016

CHAPTER II

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Coastal areas host a variety of extremely dynamic ecosystems. Therefore, it is a challenging task to assess the outcomes of human influence either in form of pollution or as attempts of environmental restoration. Within the chapter two we discuss some of the challenges and solutions in monitoring environmental pollution and restoration in coastal areas.

Assessment of pollution and environmental restoration in coastal areas: challenges and solutions

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Abstract

Coastal areas are under continuous and increasing pressure from different human activities. A cocktail of contaminants (e.g. metals, hydrocarbons, pesticides, persistent organic pollutants (POPs), and others) threatens water, sediment and biota. Additionally, coastal systems are highly dynamic, integrating the physicochemical dynamics of freshwater bodies, estuaries and lagoons with the oceanographic characteristics of adjacent seas. Thus, contamination assessments and remediation of coastal ecosystems are among the most complex and current issues in ecotoxicology and environmental management. Lack of not expensive and sensitive multi-contaminant analytical procedures, difficulties in interpreting biomarkers as bioanalytical tools, and the lack of multidisciplinary approaches limit holistic environmental health assessments. Concomitantly, a controlled coastal retreat strategy known as managed realignment is becoming common mitigation tool for anthropogenic impacts. In turn, managed realignment might impact coastal biogeochemistry. This article discusses some of the current challenges and opportunities in the assessment of contaminant distribution, and its interaction with managed realignment. In conclusion, it is highlighted that representative monitoring must address the chemical contamination, biological impacts, and their interaction with the environmental features. Similarly, managed realignment might provide positive outcomes if uncertainties and impacts on contaminant remobilization are observed.

Keywords: Risk assessment, marine pollution, contaminant analysis

1. Introduction

About 70% of the global population lives in coastal areas (Bortone, 2005), where the rapid growth of cities and human activities leads to escalating anthropogenic pressure. Activities such as tourism, agriculture, fisheries, aquaculture, naval traffic and industrialism generate a broad diversity of pollutants in amounts that are potentially harmful to the environment (Zagatto, 2006). This is especially concerning because coastal systems have been used as deposits of urban and industrial wastes during the last centuries, which turned coastal and transitional waters into the final receptacle of a complex cocktail of contaminants (Kennish, 1991).

Metals, hydrocarbons, pesticides, and several POPs are among the main chemical contaminants affecting coastal ecosystems (Seeliger et al., 1988; Kennish, 1991, Neto et al., 2008). Pharmaceuticals and personal care products (PPCPs), flame retardants and new generation anti-fouling compounds have more recently joined into the list of chemicals of emerging concern. Concomitantly, researchers are increasing awareness about certain environmental stressors that were previously considered unproblematic. For example, changes in pH (due to climate change), increased water temperature (due to climate change and usage by power plants), and increased sediment load (due to watershed uses and coastal engineering) have been demonstrated to threaten aquatic systems on the long run.

Consequently, contamination assessment and remediation are among the most complex and concerning issues in terms of environmental health and ecosystem services. A representative monitoring program must address chemical contamination, biological impacts, and oceanographic variables. Therefore, the present study discusses various challenges and solutions for monitoring pollution and the mitigation of impacts on coastal areas.

2. Environmental health assessment

Numerous approaches can be used to measure environmental impacts of pollutants, and the selection of the most appropriate methodology will depend on several factors, e.g. the objectives of the study, the environmental matrix, and the analysed compounds. Monitoring tools, such as measurements of chemical concentrations, are traditionally employed to verify the level of contaminants in a given environmental compartment (air, water, soil, sediment, biota) (Borgå and Di Guardo, 2005). Different compartments will provide particular responses to contamination depending on a wide range of factors and the interactions between them. These influencing factors include the physical characteristics of each compartment and each study area, the physical-chemical properties of the analysed compounds, biological aspects, and proximity to the pollution source (Binelli et al., 2009; Parolini et al., 2010).

Contamination sources are mainly located in populated, industrialized and agricultural areas, where anthropogenic contaminants can reach the environment by urban and industrial sewage discharge, leaching from agriculture, direct spillages into soils, urban runoff and volatilization (Breivik et al., 2002; Litskas et al., 2012). Atmospheric deposition and water transport are the most important mechanism by which contaminants are transferred from the source to residential and background areas (Ruiz-Fernández et al., 2012; Argiriadis et al., 2014).

Atmospheric transport is the main way of long-range transport of contaminants and global distillation effects (Vallack et al., 1998). Since concentrations and composition of pollutants in the air are highly influenced by primary emission sources, the assessment of air contamination represents a useful tool to assess recent emissions of contaminants, distance from sources and pathways of transport of pollutants (Nriagu and Pacyna, 1988; Pozo et al., 2006).

Water represents another major mean of transportation for contaminants on local and global scales. Water bodies can temporarily store chemicals. Their contamination sources are usually leaching from soils and discharge of effluents and industrial wastewaters into river-linked tributaries, including small streams, rivers, lakes, and drinking water supplies (Sodré et al., 2010; Mahmood et al., 2014). Water quality, in terms of physical and chemical contaminant levels, is an important issue in terms of human health and environmental conditions, and could constitute a direct measure of the degree of marine pollution (Montuori et al., 2014).

After transport, contaminants' final reservoirs are coastal areas and estuaries, where sediments act as a sink for most pollutants, which accumulate and remain in the sedimentary matrix for long periods of time, from several years to decades (Sahu et al., 2009; Ruiz-Fernández et al., 2012). Consequently, sediments can represent a record of pollution levels and environmental quality of aquatic ecosystems. Surface sediments, for example, can be used to determine horizontal distribution of chemicals, relating to the distance of sources, while sediment core data represent an important tool for describing the historical input of contaminants and understanding pollution trends over time (Hong et al., 2003; Martins et al., 2010; Combi et al., 2013).

Contaminants present in air, water and sediments can influence the surrounding biota, directly or indirectly. Compounds such as POPs, metals and PPCPs have the potential to accumulate through the food web, affecting marine biota, aquatic-dependent wildlife, and human health (Borgå et al., 2001; Hong et al., 2003). Therefore, the assessment of biota contamination is essential to understand the extension and possible risks of pollution levels as well as the transference processes between compartments (Borgå and Di Guardo, 2005).

Box 1. Biological Assessment Tools

- Biomarkers (quantitative): Measurable biological responses on body fluids, cells, tissues, or organism (including behaviour) that indicate the presence of contaminants.
  - Exposure biomarkers: Report exposure to some level of contamination
  - Effect biomarkers: Denote that contamination reached level high enough to disrupt biological functions.
- Biomonitor the level of contaminants on organisms (quantitative): Type of exposure biomarker from which bioaccumulation and bioavailability of a chemical to diverse organs and organisms can be inferred.
- Toxicity tests (quantitative): Include laboratory exposure to environmental samples (water and sediment) or environmentally relevant conditions to infer toxicity to the local fauna.
- Bioindicators (qualitative): Include interpret the presence or absence of certain species according to the normal of certain environment.

Compared to other types of assessment, bioanalytical tools or biological assessment tools convey more information about health and functioning of the ecosystem. Additionally, biological assessment provides relevant information in terms of bioavailability and potential toxicity (Neto et al., 2008). A multitude of biological parameters are currently being used in ecotoxicological research to infer environmental health and contamination (Box 1). Examples are various biomarkers (Monserrat et al., 2007; Machado et al., 2013), toxicity tests (Zagatto, 2006), levels of compounds in specific tissues of biota present in the environment (i.e. usage of biomonitors) (Seeliger et al., 1988), and the presence or absence of certain species or feature (i.e. usage of bioindicators).

3. Challenges and solutions for environmental management

3.1 Contamination assessment and analysis

The quantification of chemicals in environmental samples is performed through several steps, such as sampling, extraction, purification, and instrumental analysis. A range of issues can affect the efficiency of environmental chemistry analysis, such as the usage of large volumes of solvents and reagents, which in some cases are toxic (e.g. toluene and hydrochloric acid for the extraction of organic compounds and metals, respectively), low sensitivity and selectivity, long extraction times, and high costs (Zhang et al., 2011).

Despite the efficiency of traditional extraction methods, there are limitations regarding detectable levels of contaminants (Qiu and Cai, 2010). For example, while there are numerous studies regarding contaminants in sediments and biota, especially in highly impacted areas (e.g. Koh et al., 2006; de Souza et al., 2008; Kanzari et al., 2014), a limited number of papers presents data from water samples with extremely low concentrations (Qiu and Cai, 2010).

Another issue regarding contaminant analysis is the amount of chemicals currently or historically used. Estimates suggest that more than 100 000 chemicals are currently in use for different purposes around the world (Robles-Molina et al., 2014). The existence of such a high number of compounds in the environment leads to interferences during chemical analysis. Interferences can significantly reduce or preclude the analysis of target compounds (Liu et al., 2006). Additionally, co-contaminated matrixes (matrixes contaminated with two or more groups of pollutants, e.g. metals and organics) can be complex in terms of understanding the interaction mechanisms between different compounds and environmental conditions (Sandrin and Maier, 2003).

The development and optimizations of analytical methods that are environmental friendly and capable of detecting multiple classes of compounds at very low levels (e.g. parts per trillion – ppt) is currently one of the biggest challenges in environmental chemistry (Sterzenbach et al., 1997; Lara-Martín et al., 2011; Pintado-Herrera et al., 2013). In recent decades, many advances have occurred in this sense, with the advent of new analytical techniques and the improvement of instrumental analyses. For example, new techniques have been developed for the extraction of metals and organic compounds, such as PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls).

Accelerated solvent extraction (ASE), ultrasonic agitation/sonication (US) and microwaving are alternative techniques with recognized advantages over traditional procedures (e.g. Soxhlet and *aqua regia* extraction for organic compounds and metals, respectively). Advantages of the modern techniques include less use of solvents, elimination of additional clean-up steps and concomitant extraction of numerous classes of compounds, including emerging compounds (Nieuwenhuize et al., 19991; Aguilar et al, 2014).

3.2 Biomarkers: a promising set of biomonitoring tools

Biomarkers are a common tool in current biomonitoring studies (Rose et al., 2006), and their potential as early warning indicators for contamination effects on higher biological levels is widely acknowledged (Monserrat et al., 2007). Distinct biological responses are used as biomarkers. Amongst those commonly used are the quantification of detoxification enzymes and polypeptides, oxidative stress, DNA damage, mutagenic potential and nuclear abnormalities, immunologic responses, neurotransmission, and body composition (Machado et al., 2013, Machado et al., 2014). Several metabolic pathways in various animal organs are known to be disrupted by environmental pollution, and are therefore potential biomarkers of contamination (Monserrat et al., 2007).

However, a major constrain to the usage of biomarkers is the lack or controversial information about control level of responses as well as concerns regarding dose-response curves for both individual contaminants and mixtures. Additionally, because biomarkers are restricted to the organism level, there is considerable uncertainty about how to extrapolate such metrics to higher levels of biological hierarchy (Brown et al., 2004). Thus, linking the biomolecular, biochemical, cellular, tissue, and organism level biomarkers to population and ecosystem stressors constitute a current challenge for broader biomarkers application.

3.3 Coastal restoration, contamination, and climate change

Climate change modifies the oceanographic conditions of coastal areas, and ultimately alters contamination patterns and influences coastal management. For example, climate change can lead to enhanced volatilization of POPs from primary sources and re-emissions from secondary sources, such as meltwater from glaciers (Breivik et al., 2011; Geisz et al., 2008). Sea level rise and related risks of storm surge, flood, and erosion are already increasing the maintenance costs of hard engineered coastal defences. Often, hard defences are no longer economical, and coastal management policy is now redirected towards more natural and sustainable coastal restorations, such as “managed realignment” (MR), “managed retreat” or “coastal setback” (POSTnote 342). MR denotes deliberately breaching engineered defences to allow the coastline to migrate to a new line of defence landward in response to sea level rise (Andrews et al., 2006). This is currently one of the most common solutions to restore coastal systems in industrialized countries and mitigate effects of climate change and contamination.

3.4 A mitigation tool: Managed realignment

Hard defences provide a false sense of security and encourage urban development in high flood risk zones (Andrews et al., 2006). Additionally, sea walls prevent the natural migration landwards of tidal systems in response to sea level rise. This provokes “coastal squeeze” (Pethic, 2002), compromising environmental services of coastal ecosystems. Therefore, current costal management favours managed realignment, which propitiate the creation of new tidal areas, delivering several environmental services such as:

- Reduction of coastal defences (Andrews et al., 2006);

- Intertidal habitat for biodiversity;
- Environmental quality: Recycling water nutrients and functioning as store of green-house pollutants (CO_2 and CH_4);
- Flood defence: Intertidal zones are natural defences, attenuating wave height and energy, and tidal amplitude (Pethic, 2002);
- Compensation for habitat loss related to coastal squeeze as required by the EU Habitats Directive (Council, 1992);
- Landscape diversity.

Risks of managed realignment

MR practices flood soils that were previously used for various activities and might contain contaminants. In turn, the polluted soils can be eroded by water, and undergo chemical and biological changes, thereby increasing contaminant remobilization. Such remobilization of contaminants from soils to water is problematic because metals bound to sediments are considered mainly not bioavailable. However, dissolved in water they are bioavailable and potentially causing toxicity to aquatic organisms and ecosystems.

Box 2. Managed Realignment Techniques

Ordered according to increasing costs and environmental intervention.

- Do not renew existing hard defences when their functional time is over.
- Remove part of or entire existing sea walls to allow flooding.
- Reallocate coastal defences to higher grounds while minimizing their length.
- Engineer re-created intertidal systems, mudflats, saltmarshes, realigned estuaries, and tidal macro and micro channels.

Coastal restoration and management in Europe

Historic coastal protection favoured hard defences such as sea walls and groynes (Andrews et al., 2006). About half of England’s coastline is currently protected by hard defences (POSTnote 342). New management practices are being adopted in UK and Northern Europe towards managed realignment (Box 2). Important initiatives with potential to change coastal biogeochemistry at national and international levels are:

- Shoreline Management Plans cover the entire coast of England and Wales, favouring the work with natural processes, resulting in coastlines no longer being defended (POSTnote 342).
- Thames 2100 is a shoreline plan to London and the Thames estuary, including adaption to future climate conditions by 2100. It combines re-structuring dikes, resilient defences, and warning and emergency systems for flood protection (Thames 2100, 2015).
- The Netherlands- Delta Management Plan: €100 billion for the next 100 years. It creates intertidal beaches in North Coast, preserves and improves tidal flats south-western (Sheldet), and provides more room for Rhine and Meuse, with estuarine protection and a new gates in the Rhine (Delta Committee, 2015).

Managed realignment and potential contamination

MR can affect sediments and thus, the fate of metals and other sediment-associated contaminants in several ways:

- Changing redox conditions by flooding air-exposed soils (Du Laing et al., 2009).
- Increment of organic matter in flooded sediment due to non-optimal degradation, which enhances risks of organic mobilization (Du Laing et al., 2009).
- Salinity intrusion in freshwater saturated soils might increase desorption.
- Possibility of previously contaminated sediment to be eroded (Bianchi, 2007).
- Creation of a depositional habitat and sink of contaminants (Bianchi, 2007).
- Allowing new interactions with plants and benthic fauna.

3.5 Uncertainties

Climate Change Uncertainty

Climate change is unequivocal, but the extension of oceanographic changes in coasts, as sea level rise (POSTnote 363), climate variability and storms (POSTnote 400), and climate feedbacks (POSTnote 454) are uncertain. All those coastal and oceanographic shifts affect erosion and biogeochemistry, constituting open challenges for coastal pollution, projection, and management.

Political Uncertainty

- MR is still in an experimental phase, and it is not certain how long and how restored tidal marshes will take to function as natural (Andrews et al. 2006).
- Population scepticism and >£150 billion investments in structures along the UK coast pushes to the maintenance of hard defences (Pethic, 2002).
- Some policies as “Make Space for Water” launched in 2005 for England (Defra, 2005) favouring MR are still not fully implemented (POSTnote 362).
- EU Water Framework Directive (WFD, 2015) and Maritime Strategy Framework Directive (MSFD, 2015) for the “good ecological status” aren’t completely implemented.

4. Conclusions

Environmental management and pollution assessment represent complex matters, including analytical chemistry, ecotoxicology, and environmental engineering in a transdisciplinary manner. It involves a wide range of dynamic issues such as global warming, air, water, and sediment pollution, coastal erosion, control of toxic substances and human, biological, and ecological adverse effects. New tools and approaches have been constantly developed and optimised to deal with the constantly emerging challenges of this dynamic theme. Managed realignment might deliver positive outcome if uncertainties and impacts on contaminant remobilization are properly considered. Overall, it is important that new efforts and approaches regarding environmental management and pollution assessment provide multidisciplinary and integrative analyses. This would consequently lead to a better understanding of the extension of anthropogenic impacts on coastal and aquatic systems.

5. References

Aguilar, L., Williams, E. S., Brooks, B.W., Usenko, S., 2014. Development and application of a novel method for high-throughput determination of PCDD/Fs and PCBs in sediments. Environ. Toxicol. Chem. 33, 1529–36.

Andrews, J.E., Burgess, D., Cave, R.R., Coomber, E.G., Jickells, T.D., Parkes, D.J., Turner, R.K. 2006. Biogeochemical value of managed realignment, Humber estuary, UK. Sci. Tot. Env. 371, 19-30.

Argiriadis, E., Rada, E.C., Vecchiato, M., Zambon, S., Ionescu, G., Schiavon, M., Ragazzi, M., Gambaro, A., 2014. Assessing the influence of local sources on POPs in atmospheric depositions and sediments near Trento (Italy). Atmos. Environ. 98, 32–40.

Bianchi, T.S. 2007. Biogeochemistry of Estuaries. Oxford University Press, New York.

Binelli, A., Sarkar, S.K., Chatterjee, M., Riva, C., Parolini, M., Bhattacharya, B., 2009. Congener profiles of polychlorinated biphenyls in core sediments of Sunderban mangrove wetland (NE India) and their ecotoxicological significance. Environ. Monit. Assess. 153, 221–234.

Borgå, K., Di Guardo, A., 2005. Comparing measured and predicted PCB concentrations in Arctic seawater and marine biota. Sci. Total Environ. 342, 281–300.

Borgå, K., Gabrielsen, G.W., Skaare, J.U., 2001. Biomagnification of organochlorines along a Barents Sea food chain. Environ. Pollut. 113, 187–198.

Bortone, S.A. 2005. Estuarine Indicators. Florida, CRC Press.

Breivik, K., Gioia, R., Chakraborty, P., Zhang, G., Jones, K.C., 2011. Are reductions in industrial organic contaminants emissions in rich countries achieved partly by export of toxic wastes? Environ. Sci. Technol. 45, 9154–60.

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2002. Towards a global historical emission inventory for selected PCB congeners - a mass balance approach 2. Emissions. Sci. Total Environ. 290, 296–307.

Brown, R.J., Galloway, T.S., Lowe, D., Browne, M.A., Dissanayake, A., Jones, M.B., Depledge, M.H. 2004. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat. Toxicol., 66:267–278

Combi, T., Taniguchi, S., Lima Ferreira, P.A., Mansur, A.V., Figueira, R.C.L., Mahiques, M.M., Montone, R.C., Martins, C.C., 2013. Sources and Temporal Patterns of Polychlorinated Biphenyls Around a Large South American Grain-Shipping Port (Paranaguá Estuarine System, Brazil). Arch. Environ. Contam. Toxicol. 64, 573–582.

Council. 1992. Council Directive 92/43/EEC of May 1992 on the conservation of natural habitats and wild fauna and flora. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31992L0043&from=EN

Defra. 2005. Making space for water: Taking forward a new government strategy for flood and coastal erosion risk management in England. http://coastaladaptationresources.org/PDF-files/1329-Making-space-for-water.pdf

Delta Committee (accessed in July 2015): http://www.deltacommissie.com/en/film

de Souza, A.S., Torres, J.P.M., Meire, R.O., Neves, R.C., Couri, M.S., Serejo, C.S., 2008. Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in sediments and crabs (*Chasmagnathus granulata*, Dana, 1851) from mangroves of Guanabara Bay, Rio de Janeiro State, Brazil. Chemosphere 73, S186–92.

Du Laing, G., Meers, E., Dewispelaere, M., Rinklebe, J., Vandecasteele, B., Verloo, M.G., Tack, F.M.G. 2009. Effect of water table level on metal mobility at different depths in wetland soils of the Scheldt Estuary (Belgium). Water Air Soil Poll. 202, 353–357.

EU Water Framework Directive (WFD) (accessed in July 2015): http://ec.europa.eu/environment/water/water-framework/index_en.html

Förstner U., Wittmann G.T.W. 1979. Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin Heidelberg New York.

Geisz, H.N., Dickhut, R.M., Cochran, M. A., Fraser, W.R., Ducklow, H.W., 2008. Melting Glaciers: A Probable Source of DDT to the Antarctic Marine Ecosystem. Environ. Sci. Technol. 42, 3958–3962.

Hong, S.H., Yim, U.H., Shim, W.J., Oh, J.R., Lee, I.S., 2003. Horizontal and vertical distribution of PCBs and chlorinated pesticides in sediments from Masan Bay, Korea. Mar. Pollut. Bull. 46, 244–53.

John Pethic. 2002. Estuarine and Tidal Wetland Restoration in the United Kingdom: Policy Versus Practice. Rest. Ecol. 10, 431-437.

Kanzari, F., Syakti, a D., Asia, L., Malleret, L., Piram, a, Mille, G., Doumenq, P., 2014. Distributions and sources of persistent organic pollutants (aliphatic hydrocarbons, PAHs, PCBs and pesticides) in surface sediments of an industrialized urban river (Huveaune), France. Sci. Total Environ. 478, 141–51.

Kennish, M.J. 1991. Ecology of Estuaries: Anthropogenic Effects. Florida, CRC Press, 494p.

Koh, C.-H., Khim, J.S., Villeneuve, D.L., Kannan, K., Giesy, J.P., 2006. Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 1. Instrumental analyses. Environ. Pollut. 142, 39–47.

Lara-Martín, P. a, González-Mazo, E., Brownawell, B.J., 2011. Multi-residue method for the analysis of synthetic surfactants and their degradation metabolites in aquatic systems by liquid chromatography-time-of-flight-mass spectrometry. J. Chromatogr. A 1218, 4799–807.

Litskas, V.D., Dosis, I.G., Karamanlis, X.N., Kamarianos, a P., 2012. Occurrence of priority organic pollutants in Strymon river catchment, Greece: inland, transitional, and coastal waters. Environ. Sci. Pollut. Res. Int.

Liu, H., Zhang, Q., Cai, Z., Li, A., Wang, Y., Jiang, G., 2006. Separation of polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzo-furans in environmental samples using silica gel and florisil fractionation chromatography. Anal. Chim. Acta 557, 314–320.

Machado, A.A.S., Hoff, M.L.M., Klein, R.D., Cardozo, J.G., Giacomin, M.M., Pinho, G.L.L., Bianchini, A. 2013. Biomarkers of waterborne copper exposure in the guppy Poecilia vivipara acclimated to salt water. Aquat. toxicol. 138-139, 60–69.

Machado, A.A.S., Wood, C.M., Bianchini, A., Gillis, P.A. 2014. Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. Ecotoxicology. 23, 1345–1358.

Mahmood, A., Malik, R.N., Li, J., Zhang, G., 2014. Levels, distribution profile, and risk assessment of polychlorinated biphenyls (PCBs) in water and sediment from two tributaries of the River Chenab, Pakistan. Environ. Sci. Pollut. Res. Int. 21, 7847–55.

Marine Strategy Framework Directive (MSFD) (accessed in July 2015): http://ec.europa.eu/environment/marine/eu-coast-and-marine-policy/marine-strategy-framework-directive/index_en.htm

Martins, C.C., Bícego, M.C., Rose, N.L., Taniguchi, S., Lourenço, R. a., Figueira, R.C.L., Mahiques, M.M., Montone, R.C., 2010. Historical record of polycyclic aromatic hydrocarbons (PAHs) and spheroidal carbonaceous particles (SCPs) in marine sediment cores from Admiralty Bay, King George Island, Antarctica. Environ. Pollut. 158, 192–200.

Monserrat, J.M., Martinez, J.M., Geracitano, L.A., Amado, L.L., Martins, C.M.G., Pinho, G.L.L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J., Bianchini, A. 2007. Pollution biomarkers in estuarine animals: Critical review and new perspectives. Comp. Biochem. Physiol. C. 146, 221–234.

Montuori, P., Cirillo, T., Fasano, E., Nardone, A., Esposito, F., Triassi, M., 2014. Spatial distribution and partitioning of polychlorinated biphenyl and organochlorine pesticide in water and sediment from Sarno River and Estuary, southern Italy. Environ. Sci. Pollut. Res. Int. 21, 5023–35.

Neto, J.A.B., Wallner-Kersanach, M., Patchineelam, S.M. 2008. Poluição Marinha. Interciência, Rio de Janeiro.

Nieuwenhuize, J., Poley-Vos, C. H., van den Akker, A.H., van Delft, W. 1991 Comparison of microwave and conventional extraction techniques for the determination of metals in soil, sediments and sludge samples by atomic spectrometry. Analyst. 116, 347–351.

Nriagu, J.O.; Pacyna, J.M. 1998. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature. 333, 134–139.

Parolini, M., Binelli, A., Matozzo, V., Marin, M.G., 2010. Persistent organic pollutants in sediments from the Lagoon of Venice - a possible hazard for sediment-dwelling organisms. J. Soils Sediments. 10, 1362–1379.

Pethick, J. 2002. Estuarine and tidal wetland restoration in the Uneted Kingdom: Policy versus practice. Rest. Ecol. 10, 431–437.

Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A., 2013. Environmentally friendly analysis of emerging contaminants by pressurized hot water extraction-stir bar sorptive extraction-derivatization and gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 405, 401–11.

Pozo, K., Harner, T., Wania, F., Muir, D.C.G., Jones, K.C., Barrie, L.A., 2006. Toward a global network for persistent organic pollutants in air: Results from the GAPS study. Environ. Sci. Technol. 40, 4867–4873.

Postnote 342. 2009. Coastal management. Parlamentary Office of Science and Technology. Houses of Parliament, London, UK.

Postnote 362. 2010. Resilience of UK infrastructure. Parlamentary Office of Science and Technology. Houses of Parliament, London, UK.

Postnote 363. 2010. Sea level rise. Parlamentary Office of Science and Technology. Houses of Parliament, London, UK.

Postnote 400. 2012. Climate variability and weather. Parlamentary Office of Science and Technology. Houses of Parliament, London, UK.

Postnote 454. 2014. Risks from climate feedbacks. Parlamentary Office of Science and Technology. Houses of Parliament, London, UK.

Qiu, C., Cai, M., 2010. Ultra trace analysis of 17 organochlorine pesticides in water samples from the Arctic based on the combination of solid-phase extraction and headspace solid-phase microextraction-gas chromatography-electron-capture detector. J. Chromatogr. A. 1217, 1191–1202.

Robles-Molina, J., Lara-Ortega, F.J., Gilbert-López, B., García-Reyes, J.F., Molina-Díaz, A., 2014. Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry. J. Chromatogr. A. 1350, 30–43.

Rose, W.L., Nisbet, R.M., Green, P.G., Norris, S., Fan, T., Smith, E.H., Cherr, G.N., Anderson, S.L. 2006. Unsing an integratred approach to link biomarker and physiological stress to growth impairment of cadmium-exposed larval topsmelt. Aquat. Toxicol. 80, 298–308.

Ruiz-Fernández, A.C., Sprovieri, M., Piazza, R., Frignani, M., Sanchez-Cabeza, J.-A., Feo, M.L., Bellucci, L.G., Vecchiato, M., Pérez-Bernal, L.H., Páez-Osuna, F., 2012. 210Pb-derived history of PAH and PCB accumulation in sediments of a tropical inner lagoon (Las Matas, Gulf of Mexico) near a major oil refinery. Geochim. Cosmochim. Acta. 82, 136–153.

Sahu, S.K., Ajmal, P.Y., Pandit, G.G., Puranik, V.D., 2009. Vertical distribution of polychlorinated biphenyl congeners in sediment core fromThane Creek area of Mumbai, India. J. Hazard. Mater. 164, 1573–1579.

Sandrin, T.R., Maier, R.M., 2003. Impact of Metals on the Biodegradation of Organic Pollutants. Environ. Health Perspect. 111, 1093–1101.

Seeliger, U., Lacerda, L.D., Patchineelam, S.R. 1988. Metals in Coastal Environments of Latin America. Berlin, Springer-Verlag, Heidellberg.

Sodré, F.F., Locatelli, M.A.F., Jardim, W.F., 2010. Occurrence of emerging contaminants in Brazilian drinking waters: A sewage-to-tap issue. Water Air Soil Pollut. 206, 57–67.

Sterzenbach, D., Wenclawiak, B.W., Weigelt, V., 1997. Determination of Chlorinated Hydrocarbons in Marine Sediments at the Part-per-Trillion Level with Supercritical Fluid Extraction. Anal. Chem. 69, 831–836.

Thames 2100 (accessed in July 2015): https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/322061/LIT7540_43858f.pdf

Vallack, H.W., Bakker, D.J., Brandt, I., Brostro, E., Brouwer, A., Bull, K.R., Gough, C., Guardans, R., Holoubek, I., Mccutcheon, P., Mocarelli, P., Taalman, R.D.F., 1998. Controlling persistent organic pollutants – what next ? Environ. Toxicol. Pharmacol. 6, 143–175.

Zhang, P., Ge, L., Zhou, C., Yao, Z., 2011. Evaluating the performances of accelerated-solvent extraction, microwave-assisted extraction, and ultrasonic-assisted extraction for determining PCBs, HCHs and DDTs in sediments. Chinese J. Oceanol. Limnol. 29, 1103–1112.

Zagatto, P.A. 2006. Ecotoxicologia Aquática: Princípios e Aplicações. São Carlos, Ed. RiMa.

PART 2

The problematic of metal pollution in estuaries

CHAPTER III

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Perhaps one of the most studied and still problematic issues in terms of coastal and marine pollution is metal contamination in estuaries. These transitional systems often concentrate metals due to several physical, chemical and biological properties intrinsic to the estuarine function. Therefore, estuarine processes affecting the distribution and effect of metals might be complex and multidisciplinary in nature. Chapter three presents a transdisciplinary review on the fate and effects of metals in estuaries.

Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity

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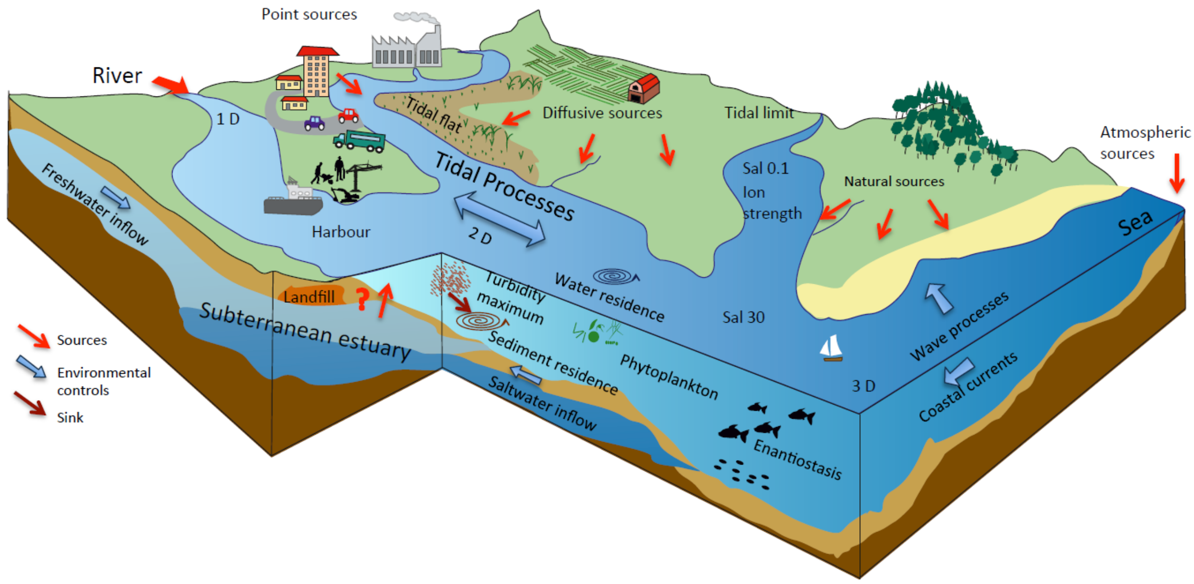
Visual Abstract



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Abstract

Metal pollution is a global problem in estuaries due to the legacy of historic contamination and the currently increasing metal emissions. However, the establishment of water and sediment standards or management actions in brackish systems has been difficult because of the inherent transdisciplinary nature of estuarine processes. According to the European Commission, integrative comprehension of fate and effects of contaminants in different compartments of these transitional environments (estuarine sediment, water, biota) is still required to better establish, assess and monitor the good ecological status targeted by the Water Framework Directive. Thus, the present study proposes a holistic overview and conceptual model for the environmental fate of metals and their toxicity effects on aquatic organisms in estuaries. This includes the analysis and integration of biogeochemical processes and parameters, metal chemistry and organism physiology. Sources of particulate and dissolved metal, hydrodynamics, water chemistry, and mechanisms of toxicity are discussed jointly in a multidisciplinary manner. It is also hypothesized how these different drivers of metal behaviour might interact and affect metal concentrations in diverse media, and the knowledge gaps and remaining research challenges are pointed. Ultimately, estuarine physicochemical gradients, biogeochemical processes, and organism physiology are jointly coordinating the fate and potential effects of metals in estuaries, and both realistic model approaches and attempts to postulate site-specific water criteria or water/sediment standards must consider such interdisciplinary interactions.

Keywords: metal fate, biogeochemistry, estuarine processes, organism physiology, metal toxicity.

1 Introduction

Estuaries are defined as water bodies that connect land and ocean and extend from fully marine conditions to the effective limit of tidal influence, and where seawater is diluted by freshwater inflow (Hobbie, 2000). These environments have traditionally been zones of intense human occupation. They provide a multitude of ecosystem services such as drinking freshwater supply, fisheries, climate regulation, sheltered access to coastal water, coastal protection, water purification and waste treatment (Millennium Ecosystem Assessment, 2005). Estuaries also serve as nursery areas for several species, provide habitat to a high diversity of organisms for the whole or part of their life cycle, and are characterized by a high biological productivity. However, estuaries have also been used for the dilution and disposal of waste worldwide (Kennish et al. 1991; Spencer et al. 2006b) which contributes to their deterioration.

In this context, trace metals are pollutants of concern (Kinne, 1984; Nemerow, 1991; Fairbrother et al. 2007). Although metals are naturally ubiquitous in aquatic systems (usually within µg.L^{-1}-range in surface waters), they are increasingly present as a result of anthropogenic activities. Förstner and Wittman (1979) concluded that the world's six most heavily polluted aquatic environments by trace metals are estuaries. In countries with long historic industrialization, such as United Kingdom, Germany and Netherlands, thousands of tons of metals were systematically deposited in the estuarine and coastal areas (Förstner and Wittman, 1979). This legacy of contamination is currently aggravated via freshwater input, increasing urbanization and discharges of domestic effluents, industry, fossil fuel burning, mining, groundwater use, surface runoff and soil erosion, and mobilization of historic contaminated sediment (Phillips, 1980; Heath, 1995; Deboudt et al. 2004; Paytan et al. 2009; Bai et al. 2015). Additionally, these contaminants are persistent in the environment, and all metals are potentially bioavailable and toxic to aquatic biota at high concentrations (Kennish et al.1991; Wood et al. 2012a, b; Machado et al. 2014b).

Integrative models of behaviour and threats of metals are required to better set environmental quality standards and goals. Currently, North American scientists put significant effort into better understanding metal transport, mobilization and toxicity and derive scientifically defensible site-specific water quality criteria for metals in salt water environments – as has already been successfully established in freshwater (Paquin et al. 2003). Whilst in Europe, a lack of knowledge of the ecological status and function of transitional waters is hindering the setting of standard baselines with consequences for the implementation of the Water Framework Directive (European Commission, 2012b). In both cases, it is relevant to ensure that management is based on a better understanding of the main risks and pressures on these systems (Elliotta and McLusky, 2002; Millennium Ecosystem Assessment, 2005; European Commision, 2012b). At best of our knowledge, such an integrated model is still lacking within the literature. Therefore, it is fundamental to discuss metal fate and consequences for their toxicity in a holistic and systematic manner.

The aim of this work is to propose a conceptual model for the distribution, environmental fate, and toxicity of metals in estuaries, including environmental dynamics, metal chemistry and organism physiology. In a first step, the estuarine features important for the environmental dynamics of metal fate are described (section 3). Secondly, the main biogeochemical processes by which these features affect metal transport, distribution and partitioning concentrations in the different estuarine sub-compartments are introduced (sections 4, 5 and 6). Finally, the organism physiology and metal toxicity under estuarine conditions are discussed (section 7), enabling a unique transdisciplinary overview of the threats of these chemical elements in estuarine environments.

2 Scope and terminology

The physiography of estuaries has been shown to vary widely impacting physical mixing processes and consequently biogeochemistry (Bianchi, 2007). The present study thus focuses on river-dominated or tidal-river estuaries. Details on processes that are characteristic for metal behaviour and effects in estuaries were identified from scientific literature and are discussed from an ecotoxicological point of view. A broad range of information sources, which include peer-reviewed scientific articles, specified books, technical articles, and online information provided by environmental protection agencies have been considered. Notwithstanding, estuarine functioning and metal literature is extensive, and readers interested in more specific details beyond the scope of this study are encouraged to refer to further reviews (Drexler et al. 2003; Pasquin et al. 2003; Bianchi, 2007; Fairbrother et al. 2007; Monserrat et al. 2007; Wood et al. 2012a,b). Likewise, in the mosaic of gradients typical of estuaries, the relative importance of different variables and processes might vary several orders of magnitude in time and space. Quantifying the extent of such variation at whole estuary scales constitutes an open research question, and is not deeply discussed in this review.

2.1 Terminology

In aquatic systems metals are known to be simultaneously present as different chemical species (Fairbrother et al. 2007; Gonzalez et al. 2007). For the purpose of this work ‘total metal’ is defined as the concentration of metal measurable in water or in sediment after strong acid digestion, including metal precipitates, metals associated with the mineral lattice, adsorbed to sediment, adsorbed to and absorbed into particulate organic matter and dissolved metals (Förstner and Wittman, 1979). Dissolved metal is defined as the fraction of total metal that passes through 0.45 µm filter, including metal-organic matter complexes, colloids, inorganic complexes, dissolved gases and free ion forms (Bianchi, 2007; Du Laing et al. 2009a). ‘Non-filterable metal’ is the fraction of total metal that does not pass the 0.45 µm filter. In turn, ‘particulate metal’ is here referred to as the portion of non-filterable metal that is relatively mobile to the dissolved phase, measurable in water after acid strong extraction. It includes metal in inorganic precipitated or co-precipitated form (e.g. carbonates and hydrous Fe-Mn oxides), and adsorbed to sediment and organic matter particles.

‘Bioaccumulated metal’ describes the metal fraction internalized (from outside to inside through membrane or tissue) by organisms, while ‘bioavailable metal’ includes metal species that are bioaccessible and have the potential for distribution, metabolism, elimination, and bioaccumulation by a given organism (Drexler et al. 2003). The term ‘effect of metal’ on organisms denotes deleterious consequences of metal exposure, while the term ‘responses’ points to a more generic physiological alteration due to metal exposure (Machado et al. 2013).

3 The conceptual model of metal fate and effects in estuaries

In aquatic systems, potentially toxic metals are transported in dissolved or particulate form, where sediment and suspended particles play important roles in metal adsorption, desorption and dissolution, and sedimentation processes (Boyle et al. 1974; Gonzalez et al. 2007). Solubilisation, speciation, precipitation as well as diffusion and advection are also critical processes that determine metal spatial distribution (Benoit et al. 1994). All these processes are influenced by physical, chemical and biological parameters in estuaries. Thus, estuarine circulation, river and groundwater discharge, tidal flooding, sediment input and re-suspension, exchange with neighbouring environments, water properties (e.g. salinity, redox and pH) and the presence of organisms jointly determine the mobility of metals. The consequence of such interactions is often metal accumulation in estuaries (Förstner and Wittman, 1979), turning the estuarine fate of metals into a complex dynamics with interdisciplinary nature.

Moreover, metal concentrations and environmental conditions jointly determine organism physiology and toxicity (Monserrat et al. 2007). Indeed, several parameters act on metal behaviour and toxicity simultaneously. Salinity, for example, affects flocculation and hence sedimentation of fine particulate matter (Bianchi, 2007), metal speciation (Drexler et al. 2003), and animal physiology (Martins and Bianchini, 2009) at the same time. Although studies regarding metal fate and toxicity in estuarine conditions exist (Falconer and Lin, 1997; Baeyens et al. 1998; Mao et al. 2006; Trento and Alvarez, 2011; Mwanuzi and De Smedt, 1999; Liu et al. 2007; Di Toro et al. 2001), very few attempts have been made to consider the system in a broader context and to integrate physical, chemical and biological processes simultaneously (Paquin et al. 2003). Thus, the interacting aspects of metal fate in estuaries remain widely unexplored.

3.1 Physico-chemical estuarine features: Environmental gradients and non-conservative metal behaviour

Estuarine waters are characterized by strong physicochemical gradients in e.g. salinity, density, flow velocity, and suspended matter composition (Elliotta and McLusky, 2002), which are important influences on the fate of metals (Fig.1). The interaction of these environmental gradients and metal sources in estuaries normally yields a non-conservative behaviour for the majority of metal elements. Oceanic waters usually have higher salinity and lower trace metal concentrations than inland freshwaters. Thus, metals are interpreted to behave conservatively if concentrations decrease linearly with salinity increase (Boyle et al. 1974). A model from Boyle et al. (1974) predicted Fe as highly non-conservative in Merrimack Estuary due to mobilization processes discussed later. Indeed, environmental partitioning coefficients of Cd, Cu and Pb and other metals are observed to vary non-linearly under different estuarine conditions (e.g. salinity, suspended matter, chlorophyll), which confirms the non-conservative behaviour (Valenta et al. 1986; Benoit et al. 1994; Spencer and MacLeod, 2002; Wang et al. 2009).

Another important estuarine feature is river discharge, which affects mixing processes, the salinity gradient and the input of dissolved organic matter, suspended material, and phytoplankton (Liu et al. 2007; Couceiro et al. 2009; Falconer and Lin, 1997; Van der Berg et al. 2001). The input of freshwater (low density) and saltwater (high density) typically generate the estuarine circulation schematized in figure 1, with vertical salinity gradients and freshwater flushing out mainly at the surface (Kundu and Cohen, 2004). Deviations from this pattern are not unusual and well or partially mixed estuaries are commonly reported (Bianchi, 2007). Consequently, river input, tidal flows and coastal processes interact with estuarine physiography, to determine the salinity distribution within the estuary (Valle-Levinson, 2010).

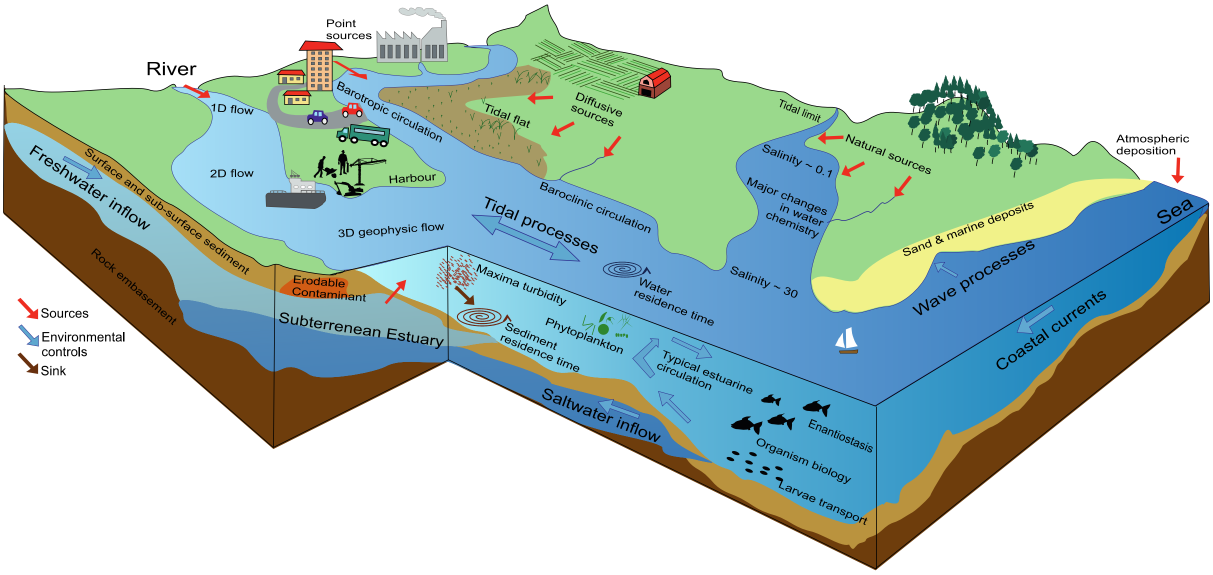

Figure 1: Conceptual model of physical, chemical and biological variables and processes for behaviour and toxicity of metals. Adapted from Bianchi (2007).

The salinity gradient is one of the main estuarine characteristics responsible for non-conservative metal behaviour. Salinity results in several major changes in water chemistry and ionic strength (Benoit et al. 1994), accounting for metal mobilization (sections 4-5). Salinity also significantly enhances the water pH buffering capacity due to high concentrations of carbonate and bicarbonate ions, such that pH often increases to slightly basic conditions (~8.08-8.33) along the estuary transect in a seawards direction. Therefore, pH is expected to have a greater influence on metal behaviour at lower salinities.

Moreover, differences in salinity induce a water density gradient within the estuary that is strong enough to affect water circulation. Thus, at very low salinities the flow is mainly barotropic, e.g. dictated by relative gradient in water column elevation. However at higher salinity ranges the flow is also baroclinic, e.g. influenced by pressure caused by distinct water densities. The misalignment of barotropic and baroclinic gradients also generates vorticity increasing turbulent mixing processes and potentially metal solubilisation (Kundu and Cohen, 2004).

An important estuarine feature resulting from above discussed riverine discharge and salinity gradient is the maximum turbidity zone (Fig. 1). Maximum turbidity zones are one or more estuarine regions with high concentrations of aggregates of particles and colloids from river and other multiple sources formed by physical dynamics of sediment supply and settling velocity, tidal mixing and estuarine stratification (Hobbie, 2000). Change in ionic strength of water is known to be one of the main processes responsible for the formation of an estuarine maximum turbidity zone (Valenta et al. 1986; Bianchi 2007). In fact in many estuaries, the maximum turbidity is found at the salinity front, usually less than 5 psu (practical salinity units; Jassby et al. 1995; Lanceleur et al. 2013). Very high turbidity zones might also be consequence of dredging activities (Van den Berg et al. 2001; Spencer et al. 2006a). The strong gradient in suspended matter alters physical and chemical mobilization of metal, as discussed later for adsorption and precipitation. In addition, maximum turbidity is often a hotspot for microbial metabolic processes, and has major biogeochemical impacts on organic matter of estuaries.

In terms of metal contamination, special focus has also to be put on the subterranean estuary, in which gradients that affect metal mobilization also occur. In a typical unconfined subterranean estuarine system saltwater intrusion occurs from sea, while freshwater flows from continental side (Fig. 1). The location of the salinity front in these underground systems depends upon freshwater inflow and sea level, i.e. the hydraulic gradient between the aquifer and the sea (average sea level, tides, surges) (Moore, 1999; Fleury et al. 2007), and also upon the geological structure, sediment permeability and hydraulic conductivity (Bianchi, 2007).

3.2 Sources and emission pathways of metals to estuaries

The main sources of metal to estuarine systems are usually riverine particulate and dissolved metal, point (harbour activities, urban centre and runoff, and sewage and industrial effluents) and diffuse natural and anthropogenic sources (runoff from road surface, and agricultural landscapes), atmospheric deposition, and groundwater enrichment (Förstner and Wittman, 1979; Nemerow, 1991) (Fig. 1). According to Zwolsman et al. (1993) riverine input of Cd, Cu, Pb and Zn to tidal flats of Scheldt estuary was significantly larger (97.5% to 99.5%) than atmospheric deposition (2.5% to 0.5%). Notwithstanding, Deboudt et al. (2004) added that atmospheric-derived metals might be dominant on the coast, thus potentially affecting metal in the lower estuary. Indeed, atmospheric wet deposition might account for 20-70% of total metal input to coastal areas in Western Europe (Deboudt et al. 2004).

Since groundwater inflow is usually orders of magnitude lower than surface discharge, subterranean metal input was often considered to be negligible. In fact, some models have represented trace metals in estuaries by only considering riverine sources (Boyle et al. 1974; Falconer and Lin, 1997), assuming groundwater accounting for less than 1% of metals discharged (Förstner and Wittman 1979). However, more recently the term subterranean estuary has gained relevance for estuarine and coastal biogeochemistry (Moore, 1999). Bone et al. (2007) demonstrated the role of groundwater as the major source of Hg in the Waquoit Bay-Massachusetts (0.47-1.9 nmol Hg $m^{-2}day^{-1}$). Although the volume of groundwater advection to the surface water might be low compared to the river-derived flow, percolation through sediments that can normally store metals up to 100.000 more than surface waters increases proportionally metal concentrations in groundwater (Förstner and Wittman, 1979), and results in significant mass transfer (Bone et al. 2007). Also, the flow in subterranean estuaries changes at different temporal scales than their river-fed counterparts and coastal circulation. Such subterranean variation in freshwater-saltwater interface changes the vertical location of the pycnocline and, subsequently the flow paths of solutes (Mao et al. 2006). This implies that the relative importance of surface and groundwater metal sources may alternate.

An additional potential source of metal to both surface and subterranean estuaries is the subsurface of contaminated sediments and landfills, which are common in industrialized countries. The leachate of coastal landfills can carry metals to the neighbouring groundwater and eventually surface waters. Leachate contamination is an emerging threat in historic deposits challenged by sea level rise and consequent coastal erosion and coastal hydrodynamics shifts (Spencer and O'Shea, 2014), groundwater salinization (Bone et al. 2007), presence of colloids and organic matter, and low pH (Jensen et al. 1999; Sañudo-Wilhelmy et al. 2002), all common stressors in estuarine landfills.

4 Physical processes: metal transport in estuaries

4.1 Changes in flow affecting metal distribution

At the riverine end, water flow and sediment transport are primarily determined by barotropic gradients, i.e. flow velocities are proportional to gradients of water surface elevation. Thus, the resulting metal transport is mainly one-dimensional along the river course on a large (several km) scale (Fig. 2). As water flows into the part of the estuary that is more affected by tidal flow, the mass transport tends to occur in two directions (Geyer and MacCready, 2014), where either vertical or horizontal stratification occurs (Kundu and Cohen, 2004). This bi-directional stratified flow is usually associated with gradients of salinity and acceleration of particles transported in the water column, which affect sedimentation, partitioning and metal mobilization, and ultimately the transport of metals in the water column (Wu et al. 2005). Moreover, the density stratification produced by salinity gradients affects turbulence. Salt wedge intrusion and vertical stratification may reduce turbulent mixing by increasing water column stability, i.e. higher Brunt-Väisälä frequency (Kundu and Cohen, 2004). Differently, horizontal salinity stratification might increase mixing due to misalignment of baroclinic and barotropic forces. Both processes potentially affect redistribution of metals within the water column because stronger turbulence causes disaggregation of large cohesive flocs by means of more frequent collisions, therefore enhancing metal transport and dissolution.

In the lower estuary, circulation might be reasonably described by considering three-dimensional hydrodynamics only (Fig. 2). Estuaries are typically funnel-shaped, so the horizontal scales can increase several orders of magnitude moving seaward with respect to the upstream river, causing a reduction in the Rossby number (quotient between velocity and the product of the horizontal scale of movement and Earth’s rotational velocity) (Azevedo et al. 2008). As a consequence, a higher influence of Earth’s rotation in the circulation of low velocity and large horizontal scale flows is observed at high latitudes. Thus, in the most coastal estuary the flow is geophysical, and the circulation interacts with Coriolis acceleration influencing the dispersion of estuarine contaminant plumes (Falconer and Lin, 1997). In large, open estuaries this effect determines the location of preferential sediment-associated metal accumulation both within and outside the estuarine system (Bianchi, 2007). The resultant transport, also known as Ekman transport, deviates to the right or the left (north or south hemisphere, respectively) of the predominant flow direction (Kundu and Cohen, 2004), and can produce regions of upwelling and downwelling along the coast and within the estuary.

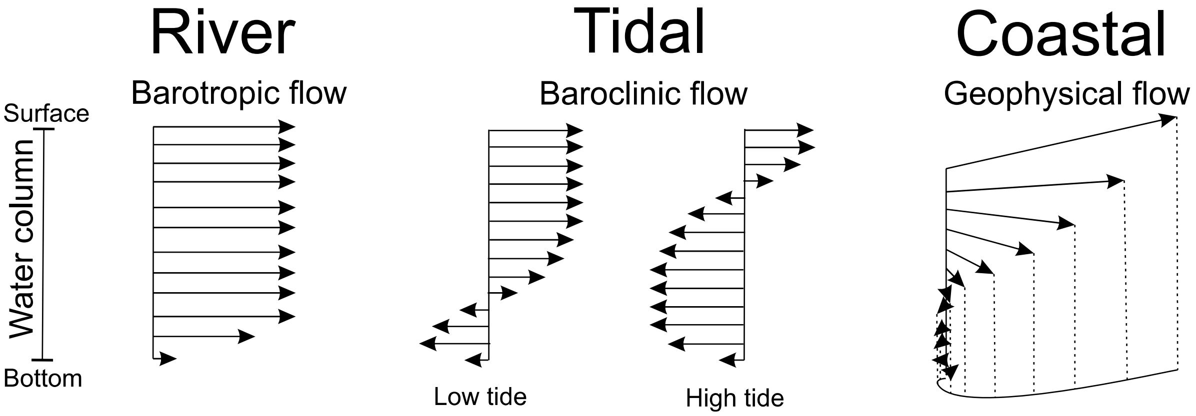

Figure 2: Comparison of river, tidal and coastal processes determining estuarine circulation, and mass transport of dissolved and particulate metal. Note: bi-directional tidal flow might occur either vertically, horizontally, or both.

Due to longitudinal salinity gradients other transport mechanisms can be identified in estuaries in addition to gravitational mixing. Among these tidal pumping can be observed which is associated with residual circulation that results from the presence of tidal loops with ebb- and flood-dominated channels. Also tidal trapping is a transport mechanism whereby the asymmetric flow over lateral shallower areas and the effect of halophytic vegetation determine a residual particle transport (Savenije, 2012). These processes are usually summarized in the one-dimensional concept of tidal dispersion, a process describing the increased longitudinal dispersion of tracers along the estuary (Geyer and MacCready, 2014).

Ultimately, the interaction of river flow and tidal cycles generates a rotational circulation that increases water and sediment residence times within the estuary (Valenta et al. 1986; Kundu and Cohen, 2004). The respective extent varies strongly with tidal amplitude and frequency, estuary geomorphology and riverine discharge, and usually results in the accumulation of particulate metal within the estuary and buffering metal input to marine coastal systems.

4.2 Changes in flow that affect metal concentration and partitioning

Not considering marine processes outside the estuary, low river discharge is usually associated with low suspended particulate matter input into the estuary, resulting in lower levels of particulate metals (Falconer and Lin, 1997; Wang et al. 2009). Under low fluvial discharge salinity increases due to higher relative contribution of seawater within the estuary. This causes dissolution and desorption of metals from particulate matter (Fig. 3, see section 4.3 for sorption) (Mwanuzi and Smedt, 1999). Increasing river discharge, in contrast, implies higher suspended material input (Couceiro et al. 2009), potential for re-suspension of settled particles (Savenije, 2012), and decreased salinity. This can intensify adsorption and precipitation processes, resulting in a maximum of particulate and a minimum of dissolved metal in the water column (Fig. 3). However, with higher riverine discharge the riverine influence is larger throughout the estuary and the flow pattern is modified such that the relative importance of tidal processes is decreased (Cai et al. 2014). This can trigger a reduction in water and particle residence times including flushing of contaminants out of the estuary. In such a situation, smaller fractions of adsorbed metal might also occur due to the increment of coarse material with lower sorptive capacity, while increasing river turbulence might promote the solubility of some metal precipitates (Förstner and Wittmann, 1978), i.e. these are processes contributing to an increase in the relative importance of the dissolved fraction (Benoit et al. 1994). Also at very high concentrations of suspended material the dissolved metal correlates with colloid concentrations, which is ultimately dependent on total suspended matter concentration (Benoit et al. 1994). Although major differences in dissolved and particulate metal concentrations have been reported as varying with discharge, the general effect is often only a slight reduction in total water metal concentration (Förstner and Wittmann, 1978; Wang et al. 2009). The extent of the influence of these variables and processes at whole estuary scales remains yet to be investigated.

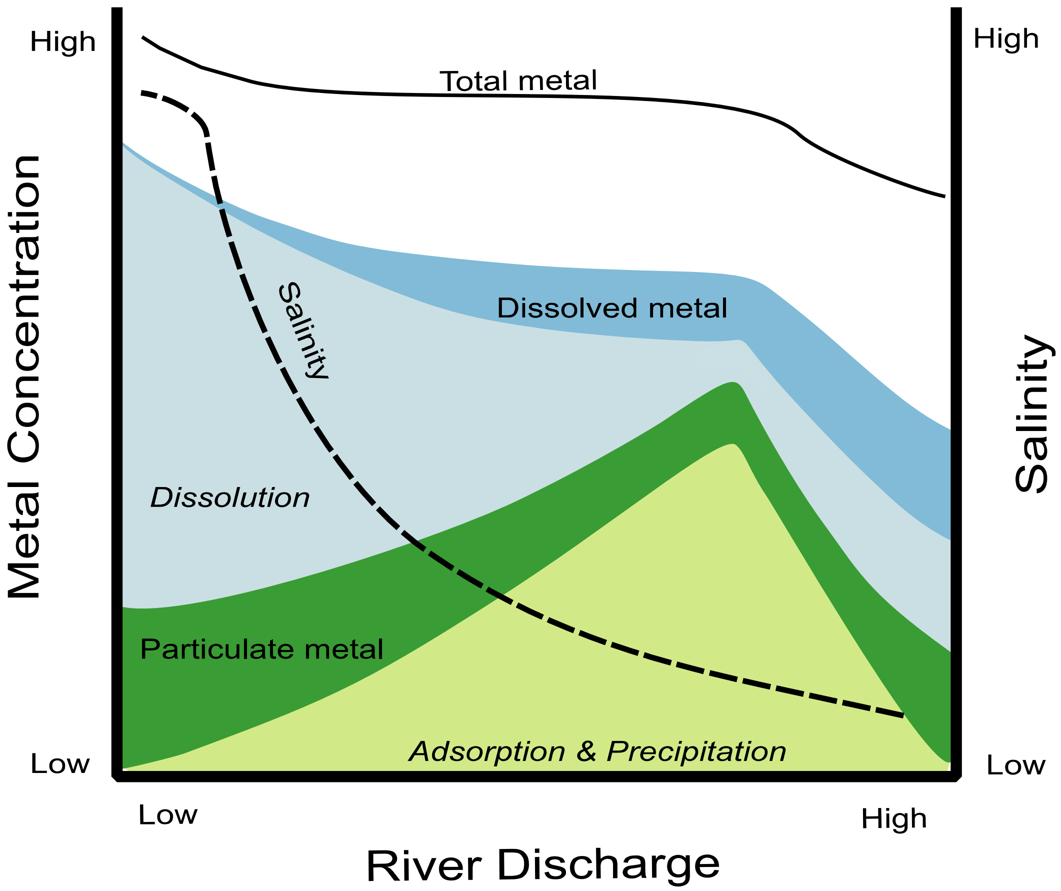

Figure 3: Conceptual river discharge effects on particulate (dark green area), dissolved (dark blue area) and total metal (continuous line) concentrations, as well as on dissolution (light blue area), adsorption and precipitation (light green area) processes. The sketch is based on Wilson model (1976) for river discharge and metal concentration, and modified to address metal interaction with salinity (dashed line) under estuarine conditions.

4.3 Sorption and desorption: The physico-chemical interactions of sorbents and salinity

The absolute distribution of metals between the particulate and the dissolved phase predominantly depends on the availability of sorbents, and results from the operation of surface forces, i.e. usually the attraction of negatively charged metal ions to positively charged surfaces (Förstner and Wittmaann, 1979). Consequently, highest concentrations of suspended particles and total and particulate metal concentrations are found within the maximum turbidity zone, where dissolved metal concentrations are low (Gonzalez et al. 2007), as observed in environmental samples of maximum turbidity for Ag (Lanceleur et al. 2013), Cu, Cd, Pb and Zn (Van den Berg et al. 2001; Müller and Förstner, 1975). Higher sorption, and consequently higher metal inventories and potential to water purification services are also expected in tidal flats or estuarine areas of deposition of finer sediments (Zwolsman et al. 1993).

The partitioning of dissolved metal phases is also affected by salinity (Förstner and Wittmann, 1979). Metal desorption from sediments and suspended matter increases with increasing salinity (Mwanuzi and De Smedt, 1999) due to complexation of metals such as Cd with chloride and sulphate forming soluble inorganic complexes (Greger et al. 1995). At the same time, cations, notably Ca and Na, compete with metals for adsorption sites displacing both weakly and moderately sorbed metals such as Cr, Cu, Zn and Pb (Fairbrother et al. 2007). For example, waterborne Cd has been reported up to 4-fold higher if salinity changes from 0 to 10 psu (Greger et al. 1995). Thus, gradients of suspended matter concentration, sediment types, and salinity are decisive for metal mobility within the estuary.

4.4 Sedimentation and metal removal from surface waters

Metal adsorbed to particulate material can settle out of suspension, providing one of the main sinks of metals in the estuarine system. Gradients that are responsible for water column stratification also affect sedimentation, precipitation and flocculation of particles and colloids (Förstner and Wittman, 1979). In turn, high turbidity areas contribute to enlarge sub-surface contaminated deposits. Thus, residence time and mobilization of particulate metal in estuaries are strongly influenced by the turbidity maximum. Sorption of metals to depositing sediments is a major removal mechanism for these contaminants from the overlying waters (Mwanuzi and Smedt 1999), providing important ecosystem service in terms of water quality improvement (Millennium Ecosystem Assessment, 2005). This transfer of metals from the aqueous to the sediment compartment may be a temporary removal from the water phase but not from the whole estuarine system.

5 Metal remobilization processes

Metals sorbed to bottom sediments do not necessarily stay in that condition, but may be released due to resuspension or desorption and act as a diffuse pollution source (Zwolsman et al. 1993; Mwanuzi and Smedt, 1999). The sediment texture, hydrological regime, salinity and organic content are responsible for the mobility and availability of metals in the superficial sediment layer especially in inter-tidal environments (Du Laing et al. 2009b). Especially in tidal flats, autochthonous (in situ precipitated) metals are constantly remobilized by interaction with organic matter, water level, redox conditions and water chemistry changes, and diagenesis (Förstner and Wittman, 1979) (Fig. 4).

5.1 Chemical metal remobilization

In freshwater saturated sediments, the mobility of metals is strongly determined by metal-sulphide interaction and Mn or Fe oxide co-precipitation (Paquin et al. 2003), or phosphate and organic matter interactions (Förstner and Wittman, 1979) since under specific redox conditions such compounds are able to sequester metal ions from solution by reducing their reactivity. Under saltwater saturated conditions, ionic interactions with carbonate oxides, oxide-hydroxides, silicates, and chloride gain additional relevance for metal mobilization (Fairbrother et al. 2007). In this case, water filtration into sedimentary layers introduces ions (such as Ca, Cl, K, Mg, Na, SO_4) which leads to the mobilization of sediment bound metal to pore water and promotes the reactive transport of these elements (Fig. 4).

In sediment layers also redox conditions determine metal remobilization (Zwolsman et al. 1993). Under oxidizing conditions the co-precipitation and adsorption of metals with Fe-Mn oxides acts as sink of dissolved metal (Fairbrother et al. 2007; Lu et al. 2014). At more reductive conditions, first Mn and then Fe are reduced, both releasing adsorbed and co-precipitated metals including Co, Ni, Cr, Cd, Cu, Pb and Zn (Zwolsman et al. 1993). This release is amplified by sulphate-metal interaction, increasing pore or surface waterborne metal (Du Laing et al. 2009b). At even stronger reductive conditions sulphate is reduced to sulphide, which can complex metals and reduces mobility and toxicity (Du Laing et al. 2009c; Lu et al. 2014).

5.2 Chemical and biologically mediated metal remobilization

For those metals that form stable methyl species (e.g. Hg, As, Cr), salinity is also expected to promote bacterial methylation by providing additional sulphate, which is the substrate for sulphate reducing bacteria (Zhang et al. 2014). In turn, methylation increases both metal toxicity and mobility by shifting metal affinity to organic matter. Notwithstanding, reduction on metal bioavailability and further production of sulphide might play a role at decreasing methylation in fully saline conditions (Fairbrother et al. 2007).

Particulate metals are also released as a result of chemical changes caused by aquatic plants (Greger et al. 1995) and benthic organisms (French and Turner, 2008). The organic matter provided by biota detritus and roots exudates plays an important role as food source for microorganisms, which catalyse a series of redox reactions in the presence of electron acceptors. Together with sulphate, Fe and Mn, organic matter in dissolved, colloidal and particulate forms provide redox-buffering capacity to sediments (Wright 1995; Pasquin et al. 2003). Organic matter also chelates metals and increases mobility (Du Laing et al. 2009c), e.g. increases of 25% in organic matter content might rise metal concentrations up to 200 times (Du Laing et al. 2009a). Frequently flooded and higher salinity areas, in general, present higher organic matter due to lower optimal redox conditions for its degradation and higher chemical stability (Du Laing et al. 2009b). Moreover, the respiration and degradation products of organic matter causes reductive conditions and changes pH altering ionic composition of pore water and overall increasing mobility (Lu et al. 2014). Du Laing et al (2009b) found that when organic matter is being decomposed Ca, Mn, Ni and Fe are released upon CO_2 accumulation and Fe/Mn oxide reduction if no sulphide is present.

A recent study suggested that organic matter might be prone to sequential oxidation and reduction cycles in the environment (Klüpfel et al. 2014). These results have implications on metal behaviour because oxidized and reduced organic matter present lower and higher metal binding capacities, respectively. Thus, during organic matter oxidation metal would be released and susceptible to Fe-Mn-oxi-precipitation or co-precipitation (Fig. 4.2). At reductive conditions, in contrast, organic matter could act as catalytic factor for metal mobilization through metal chelation.

5.3 Chemical and physically mediated metal remobilization

In the inter-tidal flats reductive and oxidative conditions are cyclic with tides what implies an additional type of organic matter-mediated metal remobilization and precipitation. Similarly, the consecutive high and low tide cycles also affect inorganic precipitation of metals in surface and sub-surface sediments (Fig. 4.3). Even for supra-tidal areas, fluctuation in groundwater table level causes the migration of organic matter among layers, changes ion compositions and affects the preferential region of metal deposition (Du Laing et al. 2009c). Experimental results of the interaction of water table level, organic matter and pore water composition across sedimentary layers showed that Cd, Cu and Zn are deposited right above the water level, while Ni is deposited right below (Du Laing et al. 2009b). Ultimately, the physical component of water level oscillation and tidal current shear implies sediment re-suspension (Hawkins et al. 1996) and is an important factor in the release of metals from particles and from pore solutions (Lindberg et al. 1975).

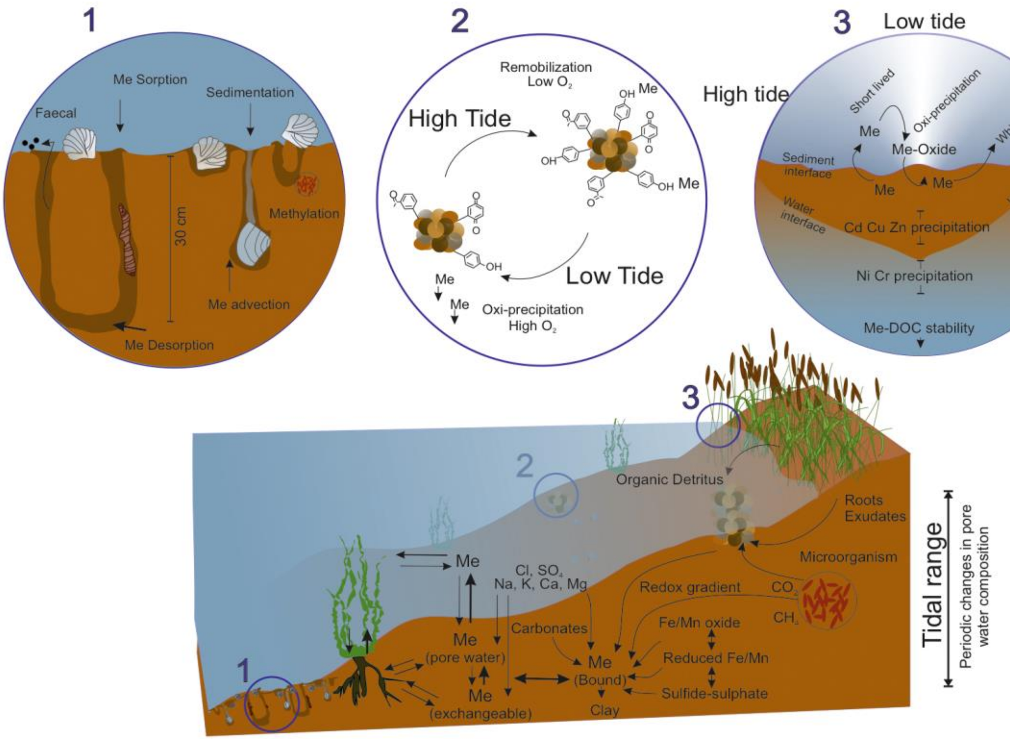

Figure 4: Physical, chemical and biological variables and processes affecting the behaviour of trace metals in tidal flats. Subplots represent processes associated to benthic invertebrates in sub-tidal zone (1), organic matter and redox cycles in inter-tidal zone (2) and water table and metal precipitation in inter-tidal zone (3). Inspired by Greger et al (1995), Du Laing et al. (2009c), and Klüpfel et al. (2014).

6 Interactions with biological processes

6.1 Physical, chemical and biological interactions for metal remobilization

Physical water intrusion favours advection of exchangeable and pore water metal to the water column or bioaccumulation by roots of aquatic macrophytes (Fig. 4). In turn, roots transfer metal to above-ground biomass which constitutes a route of particulate organic metal to the water column (Greger et al. 1995). The interaction of roots and bacteria is also able to modify the microenvironment (up to 1 cm around roots) increasing the metal amount in the exchangeable weakly bound fraction (Du Laing et al. 2009c). This might have a great impact on metal remobilization if considered the remarkable portion of surface sediments covered by their dense root system.

In the tidal flat, benthic fauna also alters metal biogeochemistry in sediments because some of the metabolites produced (as CO_2, CH_4, NH_2, PO_4) are chemical species that increase metal mobility (Du Laing et al. 2009b). Organic residues and abandoned spaces within the sediment might be hotspots for microbial activity affecting redox conditions as well as methylation processes (Fairbrother et al. 2007). Maybe even more importantly, in bioturbated sediments the thickness of the layer of exchange and advection of water and sediment might be increased from <1 cm to ~30 cm (Fairbrother et al. 2007) (Fig. 4.1). The burrowing organisms form preferential channels for groundwater flow, which, depending on sediment permeability, can reach up to $7.0\ 10^{-6}$ m s^{-1} (Brand et al. 2013) and thus promote metal advection. Brand et al. (2013) pointed that transport of oxygen-containing surface water into the deeper zones of the sediment by burrowing organisms could trigger a sequence of redox reactions with potential impacts on Fe behaviour and solute transport. Indeed, French and Turner (2008) found that platinum metals introduced via water show increased sorption behaviour when sediments are colonized by the deposit feeder *Arenicola marina*. On the other hand, the same species increased pore water metal concentrations up to 2 orders of magnitude by remobilizing contaminated sediment (French and Turner, 2008). Relocation of metals as faecal pellets might also affect their distribution. Assimilation rates about 10 % of Pt from water and sediment imply that 90 % of ingested metal is transferred to faecal pellets (French and Turner, 2008). The high filtration capabilities of some molluscs also have a potential to significantly affect sedimentation of particles at whole estuary scale. Hawkins et al. (1996) found that *Mytilus edulis* from Marennes-Oléron bay (France) showed a clearance rate (water cleared of suspended matter per mussel) of up to ~5.28 L h^{-1}, from which about 93 % of filtered material was deposited as pseudofaeces, e.g. filtered from water column but not ingested. Thus while deposit-feeders polychaeta might have effects in both deposition and remobilization of metal, bivalves would affect mainly deposition. The extension of such interactions in sedimentation and metal behaviour remains to be studied however.

Finally, estuarine sediments' remobilization and their changes in pore water release might be enhanced by physical advection events, e.g. wave action, storm surge, and dredging activities. This can affect dissolved and (mainly) particulate metal concentrations within the estuary if contaminated sediments are present (Van den Berg et al. 2001). Spencer et al. (2006b) found that historically contaminated sediments from Thames and Medway estuaries (UK) represented a risk of mobilization of Cr, Cu, Pb and Zn. These authors suggested that in case of natural or anthropogenic reworking of these historic sediments, associated metal would become readily available to cause toxicity at Thames and Medway estuaries, in which bioavailability of metals vary up to 70 % of the total content.

6.2 Phytoplankton affecting partitioning and advection of metals

One of the most important biotic factors influencing the behaviour of metals in the water column is phytoplankton (Fig. 5). This group of organisms shows high metal bioconcentration (Phillips, 1980), with uptake occurring predominantly from dissolved fractions (Paquin et al. 2003). Fisher et al. (1981) reported Cu concentrations on *Asterionella japonica* of approximately 10,000 times as high as culture media. Similarly, Fisher et al. (1984) comparing *Thalassiosira pseudonana, Dunaliella tertiolecta, Emiliania huxleyi, Oscillatona woronichinii* found bioconcentration factors (BCF, volume based) about 10^4 for Ag, 10^3 for Cd, 10^4 to 10^5 Hg and 10^2 to 10^3 for Zn. Also data from Yap et al. (2004) suggests BCF (volume based) about 10^4 to 10^5 for *Isochrisysis galbana* for Cd, Cu, Pb and Zn, ratifying the role of phytoplankton on the metal distribution between aqueous, solid and organism phase in an estuarine system.

Two ways of metal-phytoplankton interaction can be distinguished: external adsorption of the metal to the cell wall and uptake through the cell wall into the cell. The surface of phytoplankton cells provides a significant area for metal adsorption, while the increase of up to 2 units of pH around photosynthesizing microalgae might favour metal oxiprecipitation on the cell wall (Braek et al. 1980). With dividing cells producing new cells and thus new reactive surface material, the particulate metal content increases linearly (Fisher et al. 1984).

Phytoplankton also passively and actively absorbs (internalizes) metals. Biological membranes are considerably impermeable to electrically charged and highly polar neutral and organic metal species. However, passive absorption happens when metals cross these biological membranes diffusing into the organisms, which might occur with neutrally charged metals (e.g. Hg^0, $HgCl_2$, AgCl, Cu(I)Cl) or organic-metal complexes (Sunda and Huntsman, 1998). The resulting diffusion process of metals in this case is forced by external/internal gradient concentrations according to Fick's law. On the other hand, active absorption is more common for most of metal ions and polar metal-organic complexes. This uptake is performed by specific membrane transporters like channels and ion exchange pumps. It might thus occur more independently of gradient concentrations as a saturation-like curve response ruled by kinetics and thermodynamic metal/transporter interactions constants (Sunda and Huntsman, 1998; Drexter et al. 2003) and transporter density in cell membrane (Fisher et al. 1984).

Under steady-state conditions, for both processes (adsorption and absorption of metals by phytoplankton) the cellular metal concentration equals the cellular uptake divided by the specific growth rate (Sunda and Huntsman, 1998). Considering that phytoplankton is usually included on suspended matter measurements and its metal cellular content remains relatively constant after metal exposure under steady-state (Fisher et al. 1984), both adsorption and absorption increase particulate metal and alter partitioning of metal between water and suspended matter. This is significant at environmental level, for instance the distribution coefficient (L kg^{-1}) for particulate and dissolved Cd concentration was reported to vary from $17 \cdot 10^3$ up to $100 \cdot 10^3$, with highest anomalies of particulate Cd attributed to seasonal phytoplankton bloom in Scheldt Estuary (Valenta et al. 1986). Wang et al. (2009) similarly observed inverse relationship of dissolved copper and chlorophyll in the Changjiang Estuary during phytoplankton bloom.

If not consumed, phytoplankton-bound metal will be regulated by residence times and fate of particles and organic matter in the estuary, and similarly be subject to sedimentation or advection to coastal areas. In this sense, the sedimentation of dead phytoplankton might contribute to additional particulate metal input to surface sediments since dead cells might accumulate even more metal than living ones (Fisher et al. 1984). In fact, Valenta et al. (1986) noticed an efficient removal of Cd, Cu, and Pb from water column taking place during the months of sedimentation of plankton detritus.

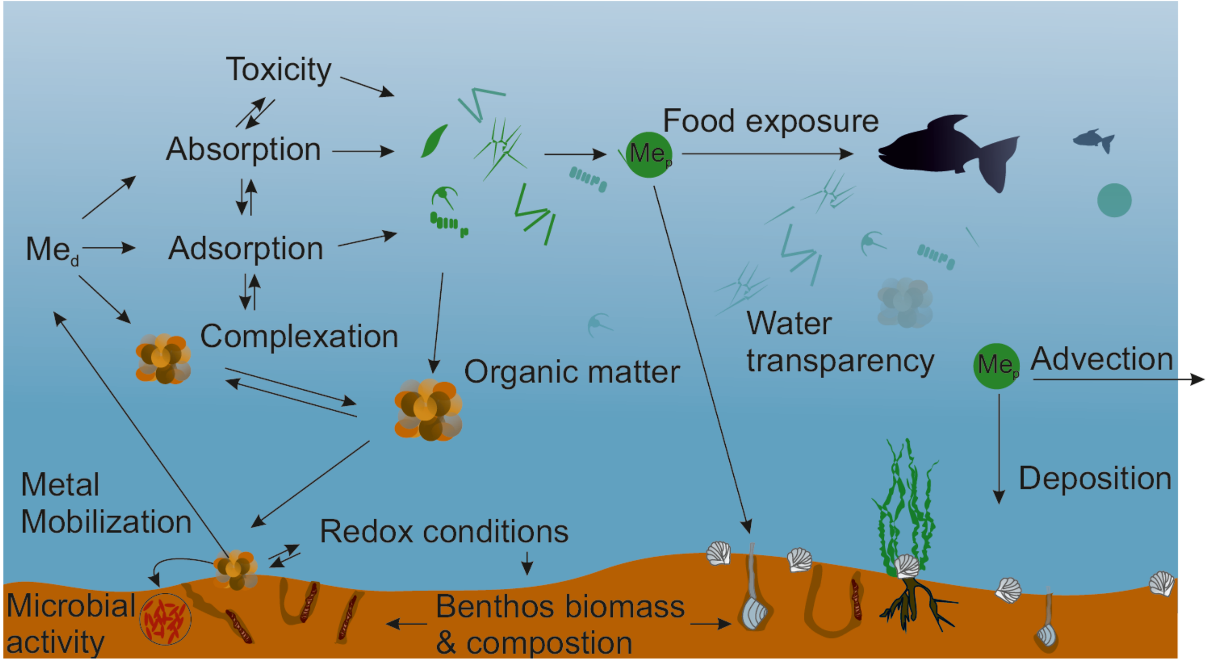

Figure 5: Conceptual model of phytoplankton consequences on dissolved (Me$_d$) and particulate (Me$_p$) metal and organisms exposure route on estuaries.

7 Organism physiology and effects of metals

7.1 Phytoplankton and environmental toxicity of metals

The important role of phytoplankton in transferring metal from the dissolved to the particulate fraction, i.e. decreasing dissolved concentrations, does not represent necessarily a decrease in metal bioavailability (Fig. 5). In fact, it implies potential increment in food transfer of metals to higher trophic levels (Sunda and Huntsman, 1998). Thus, phytoplankton alters metal chemistry and exposure routes to other organisms simultaneously. Phillips (1980) noticed that metal concentrations in the blue mussel *Mytillus edulis* (Swedish coast), pike (Stockholm archipelago), and the scallops *Pecten maximus* and *Chlamys opercularis* (English Channel) were related to metal levels rather in phytoplankton than in water. It is evident that dietary accumulation of metals is at least as important as metal uptake from the aqueous phase and in many cases dominates metal accumulation, e.g. in bivalves from marine environments (Ahlf et al. 2009). King et al. (2005) compared metal bioaccumulation by the deposit-feeding amphipod *Melita plumulosa* from water, sediment and microalgae sources under laboratory conditions. They report that feeding rates of 0.19 g algae g^{-1} organism d^{-1} implied about 95 and 75% of total Cd and Cu uptake, respectively. The same authors found that the rate of Cd and Cu accumulation from sediments was actually increased when microalgae were present due to higher feeding rates.

Although mechanisms and consequences of diet-borne exposure are widely unknown, the consensus is that it has the potential to cause toxicity (Fairbrother et al. 2007). Comparing contaminated waterborne and diet-borne effects of Cu exposure to the copepod *Acartia tonsa*, Pinho et al. (2007) found that feeding over Cu contaminated microalga *Thalassiosira weissflogii* overall improved nourishment conditions, and only highly contaminated food caused more adverse than benefiting effects. In contrast, Moreno-Garrido et al. (1999) reports that reproduction of the planktonic rotifer *Brachionus plicatilis,* which under seawater control conditions occurred within three to four days, was delayed by one to two days when this rotifer was fed with four different Cu-contaminated microalgal species.

It has been questioned even if the same underlying toxicity mechanism is occurring for metal absorbed from water (dissolved) and for metal absorbed from suspended material (particulate). Simpson and King (2005) found that Cu uptake by *M. plumulosa* and the bivalve *Tellina deltoidalis* was higher from dissolved metal compared to suspended matter metal, while toxicity was higher for particulate-exposed individuals. According to them, the uncoupling of bioaccumulated metal and toxicity regarding the different exposure routes suggests distinct toxicity mechanisms for food and waterborne related metals.

Phytoplankton growth also has indirect important effects on metal behaviour and toxicity by affecting organic matter. Phytoplankton exudates and decomposition increase significantly the dissolved organic matter in water column (Cloern, 2001), potentially increasing organic-metal complexation and solubilisation (Fig. 5). Additionally, there is evidence for higher excretion of organic matter by metal-treated phytoplankton populations (Bentley-Mowat and Reid, 1977). In terms of implication for toxicity, freshwater organic matter has a shielding effect by chelating metals and reduced bioavailability and toxicity (Di Toro et al. 2001). The protective effect of humic and fulvic acids on toxicity of copper in freshwater, for instance, is well acknowledged (Paquin et al. 2003). However, in terms of saltwater, this issue is still unsolved. Studies available so far point to a more specific response of organic matter, organism, metal toxicity and salinity than overall trends. Sanchez et al. (2007) found that humic acids actually increased absorption of Pb by gills of *Mytilus edulis* and toxicity to the purple sea urchin *Paracentrotus lividus* larvae, even when 25-75 % of this metal was bound to organic forms.

Phytoplankton-derived organic matter can further deposit as particulate or colloidal matter in sediments, where mineralization processes take place and favour reductive conditions, which affect microbial activity and fauna in the sediment (Du Laing et al. 2009c) and lead to a remobilization of metals. In this case, shifts in oxygen availability are known to cause changes in the benthic community composition, i.e. to reduce biodiversity and increase biomass and to change the community towards the direction of polychaeta species (Cloern 2001). Polychaetes, in turn, might affect metal desorption from sediments (Fig. 4.1) (French and Turner, 2008). Ultimately, phytoplankton growth and organic enrichment of water column decrease water transparency, and consequently the light availability of metals to submerged plants, limiting plant growth and transfer of particulate metal as discussed above (Fig. 5).

7.2 Metal impact on estuarine biota

In contrast to freshwater systems, where nutrients are the main factor limiting primary production, the growth of phytoplankton in coastal zones is primarily limited by the interplay of nutrient loading, filter capacity of the system, contaminants (Fig. 5), light availability, and ecological interactions (Cloern, 2001). For instance, growth reductions of up to 50 % related to exposure to less than 90 µg. L^{-1} of Cu or Zn have been demonstrated for cosmopolitan phytoplankton species (Fisher et al. 1981; Machado et al. 2014a). In fact, laboratory metal exposure inducing phytoplankton reduced cytokinesis, photosynthesis disturbance and increasing cell size has been reported for several metal and species (Shrift, 1959; Erickson, 1972; Davies, 1974; Blankenship and Wilbur, 1975; Bentley-Mowat and Reid, 1977; Rivkin, 1979). It is clear that in contaminated estuaries cellular concentrations of metals may reach toxic levels, controlling growth and potentially resulting in alterations of phytoplankton productivity (Phillips, 1980) and species composition (Fisher et al. 1984). Its quantitative relevance under environmental conditions remains to be clarified, however (Cloern, 2001).

Beyond phytoplankton, metals might also cause toxicity to other organisms according to the physiological adaptations of organisms to the estuarine environment as discussed below (Monserrat et al. 2007).

7.3 Physiology interfering the mechanisms of metal toxicity in estuaries

Most of the knowledge regarding metal toxicity mechanisms to aquatic organisms has been established in freshwaters. From these studies, especially for the metals Ag, Cd, Cu, Pb, and Zn the concept has emerged that metals react with organism's ligands at organism/environment interface similarly to metal interaction with abiotic chemical ligands (Paquin et al. 2003). Once absorbed, metals could either be located in a non-toxic pool or bound to the organ or molecule target of toxicity, often referred to as site of action (Drexler et al. 2003). The current most accepted idea is that toxicity is proportional to the metal accumulated at this toxicity target. A classic example is the correlation between Cu in the gills (as toxicant), Na uptake (as toxicity mechanism), NaK-ATPase inhibition (as site of action), and mortality (as toxicity endpoint) in rainbow trout after Cu waterborne exposure (Grosell et al. 2002). Indeed, effects on ion homeostasis have been demonstrated for several metals and freshwater organisms in both laboratory and field exposures (Heath, 1995; Wright, 1995; Grosell et al. 2002; Wood et al. 2012a,b; Machado et al. 2014b). Thus, it has been suggested that the common mechanism of metal toxicity is related to ion and osmotic disturbances driven by ion gradients between the organism's plasma and environmental water (Alsop and Wood, 2011). These concepts have been successfully demonstrated for Ag, Cd, Cu and Zn in several freshwater species, and broadly propagated as Biotic Ligand Models (Paquin et al. 2003).

However, metal toxicity to estuarine organisms might be more complex because the physiology of the organisms changes with increasing salinity. For instance, freshwater fishes are hypertonic compared to their environment (Alsop and Wood, 2011). These hyper-osmoregulating fish avoid drinking water, produce high amounts of diluted urine and uptake ions from food and especially through gills, which are the main tissues of ion regulation (Wood et al. 2012a). On the contrary, saltwater teleost fishes are hypotonic (Grosell et al. 2007), drink saltwater, produce small amounts of concentrated urine and excrete ions through gills (Wood et al. 2012a). In saltwater fishes, water is absorbed by actively producing an osmotic gradient in the intestine, which is a further important organ to ionic regulation. Thus, in an estuarine system, the importance of exposure routes as well as toxicity targets, and consequently organism sensitivity might change.

Freshwater fishes are expected to absorb waterborne metal mainly by gills, while in saltwater drinking fishes gills and gut might be important (Wood et al. 2012a). Moreover, by drinking water, organisms ingest higher amounts of particulate metal that could later be mobilized under the acidic conditions of the digestive tract. Indeed, metal sensibility and toxicity mechanisms that have been traditionally attributed to and modelled based on dissolved fractions in freshwater (Ahlf et al. 2009) might require reconsideration for saltwater organisms. As mentioned earlier, dissolved and particulate metal appear to have distinct accumulation rates and, eventually, toxicity mechanisms. Simpson and King (2005) observed faster Cu uptake by gills, but higher toxicity (lower accumulation at LC50- concentration lethal for 50% of organisms) when accumulated by intestine.

Additionally, some groups of organisms can change from osmo-regulatory to osmo-conforming behaviour as a function of salinity. This is the case for several crustaceans like the blue crab *Callinectes sapidus*. In lower salinities (0-20 psu) the organism is hyper-osmoregulating, i.e. its hemolymph is denser than the surrounding water. At higher salinities (>20 psu) the crabs are osmoconforming, i.e. their hemolymph has approximately the same osmolality of the surrounding water. In fact, changes in salinity typically cause metabolic changes in crustaceans and estuarine animals (Monserrat et al. 2007). Thus, if ion disruption is the toxicity mechanism of metals, physiology may become more important than water chemistry in predicting metal toxicity under varying salinities (Grosell et al. 2007; Zimmer et al. 2012).

7.4 A conceptual model for metal toxicity in estuaries

There is an overall negative relationship between salinity and dissolved metal accumulation or toxicity (Wright, 1995), i.e. salinity reduces the availability of metals and thus diminishes their toxicity (Fig. 6.1). This protective effect against waterborne metal exposure predicted by biotic ligand models is due to the introduction of cationic competition for sites of toxicity on the organism, and due to increasing metal-complex forms (Pinho et al. 2007; Pinho and Bianchini, 2010). The mechanisms of toxicity in varying salinity are also affected by several factors. Salinity directly affects waterborne ions, increasing ion diffusive gain (from water to the organism) and decreasing ion diffusive loss (from the organism to the environment). Consequently, at higher salinities less active ion uptake is performed. On the other hand, high salinity requires higher ion excretion rates to cope with the excess of ion gained.

Both ion uptake and excretion are regulated by several enzymes and channels, which constitute direct targets for metals (Wood et al. 2012ab)(Fig. 6.1). Thus, by impairing biologically controlled pathways, metal toxicity ultimately reflects the disturbance of the ion-osmo-regulation and the physiologically required gradient of ions inside and outside the organism (Fig. 6.2). In freshwater organisms that are not exposed to metals, the internal concentration of a certain ion over time is relatively constant (Grosell et al. 2002), and concentrations of osmolytes inside the organism are much bigger than in the surrounding water. Under such conditions, all organisms hyper-osmoregulate, and active uptake is high in order to cope with ion diffusive loss while diffusive gain into the organism and ion excretion are minimal. Thus, osmolyte loss is the possible ion-osmoregulatory effect at freshwater conditions. Indeed, ion loss is often reported in freshwater organisms after metal exposure. If ion loss is higher than a critical level, the organism dies. For instance, empiric data suggest that death for 50% of some invertebrates occurs after Ag, Cd, and Cu exposure when about 30 % of Na is lost (Vitale et al. 1999; Grosell et al. 2002).

At higher salinities the situation is different since the gradients of ions inside and outside the organisms are smaller. Thus, with increasing salinity various ion-osmoregulation and ion-osmoconformation strategies coexist. The higher salinity *per se* has implications usually increasing ion diffusive gain, such that ion excretion gains physiological relevance. In this case, diverse metal effects can be expected (Fig. 6.2). Notice that lethal toxicity caused by metals might be observed even in osmoconforming organisms, suggesting that either ionic disruption is the cause of death or other toxicity mechanisms are to be discovered.

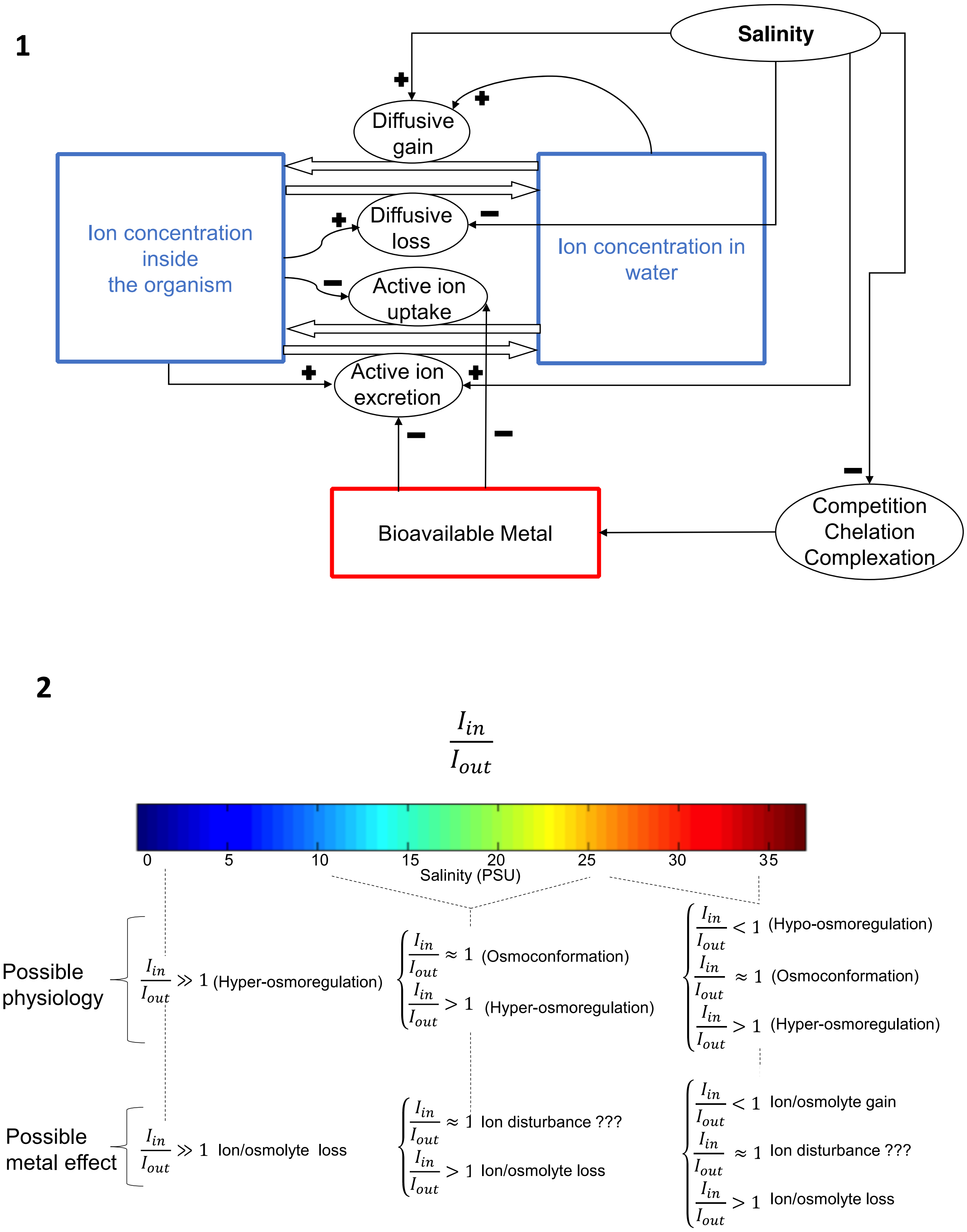

Figure 6: Conceptual model of (1) metal effects on osmoregulation with three state variables (ions inside the organism, ions in water, bioavailable metals), large arrows represent flows, light arrows indicate interrelations and impacts of variables on processes; + : reinforcing interaction (e.g. “the higher the ion concentration inside the organism the higher the active ion excretion”), - : alleviative interaction (e.g. “the higher the ion concentration inside the organism the weaker the active ion uptake”); and (2) ion-osmoregulatory behaviour and potential metal effects in salinity gradients.

The interaction of possible organism physiologies and metal effects has generated some apparent disagreement on factors accounting for metal toxicity in the scientific literature. For instance, some authors claimed that Cu toxicity in different salinities could be explained simply by changes in water chemistry and free ion activity instead organism physiology (Martins et al. 2011). Other authors stated that physiology is important to cover effects of Cu not explained by water chemistry (Wright, 1995; Grosell et al. 2007). Thus it is appropriate to mention that for estuarine organisms enantiostasis might be important to explain metal toxicity and these conflicting results. In order to keep homeostasis, organisms change the properties of interface membranes (transporters, channels, and lipid composition) (Wright, 1995) and adapt cytosolic enzymes activities among other changes, referred as enantiostasis processes (Monserrat et al. 2007). Thus, for several estuarine organisms enantiostasis drives significant changes in membrane properties or internal availability of toxic metal (Drexler et al. 2003) that affect effective metal exposure. Such changes in membrane transporters and channels shift the affinity of biotic ligands to metals, while changes in lipid composition additionally affect the membrane permeability to distinct metal species (Sunda and Huntsman, 1998). Variability of internal metal availability can also be caused by salinity-driven metallothionein adaptation (Martins and Bianchini, 2009). Indeed, Oguma and Klerks (2013) recently demonstrated in field populations of the shrimp *Paleomonetes pugio* the importance of native salinity acclimation to Cd sensibility, which might be attributed to enanstiostatic related processes affecting metal exposure, and consequently, toxicity.

Major modifications in water chemistry that affect metal speciation are expected to occur primarily under low salinity conditions, and secondary at intermediate salinities. At higher salinities relatively minor changes in metal speciation take place (Grosell et al. 2007). Concomitantly, in freshwater organisms (mandatory hyper ion-osmoregulatory physiology) only body size is a relevant component of the physiology, because it is known to affect ion turnover rates (Grosell et al. 2002). Differently, at higher salinities the physiology is more variable. Thus, although water chemistry might play a role, we conceptualize that physiological processes gain importance as salinity rises (Fig. 7). For practical purposes, physiology is here distinguished from enantiostasis derived from the memory of native water chemistry condition and related interface membrane responses. Note that such enantiostasis processes are expected to be highest in brackish water, where salinity variations are higher and more instable.

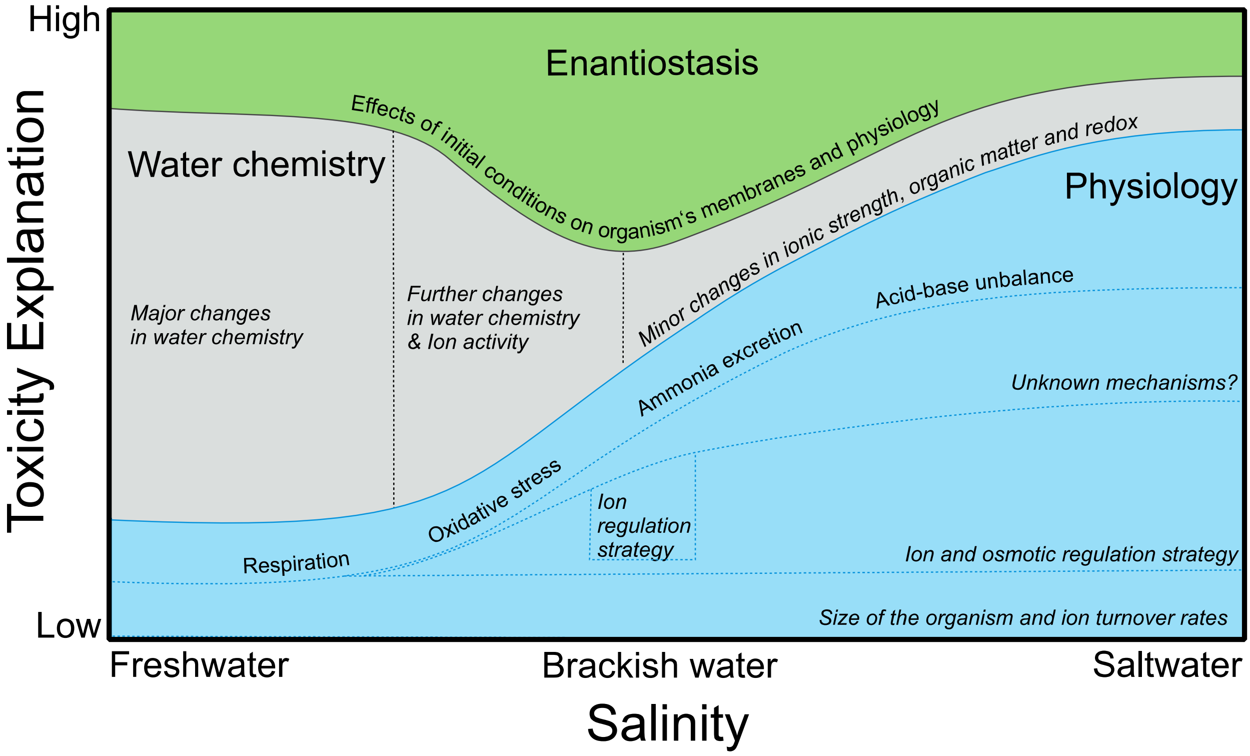

Figure 7: Conceptual idea of potential explanatory variables for metal toxicity in salinity gradients. The blue areas refer to processes related to toxicity mechanisms depending upon organisms' physiology. In grey areas some of the main water chemistry processes controlling metal in the environment are shown. In the green area is hypothesized the interactions of native water chemistry and physiology on enantiostasis processes.

Some considerations are necessary regarding metal toxicity mechanisms in saltwater. Firstly, although organisms acclimated to higher salinity are usually less sensitive to metals, the lowest sensibility often occurs at intermediate salinities when organisms are isotonic to the surrounding environment (Blanchard and Grosell, 2006; Grosell et al. 2007).

Secondly, marine species usually demonstrate lighter or non-detectable ion and osmotic disruptions when exposed to metals compared to freshwater organisms (Vitalle et al. 1999; Zimmer et al. 2012). This holds even when the free ion activity is considered (Wright, 1995; Grosell et al. 2007). Blanchard and Grosell (2006) tested the hypothesis of ion-osmotic disruption in the killifish *Fundulus heteroclitus* by Cu across salinities. These authors found no evidence of such toxicity mechanism being homogeneous as salinity changes, and only freshwater acclimated killifish toxicity indicated consistently ionic stress. Vitalle et al. (1999) observed similar results for Cd in the intertidal crab *Chasmagnatus granulata.* Thus, even if ionic and osmotic disruption is an important mechanism for metal toxicity at low salinities, other mechanisms remain to be discovered at higher salt concentrations (Fig. 7).

Some candidates for that are respiratory impairment, oxidative stress, ammonia excretion and acid-base unbalance. Although such effects have been consistent with metal exposure (Wood et al. 2010a,b; Loro et al. 2012; Zimmer et al. 2012; Machado et al. 2013; Giacomin et al. 2014; Machado et al. 2014b), their ability in providing explanation to lethal toxicity across taxa still remains to be demonstrated, and other unknown mechanisms cannot be discarded.

8 Final considerations and future directions on modelling metal fate and effects on estuaries

While this conceptual model focused mostly on metal effects under estuarine gradients, the combined environmental pressure and interactions of metal pollution with other (anthropogenic) stressors, e.g. nutrient loads and pathogens, to affect environmental health remain a complex issue to be clarified. Along this, toxic exposure in the environment occurs as mixtures, and regulations as the Water Framework Directive consider not only individual toxics but also their combined effects (European Commission, 2012a,b). In this sense, Cravo et al. (2012) found complex patterns of spatial and temporal trends for co-varying metals, hydrocarbons and tributyltin, with strong interaction of contaminants and water physicochemistry (temperature, salinity, dissolved oxygen) on driving physiological responses of the bivalve mollusc *Ruditapes decussatus*. Sures (2006, 2008) further demonstrates the influence of parasitism in either ameliorating or intensifying the effects of metal exposure. Additionally, Long et al. (1995) found that toxic sensitivity in modelling and bioassay methods differ considerably from those of field studies due to different effects of individual 28 compounds and mixtures in estuarine and marine sediments. It is yet required to identify where synergistic, additive or antagonistic effects exist (Cravo et al. 2012). This means that, especially for ecological effects, the exposure to mixtures of dissimilarly acting substances at low, but potentially relevant concentrations is a current concern, even if all substances are below the individual “predicted no observed effect concentrations” (European commission, 2012a). The first concepts of combination effects of sequential exposure to both chemical and non-chemical stressors are still continuously derived from laboratory, models and field experiments (Altenburger and Greco, 2009; Altenburger et al. 2012; Altenburger et al. 2015). Therefore, the following future main research challenges are identified regarding effects of mixtures of metals and other stressors under estuarine conditions: (1) more information on mode of action of diverse toxicants and their mixtures (Cravo et al. 2012), (2) information on effects of sequential exposure with different concentration pulses and duration (Altenburger and Greco, 2009), and (3) more robust conceptual and quantitative models for where, how often and to what extend estuarine organisms are exposed to certain mixtures over time (European Commission, 2012a).

Mathematical and conceptual models are available to address some of the specific estuarine dynamics discussed in the present work (Boyle et al. 1974; Betty et al. 1996; Baeyens et al. 1998; Di Toro et al. 2001; Paquin et al. 2003; Simpson and King, 2005; Brand et al. 2013). However, it is evident that the coupling of different models is mandatory to represent the estuarine environment as conceptualized here. Therefore, future models integrating quantitatively physical, biogeochemical and physiological processes occurring in estuaries are required for a realistic approach.

Temporal and spatial scales are key factors to the discussed metal deposition, transport and toxicity processes. The relative importance of most variables and processes varies across orders of magnitude depending on estuary dimensions, riverine discharge, coastal hydrodynamics, as well as on organism size and physiology. Water and suspended matter residence times at the surface are often in the range of weeks and months, respectively. Differently, subterranean estuary and sediments reside for much longer (months to years and decades). Metal precipitation, adsorption and dissolution processes are fast (mostly less than hours) and assumed to be instantaneous in most of the existing models, which is reasonable for the majority but not for all of the metals. Pd for instance might not be in equilibrium in estuarine systems, since time for partitioning reactions are longer than water residence time in several estuaries (French and Turner, 2008). Moreover, phytoplankton growth and consequent changes in dissolved to particulate metal affect the equilibrium partitioning in the range of days and in dependence on seasonal fluctuations. Thus, on the same time scale, fluctuations in the meaning of food-related metal exposure are expected. In this sense, the establishment of ecotoxicological and ecohydrological relevant scales constitute an additional open research topic to be addressed.

Finally, physical and chemical estuarine gradients affect biogeochemistry and organism physiology, impacting organism exposure and toxicity. The relative importance of each gradient on metal behaviour and toxicity might vary greatly and differ seasonally. The influence of salinity on partitioning coefficients can be significantly masked by oscillation in river flow, particulate and organic matter input and phytoplankton growth for instance (Valenta et al. 1986). Thus, behaviour and effects of metals in estuaries are dynamically depending upon both, environmental and biological causes, which have to be considered when establishing site-specific water quality criteria and environmental quality targets.

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References

Ahlf, W., Drost, W., Heise, S. 2009. Incorporation of metal bioavailability into regulatory frameworks- metal exposure in water and sediment. Journal of Soils and Sediments. DOI 10.1007/s11368-009-0109-6

Alsop, D., Wood, C.M. 2011. Metal uptake and acute toxicity in zebrafish: common mechanisms across multiple metals. Aquatic toxicology. 105:385-393.

Altenburger, R., Ait-Aissa, S., Antczak, P., Backhaus, T., Barcelo, D., Seiler, T.-B., Brion, F., Busch, W., Chipman, K., Alda, M.L., Umbuzeiro, G.A., Escher, B.I., Falciani, F., Faust, M., Focks, A., Hilscherova, K., Hollender, J., Hollert, H., Jager, F., Jahnke, A., Kertenkamp, A., Krauss, M., Lemkine, G.F., Munthe, J., Neumann, S., Schymanski, E.L., Scrimshaw, M., Segner, H., Slobodnik, J., Smedes, F., Kughathas, S., Teodorovic, I., Tindall, A.J., Tollefsen, K.E., Walz, K.-H., Williams, T.D., Van den Brink, P.J., Gils, J., Vrana, B., Zhang, X., Brack, W. 2015. Future water quality monitoring- Adapting tools to deal with mixtures of pollutants in water resource management. Science of the Total Environment. 512-513:540-551.

Altenburger, R., Greco, W.R. 2009. Extrapolation of concepts for dealing with multiple contamination in environmental risk assessment. Integrated Environmental Assessment and Management. 5:62-68.

Azevedo, J.L.L., Oliveira, L.R., Souza, J.F.A., Soares, I.D., Mata, M.M. 2008. Os processos de conversão de energia nos oceanos: Uma revisão do diagrama de Lorenz. Revista Brasileira de Geofísica. 26:153-172.

Baeyens, W., Monteny, F., Van Ryssen, R., Leermakers, M. 1998. A boxmodel of metal flows through the Scheldt estuary (1981-1983 and 1992-1995). Hydrobiology. 366:109-128.

Bai, J., Zhao, Q., Lu, Q., Wang, J., Reddy, R. 2015. Effects of freshwater input on trace element pollution in salt marsh soils of a typical coastal estuary, China. Journal of Hydrology. 520:186-192.

Benoit, G., Oktay-Marshall, S.D., Cantu, A., Hood, E.M., Cokeman, C.H., Corapcioglu, M.O., Santschi, P.H. 1994. Partitioning of Cu, Pb, Ag, Zn, Fe, Al, and Mn between filter-retained particles, colloids, and solution in six Texas estuaries. Marine Chemistry. 45:307.336.

Bentley-Mowat, J.A., Reid, S.M. 1977. Survival of marine phytoplankton in high concentrations of heavy metals, and uptake of copper. Journal of Experimental Marine Biology and Ecology. 26:249-264.

Betty, N.G., Turner, A., Tyler, A.O., Falconer, R.A., Millward, G.E. 1996. Modelling contaminant geochemistry in estuaries. Water Research. 30:63-74.

Bianchi, T.S. 2007. Biogeochemistry of Estuaries. Oxford University Press, New York.

Blanchard, J., Grosell, M. 2006. Copper toxicity across salinities from freshwater to seawater in the euryhaline fish Fundulus heteroclitus: Is copper an ionoregulatory toxicant in high salinities? Aquatic Toxicology. 80:131-139.

Blankenship, M.L. Wilbur K.M. 1975. Cobalt effects on cell division and calcium uptake in the coccolithophorid Cricosphaera carterae (Haptophyceae). Journal of Phycology. 2:211-219.

Bone, S.E., Charette, M.A., Lamborg, C.H., Gonneea, M.E. 2007. Has submarine groundwater discharge been overlooked as a source of mercury to coastal waters? Environmental Science and Technology. 41:3090-3095.

Boyle, E., Coller, R., Dengler, A.T., Edmont, J.M., Ng, A.C., Stallard, R.F. 1974. On the chemical mass-balance in estuaries. Geochemica et Cosmochimica Acta. 38:1719-1728.

Brand, A., Lewandowski, J., Hamann, E., Nützmann, G. 2013. Advection around ventilated U-shaped burrows: A model study. Water Resources Research. 49:2907-2917.

Brœk, G. S., Malnes, D. and Jensen, A. 1980. Heavy metal tolerance of marine phytoplankton. IV. Combined effect of zinc and cadmium on growth and uptake in some marine diatoms. Journal of Experimental Marine Biology and Ecology, 42: 39-54.

Cai, H. Savenije, H.H.G., Toffolon, M. 2014. Linking the river to the estuary: influence of river discharge on tidal damping. Hydrology and Earth Systems Science. 18:287-304.

Couceiro, F., Rauen, W., Millward, G.E., Lin, B., Turner, A., Falconer, R. 2009. Transport and reactivity of nickel in estuarine sediments: Results from a high capacity flume. Marine Chemistry. 117:71-76.

Cravo, A., Pereira, C., Gomes, T., Cardoso, C., Serafim, A., Almeida, C., Rocha, T., Lopes, B., Company, R., Medeiros, A., Norberto, R., Pereira, R., Araújo, O., Bebianno, M.J. 2012. A multibiomarker approach in the clam *Ruditapes decussatus* to assess the impact of pollution in the Ria Formosa lagoon, South Coast of Portugal. Marine Environmental Research. 75:23-34.

Davies, A. G. 1974. The growth kinetics of Isochrysis galbana in cultures containing sublethal concentrations of mercuric chloride. Journal of Marine Biological Association UK. 54:157-169.

Deboudt, K., Flament, P., Bertho, M.-L. 2004. Cd, Cu, Pb and Zn concentrations in atmospheric wet deposition at a coastal station in western Europe. Water, Air and Soil Pollution. 151: 335-359.

Di Toro, D.M., Allen, H.E., Bergan, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C. 2001. Biotic ligand model of the acute toxicity of metals. Technical basis. Environmental Toxicology and Chemistry. 20:2383–2396.

Drexler, J., Fisher, N., Henningsen, G., Lanno, R., McGeer, J., Sappington, K., Beringer, M. 2003. Issue paper on the bioavailability and bioaccumulation of metals. U.S. Environmental Protection Agency, Washington. Contract #68-C-98-148.

Du laing, G., Meers, E., Dewispelaere, M., Vandescasteele, B., Rinklebe, J., Tack, F.M.G, Verloo, M.G. 2009a. Heavy metal mobility in intertidal sediments of the Scheldt estuary: Field monitoring. Science of the Total Environment. 407:2909-2930.

Du Laing, G., Meers, E., Dewispelaere, M., Rinklebe, J., Vandecasteele, B., Verloo, M.G., Tack, F.M.G. 2009b. Effect of water table level on metal mobility at different depths in wetland soils of the Scheldt Estuary (Belgium). Water, Air and Soil Pollution. 202:353-357.

Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., Tack, F.M.G. 2009c. Trace metal behavior in estuarine and riverine floodplain soils and sediments: A review. Science of the Total Environment. 407: 3972-3985.

Elliotta, M., McLusky, D.S. 2002. The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science. 55:815-827.

Erickson, S.J. 1972. Toxicity of copper to *Thalassiosira pseudonana* in unenriched inshore seawater. Journal of Phycology. 8:318-323.

European Commission. 2012a. Communication from the Commission to the Council: The combination effects of chemical mixtures. Brussels, 31.5.2012 COM(2012) 252.

European Commission. 2012b. Report from the Commission to the European Parliament and the Council on the Implementation of the Water Framework Directive (2000/60/EC). Brussels, 14.11.2012 COM(2010) 670.

Falconer, R.A., Lin, B. 1997. Three-dimensional modelling of water quality in the Humber estuary. Water Research. 31:1092-1102.

Fairbrother, A., Wenstel, R., Sappington, K., Wood, W. 2007. Framework for metals risk assessment. Ecotoxicology and Environmental Safety. 68:145-227.

Fisher, N.S., Jones, G.J. and Nelson, D.M. 1981. Effects of copper and zinc on growth, morphology, and metabolism of *Asterionella japonica* (Cleve). Journal of Experimental Marine Biology and Ecology. 51: 57-85.

Fisher, N.S., Boh, M., Teyssi, J.-L. 1984. Accumulation and toxicity of Cd, Zn, Ag and, and Hg in four marine phytoplankters. Marine Ecology Progress Series. 18:201-213.

Fleury, P., Bakalowicz, M., Marsily, G. 2007. Submarine springs and coastal karst aquifers: A review. Journal of Hydrology. 339:79-92.

Förstner U., Wittmann G.T.W. 1979. Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin Heidelberg New York.

French, B., Turner, A. 2008. Mobilization, adsorption and bioavailability of Pt and Pd in coastal sediments: The role oft he polychaete, *Arenicola marina*. Environmental Science and Technology. 42:3543-3549.

Giacomin, M., Basso Jorge, M., Bianchini, A. 2014. Effects of copper exposure on the energy metabolism in juveniles of the marine clam *Mesodesma mactroides.* Aquatic Toxicology. 152:30-37.

Geyer, W.R., MacCready, P. 2014. The estuarine circulation. Annual Review of Fluid Mechanics. 46:175-197.

Gonzalez J-L., Thouvenin, B., Dange, C. 2007. Rôle des particules sur le comportement et la speciation de métaux traces: example du cadmium. La Houille Blanche 4:56-62

Greger, M., Kautsky, L., Sandberg, T. 1995. A tentative model of Cd uptake in *Potamogetonpectinatus* in relation to salinity. Environmental and Experimental Botany. 35:215-225.

Grosell, M., Blanchard, J., Brix, K.V., Gerdes. 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. Aquatic toxicology. 84:162-172.

Grosell, M., Nielsen, C., Bianchini, A. 2002. Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. Comparative Biochemistry and Physiology C. 133:287-303.

Heath, A.G. 1995. Water Pollution and Fish Physiology. CRC Press, Florida.

Hobbie, J.E. 2000. Estuarine Science: A synthetic approach to research and practice. Island Press, Washington, DC.

Jassby, A.D., Kimmerer W.J., Monismith, S.G., Armor, C., Cloern J.E., Powel, T.M., Schubel, J.R., Vendlinski, T.J. 1995. Isohaline position as a habitat indicator for estuarine populations. Ecological Applications. 5: 272-289.

Jensen, D.L., Ledin, A., Christensen, T.H. 1999. Speciation of heavy metals in lanfill-leacheated polluted groundwater. Water Research. 33:2642-2650.

Kennish, M.J. 1991. Ecology of Estuaries: Anthropogenic Effects. CRC Press, Florida.

King, C.K., Simpson, S.L., Smith, S.V., Stauber, J.L., Batley, G.E. 2005. Short-term accumulation of Cd and Cu from water, sediment and algae by the amphipod *Melita plumusa* and the bivalve *Tellina deltoidalis.* Marine Ecology Progress Series. 287:177-188.

Kinne, O. 1984. Marine Ecology. A comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters. Vol V. Wiley, New York.

Klüpfel, L., Keiluweit, M., Kleber, M., Sander, M. 2014. Redox properties of plant biomass-derived black carbon (Biochar). Environmental Science and Technology. 48:5601-5611.

Kundu, P.K., Cohen, I.M. 2004. Fluid Mechanics 3rd Edition. Elsevier, London, UK.

Lanceleur, L., Schäfer, J., Blanc, G., Coynel, A., Bossy, C., Baudrimont, M., Glé, C., Larrose, A., Renault, S., Strady, E. 2013. Silver behavior along the salinity gradient of the Gironde Estuary. Environmental Science and Pollution Research. 20:1352-1366.

Lindberg, S.E., Andre A.W., Harris R.C. 1975. Geochemistry of mercury in the estuarine environment. Estuarine Research Vol I, Cronin, LE (ed) New York: Academic Press, pp 64-107.

Liu, W.-C., Chang, S.-W., Jiann, K.-T., Wen, L.-S., Liu, K.-K. 2007. Modelling diagnosis of heavy metal (copper) transport in an estuary. Science of the Total Environment. 388:234-249.

Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management. 19:81-97.

Loro, V.L., Jorge, M.B., Silva, K.R., Wood, C.M. 2012. Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost *Fundulus heteroclitus*: Protective effects of salinity. Aquatic Toxicology. 110-111:187-193.

Lu, X.Q., Zhang, Y., Liu, H., Xing, M., Shao, X., Zhao, F., Li, X., Liu, Q., Yu, D., Yuan, X., Yuan, M. 2014. Influence of early diagenesis on the vertical distribution of metal forms in sediments of Bohai Bay, China. Marine Pollution Bulletin. 88:155-161.

Machado, A.A.S., Hoff, M.L.M., Klein, R.D., Cardozo, J.G., Giacomin, M.M., Pinho, G.L.L., Bianchini, A. 2013. Biomarkers of waterborne copper exposure in the guppy Poecilia vivipara acclimated to salt water. Aquatic toxicology. 138-139:60-69.

Machado, A.A.S., Gonçalves-Araujo, R., Teixeira, P.F. Tavano, V.M. Bianchini, A. 2014a. Effects of zinc on *in vivo* fluorescence, chlorophyll *a* and growth of the diatom *Conticribra weissflogii* (Thalassiosirales, Thalassiosiraceae). PANAMJAS. 9:278-287.

Machado, A.A.S., Wood, C.M., Bianchini, A., Gillis, P.A. 2014b. Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. Ecotoxicology. 23:1345-1358.

Mao, X., Prommer, H., Barry, D.A., Langevin, C.D., Panteleit, B., Li, L. 2006. Three-dimensional model for multi-component reactive transport with variable density groundwater flow. Environmental Modelling and Software. 21:615-628.

Martins, C.M.G., Bianchini, A. 2009. Metallothionein-like proteins in the blue crab *Callinectes sapidus*: Effect of the water salinity and ions. Comparative Biochemistry and Physiology: Part A. 152:366-371.

Martins, C.M.G., Barcarolli, I.F., Menezes, E.J., Giacomin, M.M., Wood, C.M., Bianchini, A. 2011. Acute toxicity, accumulation and tissue distribution of copper in the blue crab *Callinectes sapidus* acclimated to different salinities: In vivo and in vitro studies. Aquatic toxicology. 101:88-99.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Wetlands and Water Synthesis. World Resources Institute. Washington, DC. USA.

Moore, W.S. 1999. The subterranean estuary: a reaction zone of ground water and sea water. Marine Chemistry. 65:111-125.

Moreno-Garrido, I., Lubián, L.M., Soares, A.M.V.M. 1999. *In vitro* populations of rotifer *Brachionus plicatilis* Müller demonstrate inhibition when fed with copper-preaccumulating microalgae. Ecotoxicology and Environmental Safety. 44:220-225.

Müller, G., Förstner, U. 1975. Heavy metals in sediments of the Rhine and Elbe Estuaries: Mobilisation or mixing effect? Environmental Geology. 1:33-39.

Mwanuzi, F., De Smedt, F. 1999. Heavy metal distribution model under estuarine mixing. Hydrological Processes. 13:789-804.

Nemerow, N.L. 1991. Stream, Lake, Estuary, and Ocean Pollution. (2nd ed.) New York, Van Nostrand Reinhold.

Oguma, A.Y., Klerks, P.L. 2013. The role of native salinity regime on grass shrimp (*Palaemonetes pugio*) sensitivity to cadmium. Ecotoxicology. 22:279-286.

Paquin P.R., Farley, K., Santore, R.C., Kavadas, C.D., Mooney, C.G., Wu, K-B., Di Toro, D.M. 2003. Metals in Aquatic Systems: A Review of Exposure Bioaccumulation, and Toxicity Models. SETAC, Pensacola.

Paytan, A., Mackeya, K.R.M., Chena, Y., Limac, I.D., Doneyc, S.C., Mahowaldd, N., Labiosae, R., Post A.F. 2009. Toxicity of atmospheric aerosols on marine phytoplankton. Proceedings of the National Academy of Sciences. 106:4601-4605.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Applied Science Publishers, London.

Pinho, G.L.L., Pedroso, M.S., Rodrigues, S.C., Souza, S.S. Bianchini, A. 2007. Physiological effects of copper in the euryhaline copepod *Acartia tonsa*: Waterborne versus waterborne plus dietborne exposure. Aquatic Toxicology. 84:62-70.

Pinho, G.L.L., Bianchini, A. 2010. Acute copper toxicity in the euryhaline copepod Acartia tonsa: Implications fo the development of an estuarine and marine Biotic, Model. Environmental Toxicology and Chemistry. 29:1834-1840.

Rivkin, R.B. 1979. Effects of lead on growth of the marine diatom Skeletonemu cosfutum. Marine Biology. 50:239-247.

Sañudo-Wilhelmy, S.A., Rossi, F.K., Bokuniewicz, H., Paulsen, R.J. 2002. Trace metal levels in uncontaminated groundwater of a coastal watershed: Importance of colloidal forms. Environmental Science and Technology. 36:1435-1441.

Savenije, H.H.G. 2012. Salinity and Tides in Alluvial Estuaries, 2nd completely revised edition, http://salinityandtides.com.

Shrift, A. 1959. Nitrogen and sulfur changes associated with growth uncoupled from cell division in *Chlorella vulgaris.* Plant Physiology. 36:506-509.

Simpson, S.L., King, C.K. 2005. Exposure-pathway models explain causality in whole-sediment toxicity tests. Environmental Science and Technology. 39:837-843.

Spencer, K.L, Dewhurst, R.E., Penna, P. 2006a. Potential impacts of water injection dredging on water quality and ecotoxicity in Limehouse Basin, River Thames, SE England, UK. Chemosphere. 63: 509–521.

Spencer, K.L., MacLeod, C.L. 2002. Distribution and partitioning of heavy metals in estuarine sediment cores and implications for the use of sediment quality standards. Hydrology and Earth Systems Science. 6:989-998.

Spencer, K.L., MacLeod, C.L., Tuckett, A., Johnson, S.M. 2006b. Source and distribution of trace metals in the Medway and Swale estuaries, Kent, UK. Marine Pollution Bulletin. 52:226–231.

Spencer, K.L., O'Shea, F.T. 2014. The hidden threat of historical landfills on eroding and low-lying coasts. ECSA Bulletin. 63:16-17.

Sunda, W.G., Huntsman, S.A. 1998. Processes regulating cellular metal accumulation and physiological effects: Phytoplankton as model systems. Science of the Total Environment. 219:165-181.

Sures, B. 2006. How parasitism and pollution affect the physiological homeostasis of aquatic hosts. Journal of Helminthology. 80:151-157.

Sures, B. 2008. Host-parasite interactions in polluted environments. Journal of Fish Biology. 73:2133-2142.

Trento, A.E., Alvarez, A.M. 2011. A numerical model for the transpot of chromium and fine sediments. Environmental modelling and assessment. 12:551-564.

Valenta, P., Duursma, E.K., Merks, A.G.A., Rützel, H., Nürnberg, H.W. 1986. Distribution of Cd, Pb and Cu between the dissolved and particulate phase in the Eastern Scheldt and Western Scheldt Estuary. Science of the Total Environment. 53:41-76.

Van den Berg, G.A., Meijers, G.G.A., Van den Heijdt, L.M., Zwolsman, J.J.G. 2001. Dredging-related mobilisation of trace metals: a case study in The Netherlands. Water Research. 35: 1979-1986.

Vitale, A.M., Monserrat, J.M., Castilho, P., Rodriguez, E.M. 1999. Inhibitory effects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab *Chasmagnathus granulate* (Decapoda, Grapsidae). Comparative Biochemistry and Physiology C. 122:121-129.

Zhang, T., Kucharzyk, H.K., Kim, B., Deshusses, M.A., Hsu-Kim, H. 2014. Net methylation of mercury in estuarine sediment microcosms amended with dissolved nanoparticulate and microparticulate mercuric sulfides. Environmental Science and Technology. 48:9133-9141.

Zimmer, A.M., Barcarolli, I.F., Wood, C.M., Bianchini, A. 2012. Waterborne copper exposure inhibits ammonia excretion and branchial carbonic anhydrase activity in euryhaline guppies acclimated to both fresh water and sea water. Aquatic toxicology. 122-123:171-180.

Zwolsman, J.J.G., Berger, G.W., Van Eck, G.T.M. 1993. Sediment accumulation rates, historical input, postdepositional mobility and retention of major element and trace metals in salt marsh sediment of the Scheldt estuary, SW Netherlands. Marine Chemistry. 44:73-94.

Yap, C.K., Ismail, A, Omar, H. and Tan, S.G. 2004. Toxicities and tolerances of Cd, Cu, Pb and Zn in a primary producer (*Isochrysis galbana*) and in a primary consumer (*Perna viridis*). Environment International, 29: 1097-1104.

Wang, C., Wang, X., Wang, B., Zhang, C., Shi, X., Zhu, C. 2009. Level and fate of heavy metals in the Changjiang estuary and ist adjacent waters. Oceanology. 49: 64-72.

Wilson, A., L. 1976. Concentration of trace metals in river waters, a review. Tech. Rep. 16. Water Res. Cent., Medmenhan Lab. and Stevenage Lab., U.K. *in* Förstner U., Wittmann G.T.W. 1979. Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin Heidelberg New York

Wood, C.M., Farrell, A.P., Brauner, C.J. 2012a. Homeostasis and Toxicology of Essential Metals. Elsevier, San Diego USA.

Wood, C.M., Farrell, A.P., Brauner, C.J. 2012b. Homeostasis and Toxicology of Non-Essential Metals. Elsevier, San Diego USA.

Wright, D.A. 1995. Trace metal and major ion interaction in aquatic animals. Marine Pollution Bulletin. 31:8-18.

Wu, Y., Falconer, R., Lin, B. 2005. Modelling trace metal concentration distribution in estuarine waters. Estuarine, Coastal and Shelf Science. 64:699-709.

CHAPTER IV

The previous chapters explored the general physico-chemical mechanisms affecting the environmental fate and effects of pollutants at a whole estuary scale. Now, in the chapter four we look for the case of metal contamination at a local scale in which most of pollution assessments are performed. Results presented here are strongly based on laboratory and field data, complementing the more conceptual scenarios of previous chapters. Our investigation on the specific estuarine processes and parameters that impact metal distribution might contribute to better understand the environmental contamination in biogeochemically complex systems under strong anthropogenic influence.

Unravelling metal mobility under complex contaminant signatures

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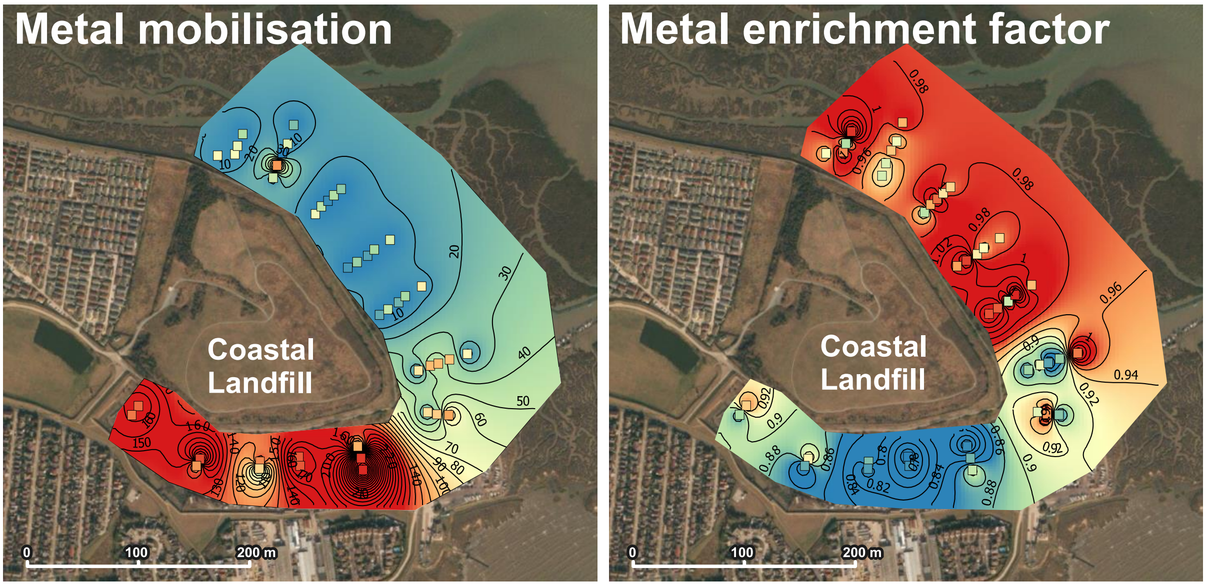
Abstract:

Metals are problematic pollutants in estuaries, where contamination arriving from multiple sources can undergo remobilisation driven by physic-chemical gradients. Environmental concentrations of metals in estuarine sediments are often higher than natural backgrounds, but show no contiguity to potential sources. Thus, estuarine mobilisation could affect contaminant signatures. This study aims (1) to evaluate the potential metal mobilisation under controlled conditions, and (2) to examine whether physico-chemical metal mobility influences pollution levels in an intertidal flat with multiple contaminant sources. Sediments from a saltmarsh adjacent to the Newlands Landfill (Thames Estuary, UK) were exposed (24 h, N = 96, 20 °C) to water at

various salinity, pH, and redox potential in the laboratory. The resultant slurry was analysed for pH, redox potential, and salinity. Major cations, Fe^{2+}, and filtered metal concentrations were analysed in the leachate. Metal remaining on sediments were also determined on Aqua Regia extracts. Salinity, pH and redox affected metal mobilization ($p < 0.001$), e.g. Fe leaching might be increased up to 1000-fold. Measurements in situ of surface (5 cm) and subsurface (up to 4 m) sediment cores revealed that landfill proximity poorly explained metal spatial distribution ($N \sim 600$, $r^2 < 0.15$). However, physicochemical parameters explain up to 97% of geochemically normalized metal concentrations in sediments. Organic matter and pH were dominant factors for most of metal concentration at the sediment surface. At subsurface, major cations (i.e. Ca, Na, Mg and K) were the most determinant parameters. Applying the empirical model obtained in the laboratory to geochemical conditions of the Newlands tidal flat, it was possible to demonstrate that Fe mobilisation regulates the fate of this (and potentially other) metal in that area. Thus, present results highlight the importance of metal mobility to control sediment pollution and estuarine fate of metals. Further studies are required to investigate whether other estuarine systems present similar high metal mobilisation as presented here.

Graphical abstract:



Highlights:

- Laboratory experiments and field survey were performed for pollution assessment.
- Contaminated estuarine sediments leached significant amounts of metals to water.
- Metal mobilisation varied up to 1000-fold depending on sediment chemistry.
- Mobility determined sediment metal levels, hindering assessment of contamination sources.
- Assessment of metal mobility in estuarine sediments is essential to infer environmental impacts.

Keywords: coastal landfill, contaminant mixtures, estuaries, pollution assessment, sediment geochemistry.

1. Introduction

Estuaries are transitional ecosystems between land and sea that provide a multitude of environmental services (Millennium Ecosystem Assessment, 2005). These areas are amongst the most biologically productive of the planet, and therefore of extreme relevance for marine and freshwater biodiversity protection. Additionally, the provisioning of privileged access to marine, freshwater and continental resources made estuarine systems preferable places for urbanization and industrialization (Kennish, 1991). In fact, 11 out of 15 current largest cities in the world are coastal, and many important large metropolitan areas of current commercial and industrial centres, such as London, New York, Shanghai are situated in estuarine systems. Therefore, estuarine environments have globally experienced both historical and current intense anthropogenic activities and consequent contamination (Chapman and Wang, 2001; Ridgway and Shmmied, 2002).

Metal contaminants have been traditionally problematic in estuaries because they trend to accumulate in those systems and present non conservative behaviour (Machado et al. 2016). Several hydrodynamic (Bai et al. 2015), anthropogenic (Machado et al. 2016) and geochemical (Betty et al. 1996) estuarine features account for the concentration of metals in estuaries. However, it is of great relevance for all of those features the interaction between metals and sediments. In fact, metal-contaminated sediments are commonly reported as potentially problematic for the quality estuarine waters and benthic organism worldwide (Bianchi, 2007). For instance, legacy contaminated sediments in estuaries from Southeast England might display up to 70% of bioavailable metals, many of which, at levels that threat environmental services and wildlife (Spencer and MacLeod, 2002). In fact, many estuaries receive substantial inputs of sediment-bound metals such as Cd, Cu, Cr, Hg, Pb, Zn (Zwolsman et al. 1993, Attrill and Thomes, 1995) that concentrate in the sediment at levels up to 1000000-fold higher than the water concentrations (Föster and Wittmann, 1979).

On the other hand, the non-conservative behaviour of metals (Machado et al. 2016) driven by physic-chemical mobilisation hampers the assessment of pollution sources and misleads inference about pollution levels. Many of the physic-chemical and biological gradients present in estuaries have potential to affect the metal speciation, partitioning, transport, and consequently spatial distributions (Zwolsman et al. 1993). For instance, the periodic flooding and air exposure of sediments on intertidal areas provoke a cyclic variation on redox, with consequent changes on speciation and cycling of several diagenetically mobile metals (Du Laing et al. 2009c). Similarly, the difference on sediment pH and salinization due to seawater inflow continuously affect metal speciation (Du Laing et al. 2009a, b). In fact, contamination within sediments cannot be considered permanent because metals may be released to the water column via diagenetic remobilisation or physical/biological reworking of sediment (Zwolsman et al. 1993, O'Shea and Spencer. 2016). As a result, complex contaminant signatures arising from multiple sources and high environmental mobility make identification of potential impacts particularly challenging.

Notwithstanding, metal pollution is still investigated in estuaries as in fully fresh or marine sediments, i.e. in terms of continuity of high contaminant levels to the contamination sources (Chapman and Wang, 2001). This might obstruct a realistic understanding of the estuarine pollution in terms of how much metal might be mobilised from the sediment to the bioavailable fraction and its impact on biota (Chapman and Wang, 2001). In the present study, laboratory experiments suggested that metal release from sediments is significant and strongly depends on the geochemistry, varying several orders of magnitude within environmentally relevant ranges of physicochemical parameters. We additionally demonstrate that metal enrichment factor, perhaps the most common proxy of metal pollution, is strongly determined by the estuarine geochemical gradients even in a area surrounded by multiple potential contamination sources. Therefore, we highlight the importance of metal mobility in estuarine sediments and suggest it to be considered for heath assessments and pollution source identification of estuarine areas.

2. Material & Methods

2.1 Terminology and definitions

For the purpose of the present study we define some terms that are broadly used in pollution science with ambiguous meaning. The term "metal immobilisation" is used here as the predominance of adsorption, absorption, precipitation and co-precipitation processes that remove metal from the water column to a solid sedimentary phase. The term "metal mobilisation" is referred here as the sum of physic-chemical and biological processes complementary to immobilisation that result into a transfer of metal as dissolved or particulate to the aqueous phase with potential to be transported by water hydrodynamics. The term "metal mobility" is thus defined as the interaction of immobilisation and mobilisation forces, in which low metal mobility implies high immobilisation whereas high metal mobility implies high mobilisation.

2.2 Study area

The study area is situated on an intertidal flat of the Thames Estuary (south east England, 58° 21’ 25” N and 18° 37’ 37” E) under influence of multiple contamination sources, including the Newlands historic landfill, docking piers, and several urban drainages (Fig. 1). In the following paragraphs we briefly describe the historic and current pollution status of Thames waters and the studied tidal flat.

The Thames Estuary is a tidal dominated estuary with strong anthropogenic influence and noticeable current and legacy metal contamination (Spencer and MacLeod, 2002; van der Wal and Pye, 2004). The area surrounding the Thames Estuary is amongst the first industrial centres in the world, and before 1960s the river was treated mostly as a lifeless ‘open sewer’ (Attrill and Thomes, 1995). Additionally, innumerous landfills were constructed on its tidal areas without much regulation and control of the material deposited (O’Shea and Spencer. 2016). In fact, low-lying coastal estuarine lands, for being prone to tidal flooding had low economic and agricultural value and hence provided suitable locations for waste disposal proximal to urban areas (O’Shea and Spencer. 2016). Such coastal landfills were often meant to be function as coastal defenses, and they are currently under risk of erosion due to a combination of sea level rise, increased frequency of high energy storm events, salinity intrusion and other related global change phenomena (Spencer and O’Shea, 2014). Despite the frequent pollution problems, the Thames Estuary was absent of comprehensive studies on metal on water or sediment until the 1990s (Attrill and Thomes, 1995). Early studies on this sense found that the Thames as a whole exhibited high concentration of most metals, with highest levels being observed in the in most developed part on the upper estuary that runs through central London and presented large numbers of storm drains (Attrill and Thomes, 1995).

It is worth to mention that salt marshes of the Thames estuaries are particularly sensitive to global changes since they are currently threatened by rapid erosion, increased wave energy, sediment loss and vegetation dissection (van der Wal and Pye, 2004). This sediment reworking enhances the potential for chemical release of sediment-bound contaminants, such as those sorbed to sediments as a result of the natural attenuation of landfill leachates (O’Shea and Spencer. 2016).

The Newlands Landfill, hereafter referred as Newlands, is a historical coastal landfill situated on the northern bank of lower portion of the Thames Estuary (Essex, UK). As many other historic coastal landfills from western Europe, Newlands was constructed with no basal or side wall engineering, which allowed release of contaminated leachate and relayed on its attenuation by surrounding sediments (O'Shea and Spencer. 2016). The site was actively receiving waste from 1954 to 1989, when it was dumped approximately 1,000,000 m^3 of diverse toxic waste (Caulmert Limited, 2011a, b). Due to the age of the Newlands, it is likely that most of contaminants were already leached to adjacent sediments and that the landfill is currently at methanogenic degradation state (O'Shea and Spencer. 2016). The Newlands is currently underneath the recreational Canvey Heights Country Park. At the south side of the Newlands there is the Oyster Creek, in which many storm water drains and docks from the Small Gains Marina and the Island Yatch Club are located.

An earlier work of ours suggested that in that intertidal area Cr, Cu, Pb and Zn were provided by anthropogenic source, with potential for these sediments to act as a present day contamination source (O'Shea and Spencer. 2016). Differently, Ca, K, Mg and Sr were found likely from a lithogenic source or association to clay minerals (O'Shea and Spencer. 2016). Therefore, these metals in addition to Fe and Mn (redox cycling metals) will be considered in the present work when discussing metal mobility. For practical constrains, only selected examples of individual metals are shown on the results and discussion session. Interested readers are encouraged to contact the authors the get additional data and figures eventually not available in the manuscript of supplementary material.

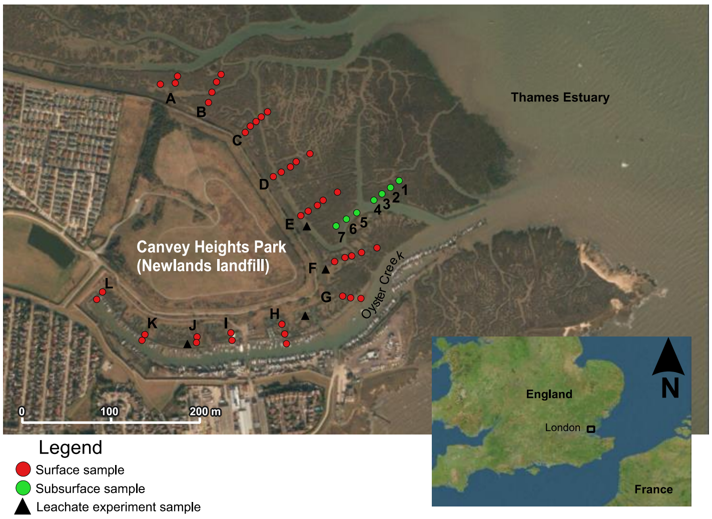


Figure 1: Schematic view of the study area: intertidal flats adjacent to the Canvey Heights Country Park (Essex, UK), and under influence of the historic Newlands landfill and Oyster Creek Docks. The transects for surface samples are denoted by uppercase letters (A-L), while subsurface samples were represented by numbers (1-7).

2.3 Laboratory experiments to quantify leaching potential

The main goal of this experiment was to quantify the potential of metal leaching on surface sediments of the tidal flats adjacent to Newlands. For that surface sediment rings (2 cm) were collected in 4 points (24 samples each) of the intertidal mud flat (Fig. 1). The samples were then wrapped with cling film, brought to the Geography laboratory of Queen Mary, University of London in refrigerated coolers, and stored at 4 °C for up to 4 weeks. Half of the samples (12 from each sampling point) were air exposed during 96 h as an attempt to increase redox gradients. These air exposures were performed in incubator at 20 ± 1 °C and humidity was kept approximately constant by placing the sediment rings over wet paper filters. No significant changes on redox potential of the slurry was observed after the air exposure, and redox potential remained with the natural variation (-229 to -1 mV).

The leachate experiment consisted to measure metal transferred from the sediment to aqueous phase at various redox, salinity and pH. The experimental slurry was prepared with 10 ± 0.1 g sediments placed on 50 mL conical centrifuge plastic tubes with 25 mL of nanopure water. The original pH of this sediment slurry was recorded with pH probe (VWR pH110), and ranged from 7.36-8.47. Four treatment of salt addition to the experimental slurry (0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt -Sigma Aldrich) were considered to investigate effects of salinization on metal mobility. The mass of marine salt added would be enough to have a salinity of leachate respectively to 0, 5, 15 and 30 if complete dissolution occurred. It was not the case since sediment buffered salinity adjustments. A summary of effect of salt addition on salinity and major ion concentration on the leachates is presented on supplementary figure A. 1, and supplementary table A. 1.

After salt addition, the original pH was modified by adding hydrochloric acid 1 N or sodium hydroxide 1N. This pH was recorded as pH initial, which ranged from 5.88-9.09. The experimental slurry (with modified salinity and pH) was then gently shaken for 24 ± 1 h at 24 ± 2 °C. After this period pH was recorded as pH final, which varied from 6.32 to 8.64. Redox potential of the slurry was also determined with a redox probe (VWR pH110). The experimental slurry was then centrifuged for 10 min at 3000 rpm, and the resulting supernatant was considered the leachate for the purpose of the present study. Salinity in the leachate was measured, and 1mL of it was immediately preserved for Fe^{2+} analysis. The remaining leachate was passed through filters with 8 µm mesh (Whatman 542) for later ICP OES analysis of total leached metal. During this filtering much of the colloidal metal was retained, as discussed in the supplementary material A (Box A. 1).

2.4 Field assessment

The sampling procedures and metal distribution on the tidal flat adjacent to Newlands has been extensively explored in O’Shea and Spencer (2016). Hence, we describe here only essential information and interested readers are referred to O’Shea and Spencer (2016). In total, 43 surface grab samples were taken every 50 m in triplicate along 12 radial transects perpendicular to the site boundary (Fig. 1). Additionally, seven sediment cores were extracted for subsurface samples at 10 m intervals along a transect perpendicular to the site boundary using a polycarbonate pipe for the first 30 cm or a Russian corer for deeper samples (Jowsey, 1966). Samples were labelled, and transported to the laboratory in refrigerated coolers, where they were kept at -12° C until required.

Subsurface were analysed at 5 cm intervals for the top 1 m, then 10 cm intervals at depths below this. Samples were then homogenized, and split, with a sub-sample used for pH, particle size and organic matter content while the other was freeze-dried for 24 hours for later metal analysis. Original pH was measured in these samples as in the leachate experiments.

The surface sediments were classified as silty clay or clayey silt (Shepard, 1954), with predominance of mud-sized (<3.9 µm) and silt-sized (3.9-63 µm) sediment fractions (O’Shea and Spencer. 2016). There was only little spatial variation on surface grain size. However, subsurface sediment shifted from clayey silt near the sediment surface to silty sand at depth (deeper than 70 cm) (O’Shea and Spencer. 2016).

2.5 Physic-chemical parameters analysed

A broad suit of geochemical parameters influencing metal mobility were measured in both leachate experiment and field assessment data. We describe here only methods, accuracy and precision for the laboratory leachate experiments, as the methods for the field assessment were similar and carefully explained in O’Shea and Spencer (2016).

2.5.1 Water percentage and Loss of ignition

Water percentage was quantified by the mass difference between overnight dried (40 °C) sediment and the initial a wet mass (24.2 ± 3.3 g). Loss of ignition (LOI) was then measured as a proxy for organic matter content on the sediment. For that a mass of 13.4 ± 2.2 g of dried sediment was ignited in a furnace (550 °C, 5 h). The difference on weight before and after ignition was interpreted in terms of percentage of organic matter combusted.

2.5.2 Aqua Regia extraction

Trace metals were extracted from all sediment samples on a hotplate using Aqua Regia (HNO3:3HCl). This method provides an insight into the potential environmental mobile and bioavailable metal. Therefore, such metal extracted is considered as environmentally available metal (National Water Research Institute, 2001) and this terminology is adopted here.

Sediment from metal leaching experiment and from field assessment were freeze-dried overnight (vacuum, -56 °C). The extraction involved placing 0.50 ± 0.02 g of sediment on Erlenmeyer flasks, adding freshly prepared Aqua Regia, and heat this mixture for in hot plate (5 h, ~ 85 °C) (Chen and Ma, 2001). The Aqua Regia was then filtered (542 Whatman, 8 µm) and made up to 50 mL with nanopure water. To assess accuracy of metal extraction additionally 2 certified materials (SUD1-Environment Canada, and the CRMLGC 6187) and method blanks were analysed in triplicate (Table 1). Also, 2 samples were measured in duplicate, which were considered together with the certified materials to assess extraction precision (Table 1). Metal extracts were kept at 4 °C until metal quantification.). All the materials in contact with metals during leachate experiment and Aqua Regia extraction were washed with nitric acid 10 % during 24 h and rinsed 3 times with nanopure water. All the chemicals used in the present study were analytical grade.

Table 1: Overall accuracy and precision for metal analyses in leachate and sediments

	ICP OES		Metal extraction	
Metal	Accuracy (%)[a]	Precision (%)[a]	Accuracy (%)[b]	Precision (%)[c]

Al	91.2	2.3	67.9	5.1
Ca	104.4	4.6	96.8	7.9
Co	102.7	1.9	86.4	4.2
Cr	103.4	2.1	94.3	3.7
Cu	101.6	0.8	97.0	6.9
Fe	107.3	1.9	94.8	2.6
K	73.6	5.3	84.2	21.5
Li	91.6	1.0	109.6	12.2
Mg	105.4	2.1	101.3	1.4
Mn	102.3	2.0	93.4	3.2
Na	80.1	9.4	89.8	19.4
Pb	104.3	2.5	95.3	3.9
Sr	103.5	1.5	81.4	3.6
Zn	101.9	3.3	92.1	6.2

[a] Accuracy and precision based on indoor standards measured 8 times

[b] Accuracy based on duplicate measurements of certified two materials (SUD1- Environment Canada; CRMLGC 6187).

[c] Precision based on duplicate measurements of certified materials (SUD1- Environment Canada; CRMLGC 6187) and two experimental samples that were also measured in duplicate.

2.5.3 *Metal quantification*

Fe^{2+} in the leachate was measured with the colorimetric method based on the reaction of Fe^{2+} with the 1,10- phenanthroline as in to Lee and Stumm (1960). Other metal concentrations on the leachate and sediments were quantified by inductively coupled plasma optical emission spectrometry (ICP OES) on a Varian Vista-Pro ICP OES instrument using internal standard settings of the Geography laboratory of Queen Mary, University of London. All samples were analysed at full concentration for trace metals and 20-fold dilution for major ions. Analytical precision and accuracy is presented on table 1. There was an effect of salinity on quantification of Li, Mn and Sr. This effect was discounted from each sample by decreased the value returned by a linear regression of measured salinity and metal concentration on blanks (supplementary table A. 2). Laboratory standards were analysed every 10 samples to measure drift and maintain analytical accuracy (quantification limits and analytical quality parameters on supplementary table A. 3

2.5.4 Geochemical normalization and enrichment factors

It is well stablished that metal concentrations in sediments vary with grain size, being necessary to normalize environmental metal concentrations (Kerten and Smedes, 2002). Estuarine intertidal areas often contain high levels of fine grained material, i.e. as clay and silt with diameters of <3.9 μm and 3.9 - 63 μm respectively. These sediments present negatively charged surfaces and have large surface/volume ratios that increase cation exchange capacity, and consequently the concentrations of sediment-bound metals (Föster and Wittmann, 1979). Such effect of grain size is long known to impact metal concentration and different types of “normalization” of metal concentration are used to interpret environmental metal concentrations (Kerten and Smedes, 2002).

Normalization consists to divide measured metal concentrations by a proxy element with environmental levels could account for the different adsorption at various grain sizes. Al and Li are amongst the most common normalizing elements (Kerten and Smedes, 2002) and were adopted in the present study. Therefore, metal concentrations presented here were divided by the Al as this metal was used in relevant estuarine geochemistry studies (Zhang and Liu, 2002). Al concentrations were normalized by Li.

Enrichment factor (EF) was also computed as this parameter is perhaps the most common indicator used for environmental assessment of metal pollution. EF was calculated empirically according to equation 1. The background concentrations for the studied area was determined as in O’Shea and Spencer. (2016), i.e. the average metal concentrations between 3.4 and 4.4 m deep of core 2.

$$Equation\ 1 \qquad EF = \frac{\left(\frac{M_s}{N_s}\right)}{\left(\frac{M_b}{N_b}\right)}$$

Where:

EF = Enrichment factor

M_s = Metal concentration in the sample

N_s = Normalizing metal in the sample

M_b = Background metal concentration for the studied area

N_b = Background normalizing metal concentration for the studied area

2.6 Statistical methods

Significant effects of geochemical parameters on metal leaching and sediment metal content in both laboratory experiments and field assessment data were detected with Kruskal-Nemenyi test with Tukey post hoc (Sachs, 1997) or linear regressions with the “lm” function from software R (R Core team 2013). All statistical analyses were performed with a = 0.05.

The empirical linear model derived from the laboratory experiments (Fig. 2) was used to estimate Fe^{2+} leaching from in situ sediments. Then, spatial distribution of Fe, and Fe^{2+} leaching maps were created on the software QGIS 2.14.1 with the interpolation of surface sediment data. Interpolation method was performed weighting by the inverse of the distance, and contour lines were extracted with the function extract raster contour lines for each 0.02 intervals for enrichment factor and each 10 intervals of metal mobilisation intervals.

3. Results and discussion

Assessment of metal pollution in estuaries has been challenging because the various geochemical gradients may affect physical and chemical forces that control metal speciation and partitioning. Shifts of metal partitioning increases metal mobility, with consequent impacts on environmental concentrations in water and sediment (Machado et al. 2016). Such geochemically driven metal mobilisation is often ignored in pollution assessment, which is especially critical in estuarine intertidal areas (Chapman and Wang, 2001). There, the fine sediments loaded with contaminants settle and then undergo diagenetic processes, chemical reactions, and periodic flooding. Since sediment concentrations are often up to 6 orders of magnitude higher than the overlying water column (Förstner and Wittmann, 1979), even a small mobilisation can release a significant quantity of metals (O’Shea and Spencer. 2016), resulting in deleterious effects on the surrounding environment (Bryan and Langston, 1992). In this sense, physic-chemical gradients often mobilize metals from sediments to the water phase, increasing metal bioavailability, and impacting aquatic fauna and flora.

In the next paragraphs we firstly explore the results from laboratory experiments on the potential for metal leaching from estuarine contaminated sediments, indicating the main drivers of metal mobilisation. Secondly, we compare the influence of sediment geochemistry and proximity to potential contamination sources to the levels of metal pollution. Finally, we exemplify the relevance of metal mobilisation to determine metal levels in that tidal flat and how ignoring metal mobilisation might mislead environmental health assessment of estuarine sediments with complex contaminant signatures.

3.1 Potentially high metal leaching in estuarine sediments

After 24 h significant amounts of metals were transferred from sediment to water as function of sediment chemistry. The metal species with highest mobilisation and influence of sediment chemistry was Fe^{2+}. Mobilisation of Fe^{2+} varied from less than 0.2 up to 267.5 µg Fe^{2+} g dry sed^{-1} d^{-1} depending mostly on pH (Fig. 2). Redox potential also affected Fe^{2+} leaching ($p < 0.001$), with maximum leaching occurring between -150 and -50 mV (Supplementary figure B. 1). Salinization presented only mild effects, with more saline slurry yielding leachates with slight higher Fe^{2+} concentration.

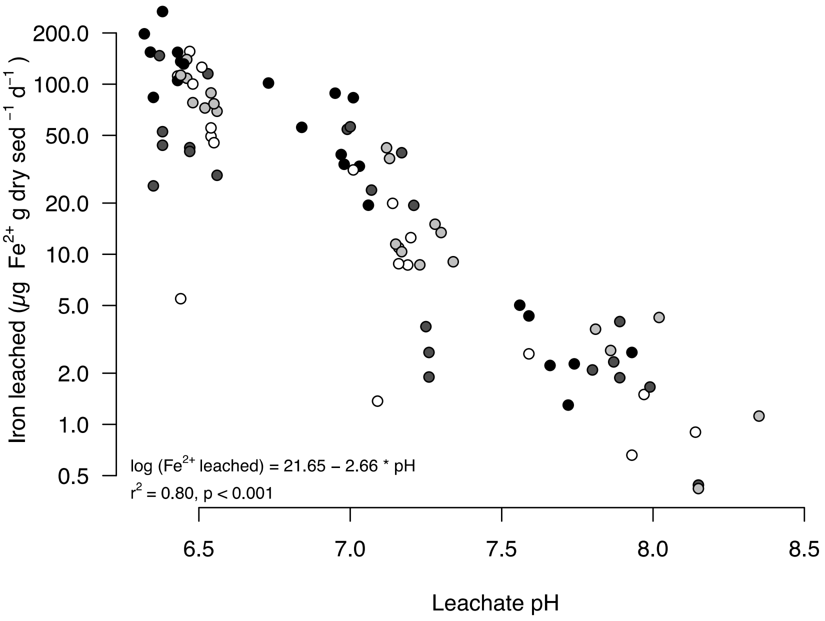


Figure 2: Effects of pH on the mobilisation of Fe^{2+}. The white, light grey, dark grey and black filled circles represent respectively 0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt added to the experimental slurry.

Al, Co, Li, Mn, Pb and Sr similarly presented strong influence of geochemical variables on metal leaching. For the total metal in the leachate filtered fraction, salinity and pH changes jointly accounted for 80% of metal leaching (Fig. 3). In this context, salinization is believed to increase metal leaching due to a combinatory effect of anions and cations in affecting the ionic forces on the slurry (Föstner and

Wittmann, 1979). Saltwater anions as chloride form soluble complexes with weakly adsorbed metals, therefore enhancing solubility (Machado et al. 2016). Saltwater cations further increase metal solubilisation by competing for adsorption sites on the sediment surfaces (Paquin et al. 2003). Likewise, acidification (positive values of pH difference, Fig. 3B) increased mobilisation by enhancing the competition of H^+ ions for metal ligands.

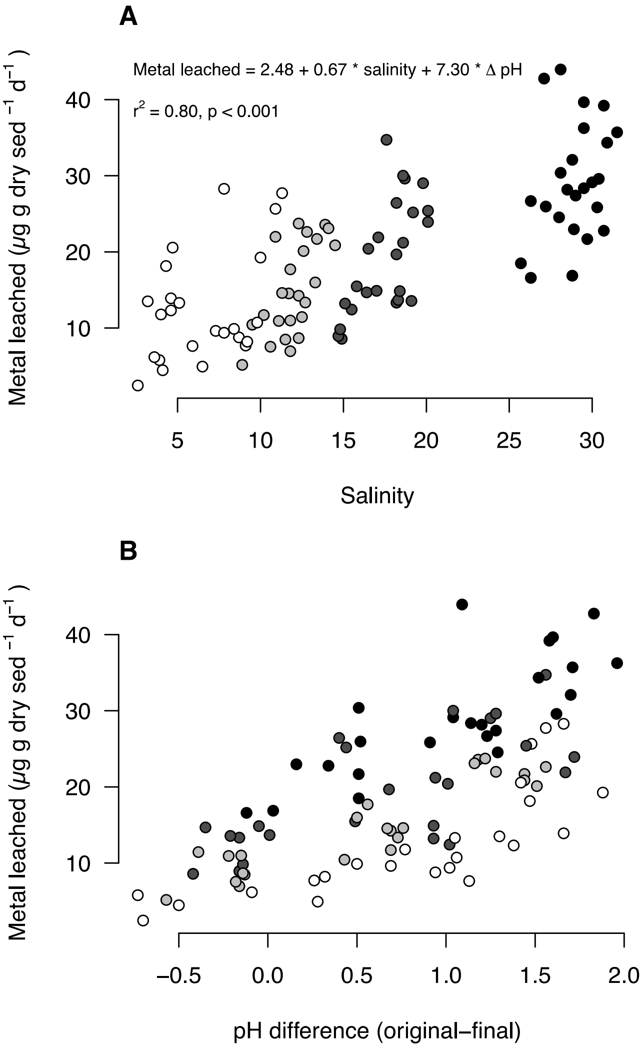


Figure 3: Effects of salinity (A) and pH changes (B) on the mobilisation of metals (∑Al, Co, Fe, Li, Mn, Pb, Sr) from sediment to the leachate. The white, light grey, dark grey and black filled circles represent respectively 0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt added to the experimental slurry.

Such notorious effects of salinization and acidification on the mobility of metals have implications in terms of global changes impacts on industrialized estuaries. Sea-level rise might cause salinization in many estuaries, which might be particularly intense for systems as the Thames Estuary, which faces a 9 mm year^{-1} sea-level rise (last 20 years average according to Environment Agency data) associated to a projected decrease in annual precipitation (Johnson et al. 2009). Additionally, sea-level rise will increase the frequency of flooding, therefore increasing the content of organic matter in the sediments (see section 3.3) and consequently affecting the pH. For instance, Du Laing et al. (2009b) found that decomposition of organic matter in sediments released CO_2 that accounted for the acidification and mobilization of Ca, Fe, Mn, and Ni at low sulphide concentrations. This remobilisation of metals might be especially concerning for many industrialized countries, in which tons of metal contaminated material was disposed in coastal environments. For instance, between 1976 and 1977 about 9,000 tons of metals (∑ Cd, Cr, Cu, Hg, Pb, and Zn) were dumped in British estuarine and coastal systems (Förstner and Wittmann, 1979). Like the Newlands, most of these contaminant receptacles relied on environmental natural attenuation of the pollution plumes. In fact, a recent review found about 5,000 coastal landfills as the Newlands under risk of flooding and/or erosion only in England and Wales (O'Shea and Spencer. 2016). Therefore, the risk of remobilisation of contaminants from historic contaminated estuarine sediments might contribute to exacerbate habitat loss in a context of global changes.

Interestingly, within the range of metal concentrations measured in the present study, the quantity of metal leached was minimally constrained by metal concentrations in the sediment (Fig. 4). For most of metals either no significant or very low correlation indexes ($r^2 < 0.1$) were found for the effect of sediment concentrations on the leached metal. In other words, the concentration of certain metal in the sediment fraction had small effects on leachate concentration, and physic-chemical mobility was the determining factor for the amount of metal mobilised.

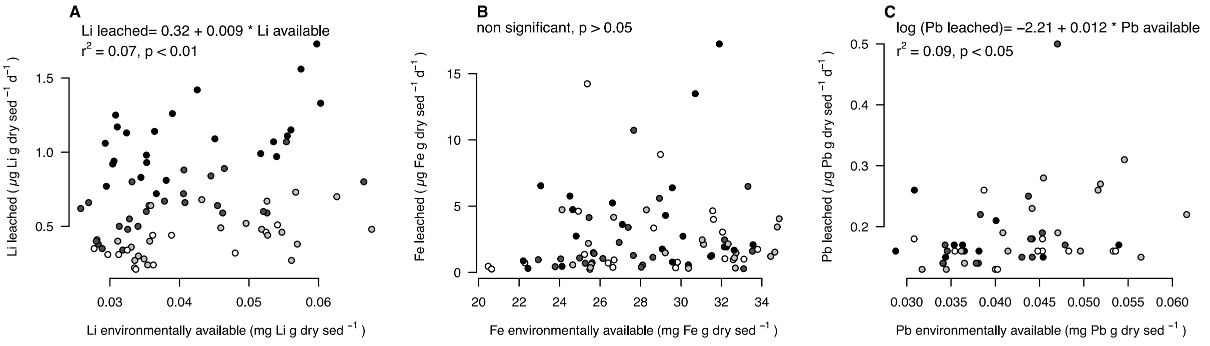


Figure 4: Effects of environmentally available metal on the mobilisation of Li (A), Fe (B), and Pb (C) as examples of lithogenic, diagenetically highly mobile, and anthropogenic metals, respectively. The white, light gray, dark gray and black filled circles represent respectively 0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt added to the experimental slurry.

3.2 *Mobilisation drives in situ metal concentrations on estuarine sediments*

From the field data it is possible to infer that spatial distributions of most metals were not contiguous to the main potential source, i.e. the Newlands or Oyster Creek docks. As exemplified for subsurface in figure 5 (A, B, C) metals levels range between natural (EF < 1) and polluted (EF > 1), and presented little influence of landfill. In fact, only low correlation values (N ~ 600, $r^2 < 0.15$, $p < 0.001$) were found between distance to the landfill and metal concentration. However, environmental concentrations of metals were not randomly distributed (Fig. 5 D, E, F). Normalised concentrations presented characteristic vertical and horizontal distributions that were significantly ($p < 0.001$) explained by geochemistry for all studied metals.

The mechanisms of metal distribution and physic-chemical mobility might vary. For instance, Mn is diagenetically mobile, i. e. trends to be remobilised in reduced conditions at depth in the profile and precipitate near the surface (Förstner and Wittmann, 1979; Du Laing et al. 2009a, b). Pb in those sediments is believed to represent the anthropogenic influence through the peak of industrialization from late 1800s to 20th century (O'Shea and Spencer. 2016). The proportionally wider scattering of Pb distribution found in the current work implies a high environmental mobility. Finally, Sr has its fate strongly determined by behaviour of Ca (Section 3.3). Thus, sediment chemistry was influential for all normalised metal concentrations through many mobility mechanisms.

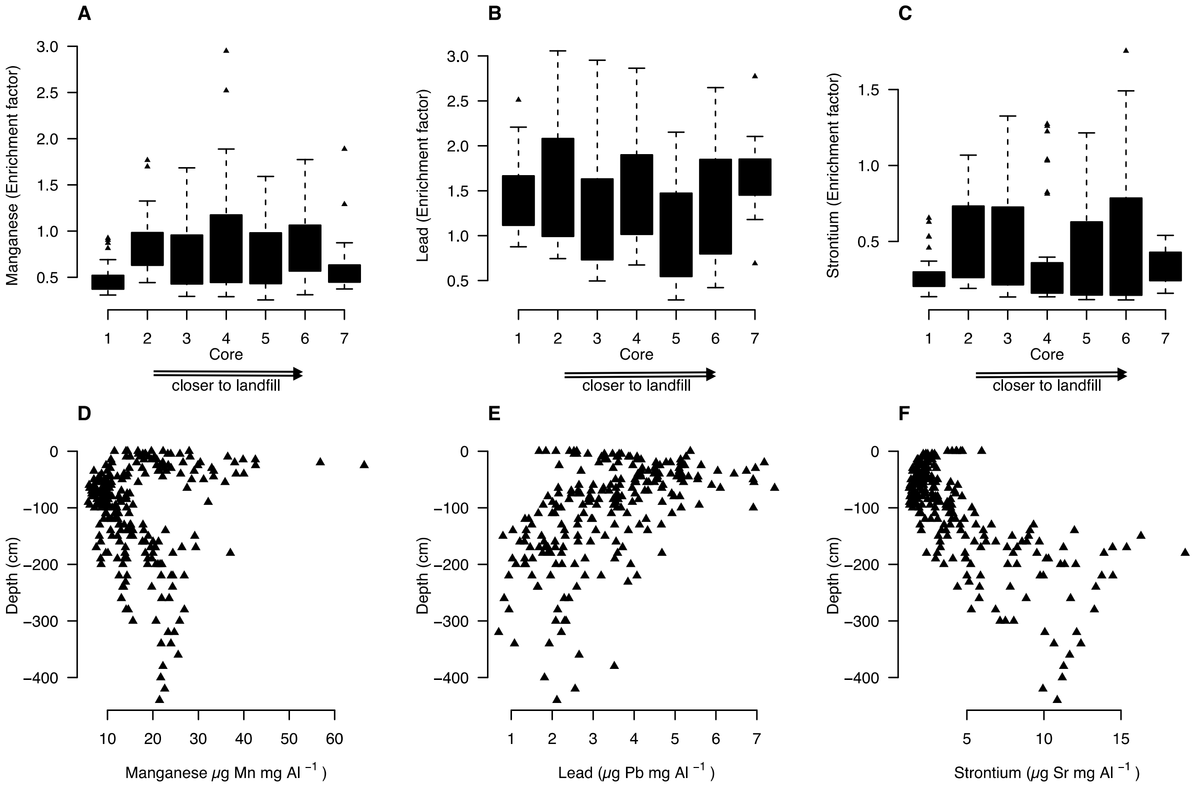


Figure 5: Environmental behaviour of selected metals on subsurface sediments. Mn was selected as an example of diagenetic mobility dominated by redox gradients. Pb was selected as it is believed as anthropogenic (O'Shea and Spencer. 2016). Sr was selected as an example of metal mobility strongly dominated by the fate Ca. A-C: Subsurface metal distributions displaying low contiguity to main potential metal contamination source (upper and lower limits of boxes represent respectively 75 % and 25 % quartiles, bar limits represent 99 % and 1 % quartiles, small triangles are extreme values). D-F: Metal distributions displaying high dependence of geochemical gradients (triangles represent individual measured data).

In the next paragraphs it is shown selected examples of metals that presented representative effects of geochemistry on their pollution levels (either increasing or decreasing). It might be worth to mention that generally the behaviour of Cu, Co, Zn and Pb displayed similar patterns. Also, Fe and Mn presented similar behaviour. Concentrations of Fe and Mn were correlated to most of trace metals ($p < 0.01$) in surface and relationships commonly faded with increasing depth. Sr and Cr presented peculiar behaviour as discussed in section 3.3.

3.3 Sediment geochemistry and pollution levels

In the present study, surface metal levels in the intertidal area were strongly influenced by pH and organic matter content ($p < 0.001$). The latter was positively correlated to water content and metal concentrations on the sediment (Fig. 6). It suggests that intertidal sediments flooded more frequently or for longer periods might present higher organic matter content, which provided important ligands for metal immobilisation. It has been noticed that increasing flooding generally enhances organic matter content on sediments due to less optimal degradation conditions (Du Laing et al. 2009b). Nevertheless, the effect of organic matter on the concentration of trace metals is commonly ignored in environmental health assessment of metal pollution. Organic matter must be taken into account when assessing metal contamination in intertidal areas. Du Laing et al. (2009a) found that a 25 % increment in organic matter might result in 200-fold increase in metal concentrations. In the present results, enrichment factor shown a much more modest sensibility to organic content. Notwithstanding, metal immobilisation by organic matter was noteworthy and ignoring it might further contribute to hamper identification on the reason of metal accumulation in estuarine sediments and lead to false positive indication of presence of anthropogenic sources.

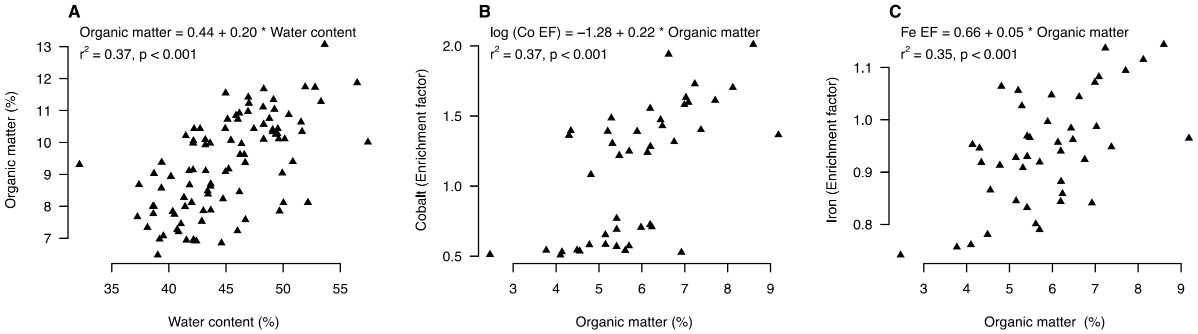


Figure 6: Environmental behaviour of organic matter and selected metals on surface sediments. A: organic matter increases with water content. B: Relationship of cobalt and organic matter. C: Relationship of iron and organic matter.

The figure 6 (B, C) exemplifies the effect of organic matter on the environmental levels of a trace potentially anthropogenic metal (Co) and a natural redox sensitive metal (Fe). In turn, Fe influences the concentration of many trace metal through precipitation and co-precipitation during the formation of oxy-hydroxide iron colloids (Fairbrother et al. 2007; Du Laing et al. 2009c). Therefore, a synergistic effect on organic matter and redox cycling metals (as Fe and Mn) on the immobilisation of trace metals cannot be discarded.

In the figure 6B it is also possible to distinguish 2 clusters of data on the relationship between organic matter and Co, which were also observed for Cu, Pb, Cr and Zn. The upper cluster (EF > 1) represent the samples taken from the intertidal areas facing the main channel to the Thames Estuary (transects A-E, Fig. 1), in which pH of the sediment was higher (O'Shea and Spencer. 2016). While the lower cluster of data (Fig. 6B, EF < 1) are sampling points under the influence of Oyster Creek (transects F-L), where a drop of approximately a unity of pH was observed. From the current results it is impossible to speculate whether the changes on pH are purely natural or have an anthropogenic influence. Notwithstanding, the gradient on sediment pH clearly influenced the metal behaviour in sediment surface samples. Enrichment factors smaller than 1 were observed for most of metal in the area of potentially higher contamination (e.g. under influence of the Newlands, adjacent docks, and with more enclosed hydrodynamic). In fact, the sum of surface levels of the various metals considered in the present study (∑ Al, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sr, Zn) could be also described as function of sediment pH (r^2= 0.46, $p < 0.001$; Supplementary material B. 2), which exemplifies the decisive effect of surface sediment pH on environmental metal levels.

The effect of pH was not consistent throughout the sediment profile. Despite the strong correlation on the surface, subsurface sediments presented weaker or even reverse relationship with pH (Fig. 7 A-B). This ambiguous effect of pH on metal concentrations on surface and subsurface reflect the different processes taking place at these two compartments. Surface sediments (top 5 cm layer) are successively exposed to air and water. Metal solubilized by lower pH values on the surface sediments can be easily transferred to the water column and removed from the intertidal area within a tidal cycle. Thus, ph-driven mobilisation in the surface sediments imply decreasing levels of metals. On the other hand, metals solubilized in the subsurface sediments move much slower. Maximum advection of water within sediment layers is of order of 10^{-6} m s^{-1} (Brand et al. 2013). Therefore, the pH-driven mobilisation in subsurface might instead transport metals to places of preferential accumulation, where interaction with strong metal binding species (e.g. sulphide) could actually increase metal levels.

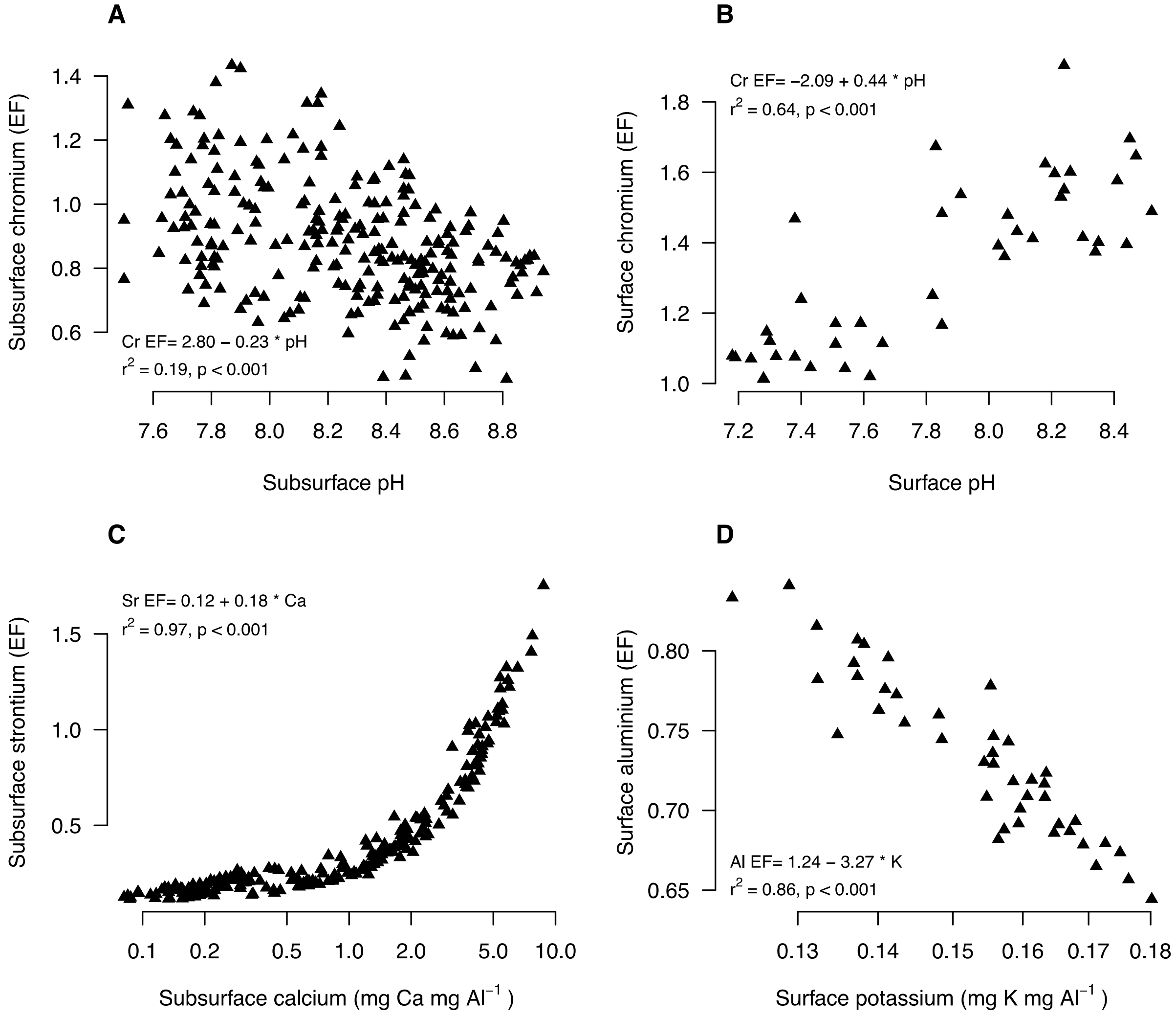


Figure 7: Geochemistry determining pollution levels, in terms of enrichment factors (EF), of trace metals on both surface and subsurface sediments. Chromium is differently influenced by pH in surface (A) and subsurface (B) sediments. Precipitation and co-precipitation (C) and competition (D) with major cations influences Sr and Al levels, respectively.

Moreover, subsurface sediments undergo several diagenetic processes that also affect metal fate. For instance, Sr distribution is strongly determined by the fate of Ca (Fig. 7C). For being and alkali Earth metal, Sr presents high affinity for carbonates (in which it can readily replace Ca) (Lerouge et al. 2010). Therefore, a decrease of pH in the sediment profile would cause decalcification, with consequent dissolution of carbonates and release of both Ca and Sr (Du Laing et al. 2009b). The relationship between Sr and Ca was also present in the surface but with smaller explanatory power ($r^2 = 0.53$, $p < 0.001$). It might be worth to mention that stronger correlation at depth were also observed for Mg, Na, K and many trace metal enrichment factors (supplementary material B.4), suggesting that diagenetic provisioning of adsorption/

co-precipitation sites, and the interaction with sea water ions might have a predominant role on the mobility of metals within the subsurface sedimentary layers. Such influence of major cations (i.e. alkaline and alkali Earth metals) on the behaviour of trace metals was weaker at surface. The only evident exception was the relationship between Al and K (Fig. 7D). At surface and subsurface geochemically normalized Al concentrations significantly ($p < 0.001$) decreased with increment of K concentrations. In any case, regarding metal pollution levels, the presence of major cations might be considered influential at the sediment surface and determining at subsurface.

Taking altogether, the results from the field assessment of metal levels also suggest that sediment geochemistry determines contamination levels of most of metals in this tidal flat and partially accounts for the complex contaminant signatures. The most geochemical influential parameters at surface were pH and organic matter content, followed by major cations and Fe-Mn. In subsurface, the diagenetic processes and slower advection favoured the higher influence of major cations on metal concentrations.

3.4 *High metal mobility in situ on estuarine sediments*

The results presented here from laboratory leachate experiment and field assessment of geochemical influence on metal levels suggest that potentially high metal mobilisation might occur within legacy contaminated estuarine sediments. Using the equation presented on figure 2 it was possible to confirm that metal mobilisation is determining the environmental concentrations of metals despite the strong anthropogenic influence in this tidal marsh (Fig. 8 A-B). Fe enrichment factors were higher in the tidal flats, away from the potential main anthropogenic sources (Fig. 8A). As mentioned earlier, several trace metals presented similar pattern. Instead denoting absence of impacts, the low enrichment factors of several metals in the area of Oyster Creek denounce the ~1000-fold higher metal mobilisation in that area. In other words, it means that area is exporting significant amounts of metals to the adjacent waters. In the case of Fe^{2+} the main driver of mobility was the gradient of sediment pH (O'Shea and Spencer. 2016) (Fig. 8B). As it has been extensively demonstrated that Fe correlates with the fate of other metals (Fairbrother et al. 2007; Machado et al. 2016; Prajith et al. 2016), it is likely that this factor also explains the variability observed in the concentrations of trace and more anthropogenic metals.

Notwithstanding, for the other metals, it is sensible to expect that salinity might also play important role.

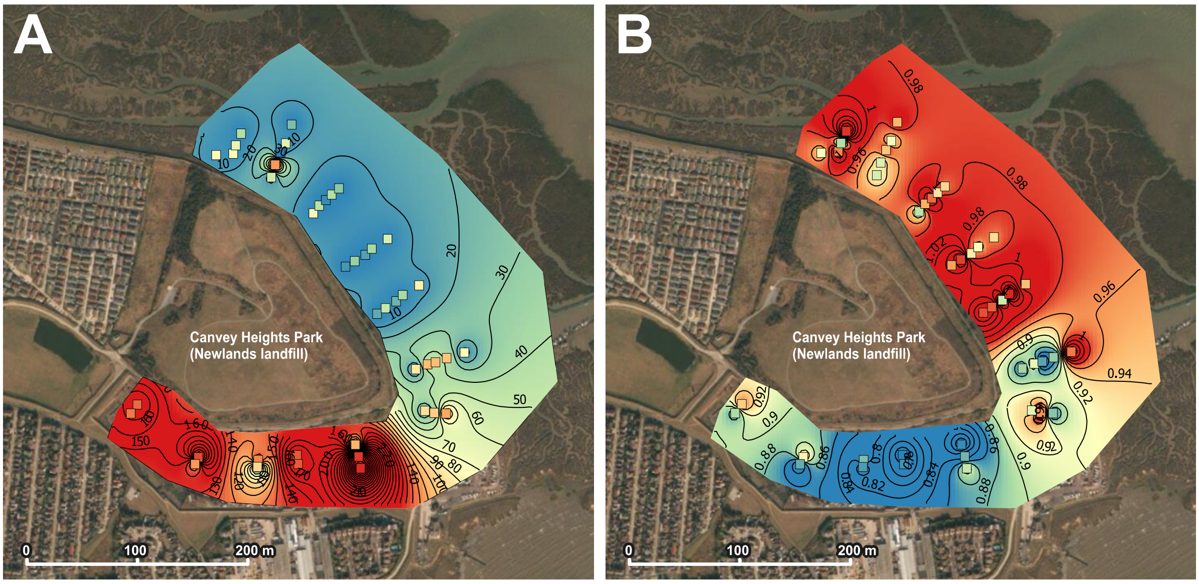


Figure 8: Effect of metal mobility on pollution levels. A: Iron leaching (µg Fe^{2+} g dry sed^{-1} d^{-1}) was negatively correlated to iron enrichment factor (B) in the sediment surface ($r^2 = 0.42$, $p < 0.001$). In both figures squares represent measured points that were interpolated for the whole area.

4. Conclusions and future directions

In a recent study, O'Shea and Spencer. (2016) identified that the Newlands was able to rise sediment metal levels within a radius of 15 m of the landfill edge. As in many estuaries, after this short influence zone, metal levels were generally high (comparable to other industrialized estuaries) but without continuity to the main anthropogenic sources. Our results demonstrate that the real impacted zone might be much larger because sediment geochemical gradients affected metal mobility and determined pollution level for all studied metals in such system. Metal mobility was found to potentially transfer significant amounts of metals from sediment to the aqueous phase in the laboratory. In the field, gradients of pH and organic matter were the most important in the surface for most of metals, while major cations were decisive on the subsurface. The comparison of leachate empirical model with environmental levels ratifies that metal mobilisation is an important variable for the pollution levels in estuarine tidal sediments. Further studies are required to verify whether the empirical models for mobilisation provided here are valid for other estuaries in order to derive a method to isolate the effect of metal mobility and allow inference of the real impact anthropogenic sources of metal contamination in estuarine environments.

5. Acknowledgements

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6. References

Attrill M.J., Thomes M. 1995. Heavy metal concentrations in sediments from the Thames Estuary, UK. Marine pollution Bulletin. 30: 742-744.

Betty, N.G., Turner, A., Tyler, A.O., Falconer, R.A., Millward, G.E. 1996. Modelling contaminant geochemistry in estuaries. Water Res. 30: 63-74.

Brand, A., Lewandowwski, J., Hamann, E., Nützmann, G. 2013. Advection around ventilated U-shaped burrows: A model study. Water Res. 49: 2907-2917.

Bianchi, T.S. Biogeochemistry of Estuaries. Oxford University Press, New York.

Bryan, G.W., Langston, W.J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ. Pollut. 74: 89-131.

Caulmert Limited. 2011a. Hadleigh Sea Wall Landfill Site. Essex County Council, Annual Monitoring Report.

Caulmert Limited. 2011b. Newlands Landfill, Annual Monitoring Report, Bangor, Caulmert Limited.

Chapman, P.M., Wang, F. 2001. Assessing sediment contamination in estuaries. Environ. Tox. Chem. 20: 3-22.

Chen, M., Ma, L.Q. 2001. Comparison of Three Aqua Regia Digestion Methods for Twenty Florida Soils. Soil Sci. Soc. Am. J. 65: 491-499.

Du Laing, G., Meers, E., Dewispelaere, M., Vandescasteele, B., Rinklebe, J., Tack, F.M.G., Verloo, M.G. 2009a. Heavy metal mobility in intertidal sediments of the Scheldt estuary: field monitoring. Sci. Total. Environ. 407: 2909-2930.

Du Laing, G., Meers, E., Dewispelaere, M., Rinklebe, J., Vandescasteele, B., Verloo, M.G., Tack, F.M.G. 2009b. Effect of water table level on metal mobility at different depth in wetland soils of the Scheldt estuary (Belgium). Water Air Soil Pollut. 202: 353-357.

Du Laing, G., Rinklebe, J., Vandescasteele, B., Meers, E., Tack, F.M.G. 2009c. Trace metal behavior in estuarine and riverine floodplain soil and sediments: a review. Sci. Total. Environ. 407: 3972-3985.

Fairbrother, A. Wenstel, R., Sappington, K., Wood, W. 2007. Framework for metals risk assessment. Ecotoxicol. Environ. Saf. 68: 145-227.

Förstner, U. Wittmann, G.T.W. 1979. Metal pollution in the Aquatic Environment. Springer-Verlag, Berlin Heidelberg New York.

Johnson, A.C., Acreman, M.C., Bunbar, M.J., Feist, S.W., Giacomello, A.M., Gozlan, R.E., Hinsley, S.A., Ibbotson, A.T., Jarvie, H,P, Jones, J.I., Longshaw, M., Maberly, S.C., Marsh, T.J., Neal, C., Newman, J.R., Nunn, M.A., Pickup, R.W., Reynard, N.S., Sullivan, C.A., Sumpter, J.P., Williams, R.J. 2009. The British river of the future: How climate change and human activity might affect two contrasting river ecosystems in England. Sci. Total Environ. 407: 4787-4798.

Jowsey, P.C. 1966. An Improved Peat Sampler. New Phytologist. 65: 245-248.

Kennish, M.J. 1991. Ecology of Estuaries: Anthropogenic Effects. CRC Press, Florida.

Kersten, M., Smedes, F. 2002. Normalization procedures for sediment contaminants in spatial and temporal trend monitoring. J. Environ. Monit. 4: 109-115.

Lee, G.F., Stumm, W. Determination of ferrous iron in the presence of ferric iron with bathophenanthroline. Journal American Water Works Association. 52: 1567-1574.

Lerouge, C., Gaucher, E.C., Tournassat, C., Negrel, P., Crouzet, C., Guerrot, C., Gautier, A., Michel, P., Vinsot, A., Buscchaert, S. 2010. Strontium distribution and origins in a natural clayey formation (Callovian-Oxfordian, Paris Basin, France): A new sequential extraction procedure. Geochimica et Cosmochimica Acta 74: 2926-2942.

Machado, A.A.S., Spencer, K., Kloas, W., Tofollon, M., Zarfl, C. 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. Sci. Total Environ. 541: 268-281.

O'Shea, F.T., Spencer, K. 2016. Metal distribution in sediments adjacent to an historical landfill. *Manuscript in preparation.*

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Wetlands and Water Systhesis. World Resources Institue, Washington, DC. USA.

National Water Research Institute. 2001. Recoverable and leachable elements in SUD-1. National Water Research Institute. Burlington, ON, Canada.

Paquin, P.R., Farley, K., Santore, R.C., Kavadas, C.D., Mooney, C.G., Wu, K.-B., Di Toro, D.M. 2003. Metals in aquatic systems: A review of exposure bioaccumulation, and toxicity models. SETAC, Pensacola.

Prajith, A., Purnachandra Rao, V., Chakraborty, P. 2016. Distribution, provenance and early diagenesis of major and trace metals in sediment cores from the Mandovi estuary, western India. Estuarine, Coastal and Shelf Science. 2016. 170: 173-185.

R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/. (Accessed 9 Nov 2013).

Ridgway, J., Shimmield, G. 2002. Estuaries as repositories of historical contamination and their impacts on shelf seas. Estuarine, Coastal and Shelf Science. 55: 903-928.

Sachs, L. Angewandte Statistik; Springer: Berlin, 1997.

Shepard, F.P. 1954. Nomenclature based on sand-silt-clay ratios. J Sed. Petrol. 5:24.

Spencer, K.L., MacLeod, C.L. 2002. Distribution and partitioning of heavy metals in estuarine sediment cores and implications for the use of sediment quality standards. Hydrol. Earth Syst. 6: 989-998.

Spencer, K.L., O'Shea, F.T. 2014. The hidden threat of historical landfills on eroding and low-lying coasts. ECSA Bull. 63: 16-17.

van der Wal, D., Pye, K. 2004. Patterns, rates and possible causes of saltmarsh erosion in the

Greater Thames area (UK). Geomorphology. 61: 373-391.

Zhang, J. Liu, C.L. 2002. Riverine composition and estuarine geochemistry of particulate metals in China- Weathering features, anthropogenic impacts and chemical fluxes. Estuarine, Coastal and Shelf Science. 54: 1051-1070.

Zwolsman, J.J.G., Berger, G.W., Van Eck, G.T.M. 1993. Sediment accumulation rates, historical input, postdepositional mobility and retention of major element and trace metals in salt march sediment of Scheldt estuary. SW Mar. Chem. 44: 73-94.

CONCLUSIONS AND FUTURE DIRECTIONS

General conclusions and future directions

There is significant evidence that human activities rival to natural geophysical forces determining the functioning of the Earth Systems (Steffen et al. 2011). The period within it happened is often referred as Anthropocene (Steffen et al. 2007), and it has pervasive implications for baseline changes related to human activity and its deleterious environmental effects on coastal and marine systems. Anthropogenic chemical (e.g. metals, persistent organic pollutants, and emerging contaminants), physical (e.g. microplastics, sediment loads, temperature), and biological (e.g. invasive species) stressors increasingly affect marine and coastal aquatic systems, pushing these environments to a new equilibrium state (Machado, 2016).

Assessing such coastal pollution is particularly challenging because a complex cocktail of contaminants threatens water, sediment and biota (Machado and Combi, 2015). Furthermore, coastal systems are highly dynamic, integrating the physicochemical dynamics of freshwater bodies, estuaries and lagoons with the oceanographic characteristics of adjacent seas (Machado et al. 2016). Thus, monitoring contamination and remediation of coastal ecosystems are among the most complex and current issues in ecotoxicology and environmental management. Many affordable, practical and sensitive multi-contaminant analytical procedures, and conceptual frameworks are still required to interpret pollution effects in a scientifically defensible environmental management perspective (Machado and Combi, 2015). Indeed, holistic approaches taking into account interactions of the intrinsic environmental features are necessary to fight chemical contamination and its biological impacts in our coasts.

In the context of providing practical tools for pollution monitoring in coastal areas, we investigated the problematic of metal contamination in estuaries. In these transitional systems between land and sea, metals arriving from multiple sources can undergo significant remobilisation driven by physic-chemical gradients (Machado et al. 2016). As result, environmental concentrations of metals in estuarine sediments are often higher than natural backgrounds, but show no contiguity to potential sources (O'Shea and Spencer, 2016; Schneider et al. 2016). Such estuarine lack of apparent continuity in the dilution from metal sources towards final receptacles might be interpreted as “non-conservative” metal behaviour (Machado et al. 2016).

We evaluated the potential metal mobilisation in the laboratory and examined the influence of physic-chemical mobility on pollution levels in an intertidal flat (Newlands Landfill, Thames Estuary, UK). Salinity, pH and redox affected metal mobilization, e.g. Fe leaching might be increased up to 1000-fold. Measurements in situ of surface (5 cm) and subsurface (up to 4 m) sediment cores revealed that proximity to main potential sources poorly explained metal spatial distribution. However, physicochemical parameters explain up to 97 % of geochemically normalized metal concentrations in sediments. Organic matter and pH were dominant factors for most of metal concentrations at the sediment surface. At subsurface, major cations (i.e. Ca, Na, Mg and K) were the most determinant parameters. Combining the results from potential leaching from the laboratory to the geochemical parameters measured in the field, it was possible to demonstrate that Fe mobilisation regulates the fate of this and potentially other metals in that intertidal area. Further studies are required to investigate whether other estuarine systems present similar high metal mobilisation as presented here.

In a broad view, the current thesis addressed the lack of practical and conceptual frameworks for monitoring chemical pollution in coastal areas, with special focus on metal contamination on estuaries. According to the European Commission, integrative comprehension of fate and effects of contaminants in different compartments of these transitional environments (estuarine sediment, water, biota) were still required to better establish, assess and monitor the good ecological status targeted by the Water Framework Directive (European Commission, 2012). Therefore, the current document generally contributed to the advance of our current understanding of pollution issues in coastal areas by providing data and multidisciplinary overviews for the fate and effects of metals in estuaries (Machado et al. 2016). The data provided here (chapter 6) and our conceptual model (Machado et al. 2016) included the analysis and integration of biogeochemical processes and parameters, metal chemistry and organism physiology. To the best of our knowledge, we concluded that estuarine physicochemical gradients, biogeochemical processes, and organism physiology are jointly coordinating the fate and potential effects of metals in estuaries, and both realistic model approaches and attempts to postulate site-specific water criteria or water/sediment standards must consider such interdisciplinary interactions.

References

European Commission. 2012. Report from the Commission to the European Parliament and the Council on the implementation of the Water Framework Directive (200/60/EC). Brussels, 14.11.2012 COM(2010)670.

Machado, A.A.S. 2016. Coastal and marine pollution in the Anthropocene: Identifying contaminants and processes. Annals of YOUMARES 6.

Machado, A.A.S., Combi, T. 2015. Assessment of pollution and environmental restoration in coastal areas: challenges and solutions. Annals of YOUMARES 6.

Machado, A.A.S., Spencer, K., Kloas, W., Toffolon, M., Zarfl, C. 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. Sci. Total Environ. 541: 268-281.

O'Shea, F.T., Spencer, K. 2016. Metal distribution in sediments adjacent to an historical landfill. *Manuscript in preparation.*

Schneider, L., Maher, W.A., Floyd, J., Potts, J., Batley, G.E., Gruber, B. 2016. Transport and fate of metal contamination in estuaries: Using a model network to predict the contributions of physical and chemical factors. Chemosphere 153: 227-236.

Steffen W., Crutzen P.J., McNeill J.R. 2007. The Anthropocene: Are Humans Now Overwhelming the Great Forces of Nature. AMBIO: A Journal of the Human Environment, 36(8): 614-621.

Steffen W., Grinevald J., Crutzen P., McNeill J. 2011. The Anthropocene: conceptual and historical perspectives. Philophical Transactions of Royal Society. A. 369: 842-867.

APPENDICES

Appendices I

List of articles published during the development of the thesis

- **MACHADO, A. A. S.,** Valyi, K., Rillig, M.C. Potential Environmental Impacts of an “Underground Revolution”. Currently accepted for publication at *Trends in Ecology and Evolution.*
- **MACHADO, A. A. S**., Spencer, K., Kloas, W., Toffolon, M., Zarfl, C. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. *Science of the Total Environment.* 2016
- Jasinska, E. J., Goss, G. G., Gillis, P. L., Van Der Kraak, G. J., Matsumoto, J., **MACHADO, A. A. S**., Giacomin, M., Moon, T. W., Massarsky, A., Gagné, F., Servos, M. R., Wilson, J., Sultana, T., Metcalfe, C. D. Assessment of biomarkers for contaminant of emerging concern on aquatic organisms downstream of a municipal wastewater discharge. *Science of the Total Environment.* 2015
- **MACHADO, A. A. S.**, Gonçalvez-Araujo, R., Teixeira, P. F., Tavano, V. M., Bianchini, A. Effects of zinc on *in vivo* fluorescence, chlorophyll *a* and growth of the diatom *Conticriba weissflogii* (Thalassiosirales, Thalassiosiraceae). *Pan-American Journal of Aquatic Sciences.* 2014
- **MACHADO, A. A. S.**, Wood, C. M., Bianchini, A., Gillis, P. L. Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. *Ecotoxicology.* 2014.
- **MACHADO, A. A. S.** Hoff, M. L. M., Klein, R. D., Cordeiro, G. J., Lencina Avila, J. M., Costa, P. G., Bianchini, A. Oxidative stress and DNA damage responses to phenanthrene exposure in the estuarine guppy *Poecilia vivipara. Marine Environmental Research.* 2014.

Appendices II

Supplementary Material for Chapter IV

SUPPLEMENTARY INFORMATION

Machado, A.A.S., O'Shea, F., Zarfl, C., Spencer, K. 2016. Unravelling metal mobilisation under complex contaminant signatures. Estuarine, Coastal and Shelf Science.

Contact: machado@igb-berlin.de

Supplementary Material A: Material and Methods

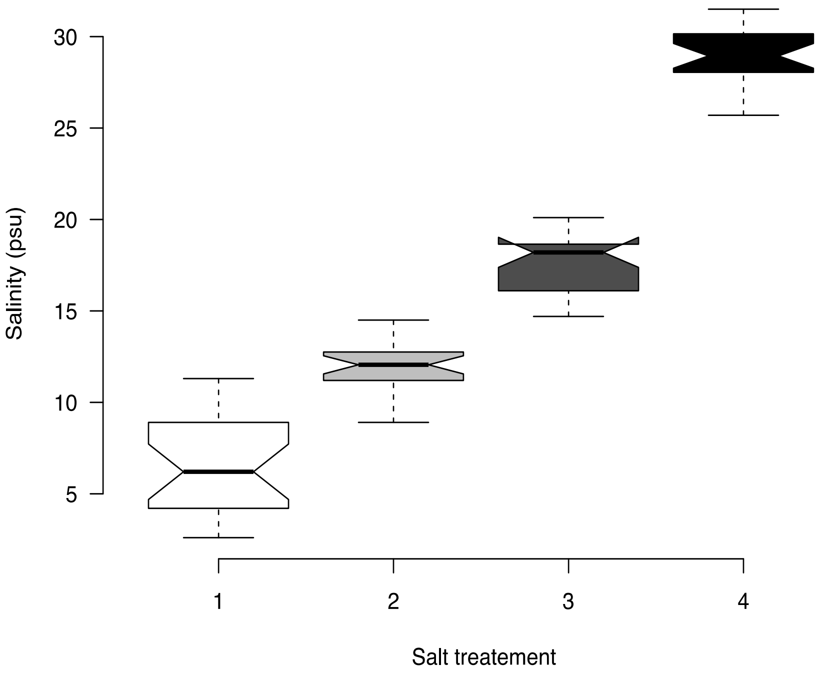

Figure A. 1: Effect of marine salt addition on the salinity of the leachate (treatments 1, 2, 3, and 4 represent respectively addition of 0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt (Sigma Aldrich) to the experimental slurry).

Table A. 1: Major metals in the artificial salt water used on leachate experiments.

Salinity	Metals (mg L^{-1})			
	Ca	K	Mg	Na
0	164.13 ± 0.99	0.09 ± 0.03	18.83 ± 0.05	24.80 ±0.29
5	156.57 ± 1.11	35.62 ± 2.55	215.19 ± 8.53	865.43 ± 16.91
15	152.34 ± 0.81	158.05 ± 9.97	524.68 ± 4.65	2596.29 ± 50.72[a]
30	277.89 ± 1.61	438.95 ± 13.15	1203.02 ± 14.40	7788.86 ± 152.16[a]

[a] Values estimated out of calibration range.

Table A. 2: Saltwater interference on the trace metal ICP quantification.

Salinity (psu)	Metal (µg L^{-1})		
	Li	Mn	Sr
0	BQL[b]	BQL[b]	BQL[b]
5	31.12 ± 0.08	BQL[b]	761.52 ± 2.16
15	90.24 ± 0.05	6.80 ± 0.01	1962.58 ± 1.39
30	207.71 ± 0.13	13.35 ± 0.01	3799.88 ± 5.46

[a] All the other metals discussed in the present work were below quantification limits in all the artificial salt water.

[b] Below quantification limits.

Table A. 3: Quantification limit and quality parameters for trace metal analysis (N=5).

Metal	Quantification limit (µg L^{-1})[a]	Accuracy (%)	Precision (%)
Al	40.00	93.7	14.4
Cd	20.00	96.3	3.8
Co	5.00	86.8	8.1
Cr	40.00	88.2	1.2
Cu	20.00	99.1	3.9
Fe	40.00	83.5	26.3
Li	20.00	86.4	4.3
Mn	5.00	88.6	3.1
Pb	20.00	97.3	20.8
Sr	40.00	99.3	2.7
Zn	40.00	92.3	56.2

[a] Quantification limit was established at lowest measured standard with accuracy error > 20%

Box A. 1. Underestimation of metal mobilisation

The present study assessed only Fe^{2+} on the unfiltered fraction of leachate. Most of metals were assessed in terms of 8 µm filtered metal, as proxy to total metal leached. There was a correlation between filtered and unfiltered iron. However, a comparison of Fe^{2+} and total Fe leached reveals that the filtering underestimated metal leached up to 100-fold. A possible explanation is that during the filtering process Fe^{2+} is quickly oxidized and starts to crystalize into colloidal forms that are retained by the filter. In fact, rapid precipitation of Fe^{2+} is well described for aerated water. Notwithstanding, such filtering is still common practice in leachate studies since it is required for ICP analyses.

Precipitation of Fe^{2+} might also affect other trace metals, which co-precipitate and adsorb to iron oxides-hydroxides. Therefore, data from this and other traditional studies on the filtered fraction must be seen with care. While some mobilisation processes might be still manifest on the filtered fraction, levels of metal leached may be greatly underestimated.

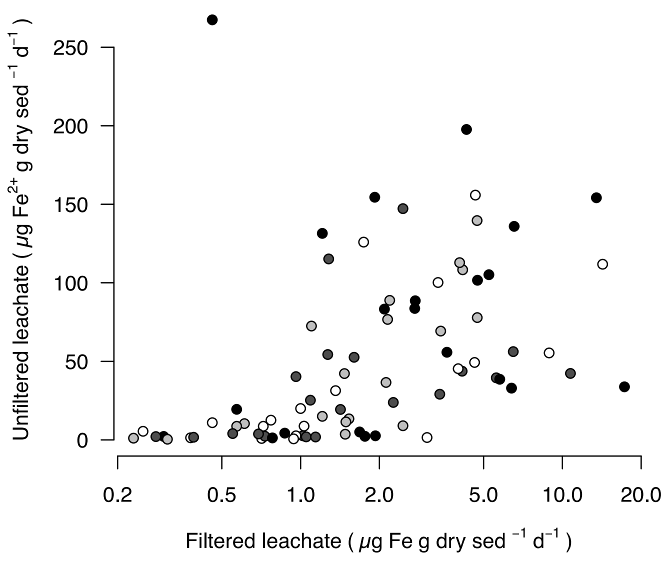

Figure: Comparison of unfiltered and 8 µm filtered Fe.

Supplementary Material B: Additional results

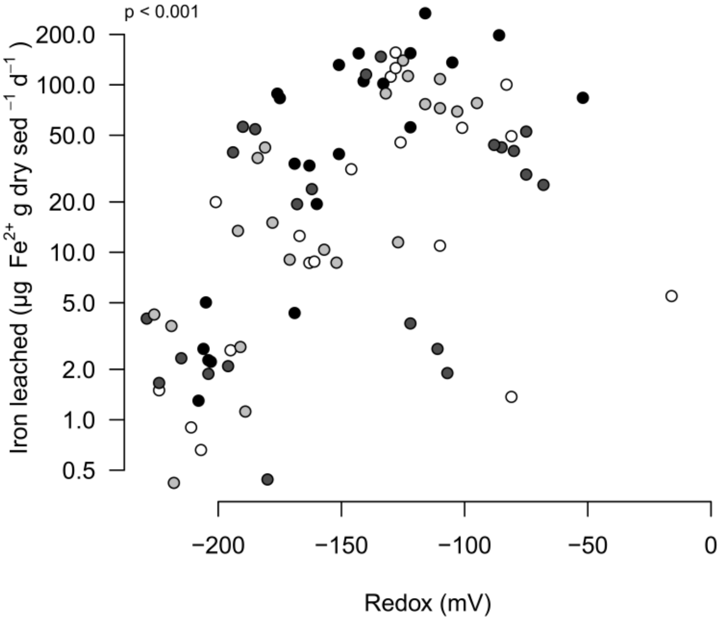

Figure B. 1: Effect of redox potential on the leaching of Fe^{2+}. Maximum mobilisation occurs between -150 and -50 mV. The white, light gray, dark gray and black filled circles represent respectively 0.000 g, 0.125 g, 0.375 g or 0.875 g of marine salt added to the experimental slurry.

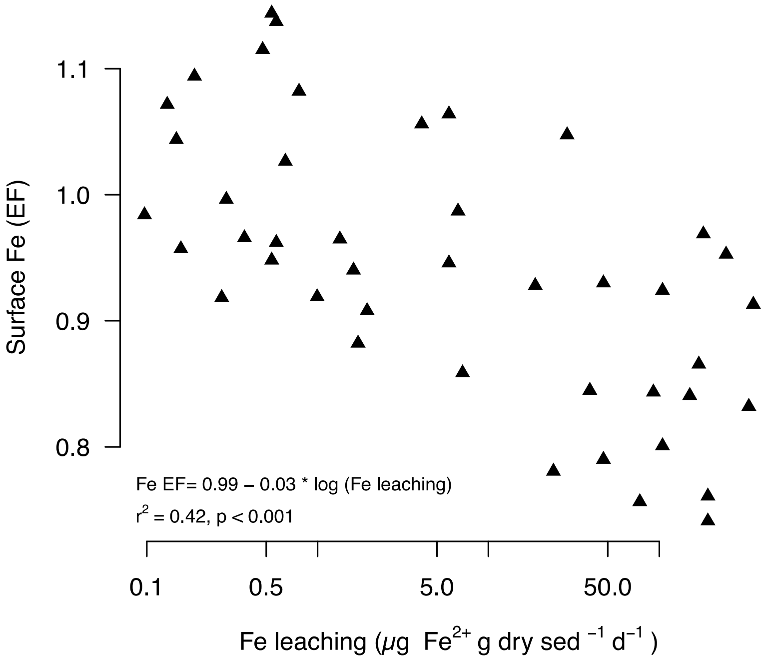

Figure B. 3: Effect of leaching of Fe^{2+} on the environmental pollution levels of surface sediments in terms of enrichment factor (EF).

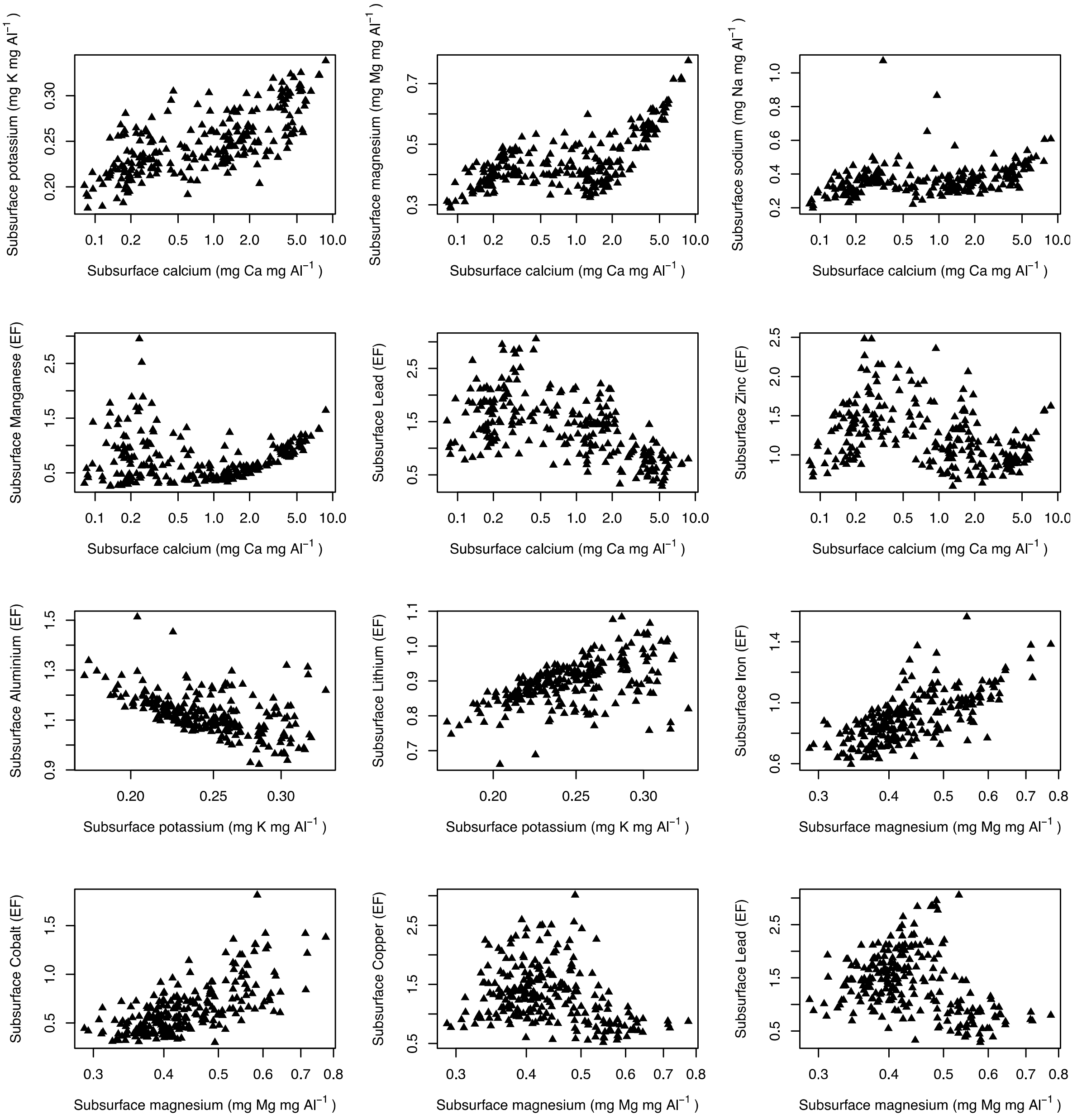

Figure B. 4: Selected relationships between geochemically normalised concentrations of alkali and alkaline Earth elements and levels of subsurface metal pollution (in terms of enrichment factor-EF).

AFTERWORD

Frontiers on aquatic ecotoxicology

In the previous chapters we addressed several important traditional contaminants and that have threathned aquatic and coastal systems. However, there is an emerging type of contamination for which traditional pollution assessment frameworks may not be appropriate for either coastal and inland water systems. In this context, ecotoxicology is relatively recent transdisciplinary field of research that deals with the challenging tasks of preventing, understanding, and monitoring threats to the environmental heath of various ecosystems. The complexity of ecotoxicological issues has been further increased due to the fact that many of the current potential pollutants do not fit to some elemental principles of environmental toxicology.

The success on assuring a healthy environment greatly depends on the reliability of core concepts and assumptions applied in ecotoxicological studies. In the next two manuscripts, we explore a case were several central ecotoxicological dogmas might be not fulfilled. The first manuscript is a letter accepted for publication on Trends in Ecology and Evolution. To prevent deleterious effects of microorganisms as prospective contaminants, this letter addresses how microorganisms might behave different from traditional contaminants and that new ecotoxicological tools are required to their proper understanding, monitoring and regulation. The second manuscript is a research article currently under review at Environmental Science and Technology. It demonstrates how a worldwide used microbiological pesticide might exhibit particular dose-response for toxicity, which might cause serious misconceptions on assessments of potential environmental effects.

We hope that the information and findings discussed here could also contribute to the future securement of environmental health.

Title:

Potential Environmental Impacts of an “Underground Revolution”

Response to: An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability S. Franz Bender, Cameron Wagg, and Marcel G.A. van der Heijden Trends in Ecology & Evolution, June 2016, Vol. 31, 440-452.

Authors: Anderson Abel de Souza Machado[1,2,3]; Kriszta Valyi[1,4]; Matthias C Rillig[1,4]

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Keywords: Biodiversity, contaminant fate, ecotoxicology, soil microecology, sustainability.

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DOI: 10.1016/j.tree.2016.10.009

Link: http://dx.doi.org/10.1016/j.tree.2016.10.009

Misleading ecotoxicological dogma: The case of DiPel and non-monotonicity.

Title: Yellow sign for a “green” pesticide: Non-monotonic dose-responses for the toxicity of a *Bacillus thuringiensis* biocide to *Daphnia magna*

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Abstract

Currently, there is a trend to increase usage of biopesticides assumed as environmentally friendly, such as *Bacillus thuringiensis* (Bt). Studies on Bt toxicity to non-target organisms reported low effects at high exposure, interpreted as negligible risk to non-target organisms. We investigated the response of the non-target *Daphnia magna* to waterborne DiPel ES, a globally used Bt formulation. Neonates and adults were 48h-exposed to a wide range of concentrations to monitor immobilization and mortality. Whole body biomarkers (body weight, protein, chitobiase, catalase, xenobiotic metabolism, acetylcholine-esterase) were measured in adults. Immobilization and mortality of neonates were affected in a non-monotonic and inverse U-shaped pattern (EC_{50s} were ~10^5-fold lower than reported by the manufacturer). The immobilization of adults presented a similar pattern, without significant mortality. Biomarkers revealed multiphasic dose-responses and suggested toxicity mechanisms affecting various physiological pathways. The main particle size in exposure media was in the size range of bacteria spore and crystal toxins. However, chemical heterogeneity was non-monotonic, changing phase at maximum of toxicity (~5 µL L^{-1}), which might explain the observed non-monotonic effects. These results point out vulnerability of a non-target organism to a biopesticide supposed to be safe, while challenging the universal applicability of the ecotoxicological central assumption of monotonicity.

Keywords: Aquatic ecotoxicology, Biomarkers, Biopesticide, Dipel, Low-dose effects, Non-monotonic response.

DOI: 10.1021/acs.est.6b03056

Link: http://dx.doi.org/10.1021/acs.est.6b03056

ABOUT THE AUTHOR

About the author

Abel Machado is a young oceanographer that has been focusing his academic interests in many multidisciplinary topics within pollution science, especially on the evaluation of fate effects of various pollutants in diverse aquatic environments and organisms. Abel obtained his bachelor degree in Oceanology (Universidade Federal do Rio Grande- FURG, Brazil) one year in advance of the regular time. During his masters, Abel was awarded an IRDC-Canada grant to work with Prof. Chris M. Wood (McMaster University, Hamilton, Canada) and the Aquatic contaminants division- Environment Canada (Burlington, Canada) with biomonitoring of the Grand River on Great Lakes basin. Abel also coordinated experiments in coastal and mining areas, focusing the effect of water contamination on organisms for the network INCT-Toxicologia Aquática sponsored by Brazilian Ministry of Science and Technology. The current Ph.D. thesis was written in the scope of SMART- Sicence for Management of Rivers and their Tidal Systems, a competitive multinational Ph.D. program funded by European Union (EACEA) under co-tutelage of Freie Universität Berlin (Germany), Queen Mary, University of London (UK), and University of Trento (Italy).

Links

https://www.researchgate.net/profile/Abel_Machado
http://lattes.cnpq.br/9987227208142353

Education

PhD River Science: joint degree corresponding to double qualification of Dr. rer. nat by Freie Universität Berlin and PhD in Physical Geography by Queen Mary, University of London. (November 2016)

MSc in Biological Oceanography. Universidade Federal do Rio Grande. Focusing the use of biological and chemical responses for environmental monitoring of salt and fresh water. (July 2012)

Bachelor in Oceanology. Universidade Federal do Rio Grande. Focusing on the effect of metals on growth of micro-crustaceans in salt water. (December 2009).

International and Professional experiences

- Guest researcher at Deltares- Delft (The Netherlands). (2015)
- Guest researcher at Leibiniz- Institute of Freshwater Ecology and Inland Fisheries. (2013- current)
- Collaborating researcher at Water Research Contaminants Division (Environment Canada, Burlington, ON, Canada). (2011)
- Exchange at Biology Graduate Program in McMaster University (Hamilton, Ontario, Canada). (2011)
- Water Chemistry trainee at Instituto Ambiental do Paraná (IAP, Curitiba, Brazil) . (2010)

Scientific articles published

- **MACHADO, A. A. S.** Coastal and marine pollution in the Anthropocene: Identifying contaminants and processes. *Annals of YOUMARES VII meeting.* 2016
- **MACHADO, A. A. S**., Spencer, K., Kloas, W., Toffolon, M., Zarfl, C. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. *Science of the Total Environment.* 2016
- Jasinska, E. J., Goss, G. G., Gillis, P. L., Van Der Kraak, G. J., Matsumoto, J., **MACHADO, A. A. S**., Giacomin, M., Moon, T. W., Massarsky, A., Gagné, F., Servos, M. R., Wilson, J., Sultana, T., Metcalfe, C. D. Assessment of biomarkers for contaminant of emerging concern on aquatic organisms downstream of a municipal wastewater discharge. *Science of the Total Environment.* 2015
- **MACHADO, A. A. S.**, Combi, T. Assessment of pollution and environmental restoration in coastal areas: challenges and solutions. *Annals of YOUMARES VI*

meeting. 2015

- **MACHADO, A. A. S.**, Gonçalvez-Araujo, R., Teixeira, P. F., Tavano, V. M., Bianchini, A. Effects of zinc on *in vivo* fluorescence, chlorophyll *a* and growth of the diatom *Conticriba weissflogii* (Thalassiosirales, Thalassiosiraceae). *Pan-American Journal of Aquatic Sciences.* 2014
- **MACHADO, A. A. S.**, Wood, C. M., Bianchini, A., Gillis, P. L. Responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures. *Ecotoxicology.* 2014.
- **MACHADO, A. A. S.** Hoff, M. L. M., Klein, R. D., Cordeiro, G. J., Lencina Avila, J. M., Costa, P. G., Bianchini, A. Oxidative stress and DNA damage responses to phenanthrene exposure in the estuarine guppy *Poecilia vivipara. Marine Environmental Research.* 2014.
- Harayashiki, C. A. Y. Junior, A. S. V., **MACHADO, A.A.S.,** Cabrera, L. C., Primel, E. G. Bianchini, A., Corcini, C. D. Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water. *Aquatic Toxicology.* 2013
- **MACHADO, A. A. S.** Hoff, M. L. M., Klein, R. D., Cardozo, J.G., Giacomin, M. M., Pinho, G. L. L., Bianchini, A. Biomarkers of waterborne copper exposure in the guppy *Poecilia vivipara* acclimated to salt water. *Aquatic Toxicology.* 2013.
- Avila, T. R., **MACHADO, A. A. S.**, Bianchini, A. Estimation of zooplankton secondary production in estuarine waters: Comparison between the enzymatic (chitobiase) method and mathematical models using crustaceans. *Journal of Experimental Marine Biology and Ecology.* 2012
- Avila, T. R., **MACHADO, A. A. S.**, Bianchini, A. Chitobiase of planktonic crustaceans from South Atlantic Coast (Southern Brazil): Characterization and influence of abiotic parameters on enzyme activity. *Journal of Experimental Marine Biology and Ecology.* 2011

Scientific article accepted for publication

- **MACHADO, A. A. S.,** Valyi, K., Rillig, M.C. Potential Environmental Impacts of an "Underground Revolution". Currently submitted to *Trends in Ecology and Evolution.*

Scientific article under review

- **MACHADO, A. A. S.,** Zarfl, C., Rehse, S, Kloas, W. Yellow sign for a green pesticide: Non-monotonic dose-response for the toxicity of a *Bacillus thuringiensis* pesticide to *Daphnia magna.* Currently under review at *Environmental Science and Technology.*

Scientific article at submission stage

- **MACHADO, A. A. S.,** O'Shea, F., Zarfl, C., Spencer, K. Unravelling metal mobility under complex contaminant signatures. To be submitted to *Estuarine, Coastal and Shelf Science.*

Scientific presentations

- Premier, V., **MACHADO, A.A.S.**, Zarfl, C., Spencer, K., Toffolon, M. Metal behavior in the Thames Estuary: Insights from modelling studies. YOUMARES VII 2016.
- **MACHADO, A. A. S.**, Zarfl, C, Kloas, W. An inverse U-shaped dose-response for toxicity of pesticide Dipel to *Daphnia magna*. SETAC Europe 25th Annual Meeting 2015.
- **MACHADO, A. A. S.**, Wood, C. M., Bianchini, A., Gillis, P. From waterborne contaminants to subcellular biomarkers, tissue bioaccumulation, and mussel health: Tracking the effects of waterborne metals throughout the biological hierarchy. SETAC Europe 25th Annual Meeting 2015.
- **MACHADO, A. A. S.**, Kloas, W., Zarfl, C. Effect of metals in estuarine organisms: modelling toxicity in physiologically changing systems. SETAC Europe 25th Annual Meeting 2015.
- **MACHADO, A. A. S.**, Spencer, K., Kloas, W. Zarfl, C. Environmental and biological factors affecting metal remobilization and toxicity in estuaries. 35th Annual Meeting Society of Environmental Toxicology and Chemistry_SETAC North America. 2014.
- Gillis, P. L., **MACHADO, A. A. S.**, Solla, S., Bianchini, A., Wood, C. M. Examining the contribution of metal exposure to observed negative effects on wild freshwater

mussels. 3rd Aquatic Toxicology Symposium. 2014.

- **MACHADO, A. A. S.**, Hoff, M. L. F., Klein, R. D., Cardozo, J. G., Giacomin, M. M., Pinho, G. L. L., Bianchini, Adalto . Copper effects on phisiology of estuarine guppy Poecilia vivipara: understanding mechanisms and potential biomarkers. 33rd Annual Meeting Society of Environmental Toxicology and Chemistry_SETAC North America. 2012.
- **MACHADO, A. A. S.**, Wood, C.M. Bianchini, A., Gillis, P.L. Using a suite of biomarkers and bioindicators to assess the effects of complex contaminant mixtures on chronically exposed wild freshwater mussels. 33rd Annual Meeting Society of Environmental Toxicology and Chemistry_SETAC North America. 2012.
- Wood, C.M., Niyogi, S., Nadella, S., Jorge, M.B, Loro, V.L., **MACHADO, A. A. S**., Giacomin, M. M., Bianchini, A., Gillis, P.L. . Metals, mixtures, fish and freshwater mussels. 2nd Aquatic Toxicology Symposium. 2012.
- Ferreira, E.C., Zacchi, F., Toledo-Silva, G., Mattos, J., **MACHADO, A. A. S.**, Dorrington, T.S., Bianchini, A., Bainy, A.C.D . Differential gene transcription in Poecilia vivipara exposed to copper. 6th World Congress/ 22 Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC). 2012.
- Ferreira, E.C., Dorrington, T.S., Zacchi, F., Toledo-Silva, G., Mattos, J., Piazza, C.E., **MACHADO, A. A. S**., Bianchini, A., Bainy, A.C.D . Differential gene transcription in Poecilia vivipara exposed to the herbicide Atrazine. 6th World Congress/ 22 Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC). 2012.
- Gomes, E. G., **MACHADO, A. A. S.**, Bianchini, Adalto . Brain acetylcholinesterase activity as diagnostic biomarker of water contamination in the guppy Poecilia vivipara. 33rd Annual Meeting Society of Environmental Toxicology and Chemistry (SETAC North America). 2012.
- Avila, Tatiana Ramos, **MACHADO, A. A. S**., Bianchini, Adalto . Influence of seawater-derived organic matter on acute Zn toxicity in the copepod Acartia tonsa in a wide range of salinities. 33rd Annual Meeting Society of Environmental Toxicology and Chemistry (SETAC North America). 2012.
- **MACHADO, A. A. S.**, Klein, R. D., Gomes, E. G., Bianchini, A. . Atrazina e Biomarcadores de Contaminação Aquática: Apontando Mecanismos de Toxicidade e Ferramentas para o Monitoramento Ambiental. XII Congresso Brasileiro de

Ecotoxicologia. 2012.

- **MACHADO, A. A. S.**, Cardozo, J. G., Gomes, E. G., Hoff, M. L. F., Bianchini, A. Biomarkers to Access Metal Exposure in Estuarine Fish. 32nd Annual Meeting of Society of Environmental Toxicology and Chemistry North America. 2011.
- Gomes, E. G., **MACHADO, A. A. S.**, Hoff, M. L. F., Bianchini, A. Avaliação da Lipoperoxidação em Peixe como Potencial Biomarcador de Contaminação Aquática. I Semana de Toxicologia Ambiental-FURG. 2011.
- Gomes, E. G., **MACHADO, A. A. S.**, Hoff, M. L. F., Bianchini, A. Lipoperoxidação induzida pela exposição a atrazina, fenantreno e cobre em Poecilia vivipara. 10ª Mostra de Produção Universitária- FURG. 2011.
- Cardozo, J. G., **MACHADO, A. A. S.**, Hoff, M. L. F., Bianchini, A. Micronúcleos em Eritrócitos de Poecilia vivipara Expostos ao Cobre, Fenantreno e Atrazina. 10ª Mostra de Produção Universitária. 2011.
- Cardozo, J. G., **MACHADO, A. A. S.**, Hoff, M. L. F., Bianchini, A. Análise de Micronúcleo em Poecilia vivipara Exposto ao Cobre. Congresso Brasileiro de Toxicologia. 2011.
- Gomes, E. G., **MACHADO, A. A. S**., Hoff, M. L. F., Bianchini, A. Lipoperoxidação como Biomarcador de Exposição ao Cobre em Poeciilia vivipara. Congresso Brasileiro de Toxicologia. 2011.
- Giacomin, M. M., **MACHADO, A. A. S.**, Bersano, J.G.F . Avaliação Preliminar da Toxicidade Aguda da Fraçâo Solúvel de um Biodiesel em Metamysidopsis elongata Atlântica (crustacea, mysidacea). IV Congresso Brasileiro de Oceanografia. 2010.
- **MACHADO, A. A. S.**, Araujo, R. G., Teixeira, P., Garcia, V. M. T., Bianchini, A. Efeitos do Zinco sobre o Crescimento de Thalassiosira weissflogi. XI Congresso Brasileiro de Ecotoxicologia. 2010.
- **MACHADO, A. A. S.**, Ávila, T. R., Bianchini, A. Toxicidade de Metais no Desenvolvimento Naupliar e Atividade da Quitobiase do Copépode Acartia tonsa. XI Congresso Brasileiro de Ecotoxicologia. 2010.
- **MACHADO, A. A. S.**, Ávila, T. R., Bianchini, A. Effects of metals on chitobiase activity: Relevance in ecological and ecotoxicological studies. 31st Annual Meeting of Society of Environmental Toxicology and Chemistry North America. 2010.
- **MACHADO, A. A. S.**, Neves, C.A. Efeitos da bioacumulção do cobre sobre parâmetros fisiológicos do camarão-branco Litopenaeus vannamei. 1º Ciclo de

Seminários de Orientação Tecnológica em Toxicologia. 2009.

- **MACHADO, A. A. S.**, Ávila, T. R., Bianchini, A. . Inibição da Atividade da Quitobiase do Copépode Acartia tonsa. XVIII Congresso de Iniciação Científica-FURG. 2009.
- Julião, H.P., **MACHADO, A. A. S.**, Martins, C.M.G., Barcaroli, I., Wasielesky, W.J., Neves, C.A. . Efeitos da Bioacumulação do Cobre no Camarão-Branco: Implicações Ecológicas e Fisiológicas. XVIII Congresso de Iniciação Científica-FURG. 2009.
- **MACHADO, A. A. S.** Efeitos de Metais em Organismos Aquáticos: Um Desafio à Pesquisa e à Gestão da Qualidade Ambiental. III Semana Acadêmica de Oceanologia-FURG. 2009
- **MACHADO, A. A. S.,** Julião, H.P., Neves, C.A., Barcaroli, I., Martins, C.M.G. . Biomarcadores De Contaminação Aquática Pelo Cobre No Camarão-Branco Litopenaeus vannamei (Boone, 1931) (Decapoda, Penaeidae). X Congresso Brasileiro de Ecotoxicologia. 2008.
- **MACHADO, A. A. S**., Ávila, T. R., Bianchini, A. Variação Temporal na Assembléia Zooplantônica na Enseada do Saco Do Justino, Rio Grande, RS. XVII Congresso de Iniciação Científica. 2008.
- **MACHADO, A. A. S.,** Ávila, T. R., Bianchini, A. Comparação da Variação Temporal na Assembléia Zooplantônica na Praia do Cassino e Enseada do Estuário da Lagoa dos Patos (Rio Grande, RS). IV SImpósio Brasileiro de Oceanografia. 2008.
- Medeiros, E. S., Storino, L.G., **MACHADO, A. A. S.**, Neves, C.A. Influência da Angiotensina II e Endotelina 2 Humanas na Frequência Cardíaca de Palaemonetes argentinus. XXII Latin-American and Fisrt Ibero-American Congress of Physiological Sciences. 2006.
- Storino, L.G., Medeiros, E. S., **MACHADO, A. A. S.**, Neves, C.A. Efeitos de Aminas, Serotoninas e Octopamina na Frequência Cardíaca do Camarão Palaemonetes argentinus. XXII Latin-American and Fisrt Ibero-American Congress of Physiological Sciences. 2006.
- MEDEIROS, E., Storino, L.G., **MACHADO, A. A. S.**, Neves, C.A. Angiotensina II e Endotelina 2 humanas na regulação da freqüencia cardíaca de modelo invertebrado- o camarão Palaemonetes argentinus. XXII Latin-American and Fisrt Ibero-American Congress of Physiological Sciences. 2006.