



Platinum biogeochemical cycles in coastal environments

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Par **Mélina ABDOU**

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Cycles biogéochimiques du platine dans les environnements côtiers

Sous la direction de Pr. Jörg SCHÄFER
et la co-direction de Pr. Gérard BLANC

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Devant la commission d'examen formée de

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Biogeochemical platinum cycles in coastal environments

Platinum (Pt) is a Technology-Critical Element (TCE) and an emerging contaminant increasingly released into all Earth compartments, but its environmental occurrence, distribution, and dynamics are under-documented. The present research combines laboratory observations and field monitoring studies on Pt biogeochemistry in coastal systems (Atlantic Ocean and northwestern Mediterranean Sea), including its distribution between seawater, particles, and living organisms. Exposure experiments with oysters facing a wide range of exposure levels provide the first Pt accumulation kinetics in marine bivalves, supporting uptake of Pt from seawater in line with field observations. At environmentally-relevant Pt levels (50 and 100 ng.L⁻¹), the positive linear relation between exposure and Pt accumulation in tissues justifies the use of oysters as integrative sentinels for seawater Pt contamination. At relatively high Pt levels (10,000 ng.L⁻¹), deleterious physiological effects (e.g. increased oxidative stress and energetic stock mobilization) occurred in oysters. Field observations at contrasting sites have provided regional background Pt concentrations in seawater in the Atlantic and Mediterranean coasts (0.05 ng.L⁻¹ and 0.08 ng.L⁻¹, respectively) and bioconcentration factors for phytoplankton ($\sim 10^4$), and bivalves (oysters and mussels $\sim 10^3$). Spatial distribution of Pt levels in seawater, plankton and bivalves suggest higher contamination along the northwestern Mediterranean coast, especially in semi-enclosed, industrialized/urbanized systems (Toulon Bay and Genoa Harbor). Historical records in sediments ($\sim 1900 - 2010$) and bivalves (1980 to present) from the Gironde watershed and the Toulon Bay showed past Pt contamination due to industrial (metallurgic, oil-refining, coal) activities, whereas the strong recent increase in Pt contamination originates from emerging Pt sources, e.g. car catalytic converters, hospital effluents, and sewage. High temporal resolution observation of Pt partitioning, together with environmental master variables (O₂, Salinity, Chl-a, nutrients etc.), suggests that in coastal systems, biogeochemical processes including phytoplankton production and degradation may play an important role in Pt behavior and fate.

Keywords: Platinum, seawater, particles, bivalves, accumulation, distribution, contamination

Cycles biogéochimiques du platine dans les environnements côtiers

Le platine (Pt) est un élément technologique critique (ETC) et un contaminant émergent dans les compartiments environnementaux. Peu de données existent concernant ses concentrations, sa distribution et sa dynamique dans le milieu naturel. Ce travail combine des résultats d'expérimentation en laboratoire à des observations de terrain afin d'étudier la biogéochimie de Pt dans les systèmes côtiers (Océan Atlantique et Méditerranée). Une expérimentation exposant des huîtres à une large gamme de concentrations de Pt a produit les premières données de cinétique d'accumulation de Pt dans les bivalves marins et reflète l'assimilation de Pt constatée *in situ*. À des niveaux d'expositions réalistes (50 et 100 ng.L⁻¹), la relation linéaire positive entre les niveaux d'exposition et celles des tissus justifie l'utilisation des huîtres comme sentinelles des niveaux de Pt dans l'eau de mer. À forts niveaux de Pt (10 000 ng.L⁻¹), des effets délétères sur l'état physiologique des huîtres (e.g. augmentation du stress oxydatif et mobilisation du stock énergétique) sont observés. À partir d'études de terrain dans des sites contrastés les bruits de fond géochimiques régionaux dans l'eau de mer des côtes atlantique et méditerranéenne (0,05 ng.L⁻¹ et 0,08 ng.L⁻¹ respectivement) et les facteurs de bioconcentration dans le phytoplancton ($\sim 10^4$) et dans les bivalves (huîtres et moules, $\sim 10^3$) ont été établis. La répartition spatiale des niveaux de Pt dans l'eau de mer, le plancton et les bivalves reflète une contamination plus élevée de la côte nord-ouest méditerranéenne, particulièrement dans les zones semi-fermées et fortement urbanisées / industrialisées (baie de Toulon et port de Gênes). Les enregistrements historiques dans les sédiments ($\sim 1900 - 2010$) et dans les bivalves (1980 à aujourd'hui) en Gironde et dans la baie de Toulon ont montré une contamination ancienne de Pt liée aux activités industrielles (métallurgie, raffinage du pétrole, charbon), tandis que la forte augmentation récente provient de sources émergentes telles que les pots catalytiques, les rejets hospitaliers et les eaux usées. L'observation à haute résolution temporelle de la distribution de Pt entre la phase dissoute et particulaire et des paramètres environnementaux majeurs (O₂, salinité, Chl-a, nutriments etc.), suggèrent l'importance des processus biogéochimiques, comprenant la production et dégradation du phytoplancton, dans le comportement et le devenir du Pt en milieu côtier.

Mots clés: Platine, eau de mer, particules, bivalves, accumulation, distribution, contamination

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General introduction

The Anthropocene has been proposed as a new geo-stratigraphic epoch, when humans have become a global factor affecting the ecosystems (Álvarez-Vázquez et al., 2017). Knowing what determines spatial distribution and density of human populations is fundamental to understand the relationships between humans and the environment (Small and Cohen, 2004). At present, human population is concentrated near coasts with ~ 60 % within 100 km of the coastline (Vitousek et al., 1997). As stated by Small and Cohen (2004), this reflects economic and strategic advantages.

Progressive anthropogenic impact on coastal areas results in changes in the biology and chemistry of seawaters that can drastically alter their ecological and biological dynamics (Oursel et al., 2013) making them highly vulnerable systems. Decreasing environmental quality also leads to significant economic and social consequences, especially when pollutant concentrations reach or even overpass limits stated by law such as the Water Framework Directive or the Marine Strategy Directive at a European level. The majority of the pollution impacts in coastal environments originate from land-based sources (Tercier-Waeber et al., 2015). Among the variety of hazardous chemical compounds released into the coastal environment, trace metals are key targets due to their absolute persistence and their high ecotoxicological potential. Trace metals are natural constituents of crustal materials which can enter aquatic environments through several processes. Industrial development and urbanization go along with increasing demand for metals, including ‘new’ metals for high-end technologies, which may end-up in aquatic systems. In addition, the related mining and metallurgic activities represent consequent potential pollution sources (Audry et al., 2004).

Among the variety of trace metals, platinum, symbolized Pt, belongs to the Platinum Group Elements. Apart from a longer history of Pt resources exploration, research on PGE cycles in the surface environment has been performed for ~ three decades by a limited number of researchers, and their distribution and concentration in natural ecosystems and especially in marine environments still remain relatively under-documented. Up to now, a clear risk assessment for PGEs cannot be done considering the scarcity of field studies (Ruchter et al., 2015). This lack of relevant environmental data might be partly due to challenging analytical methods for PGE determination at environmental concentration ranges, added to the fact that PGE are not analyzed in standardized monitoring programs (Ruchter et al., 2015). It is now clear that anthropogenic disturbance of Pt natural geochemical cycles occurs in all Earth compartments with increasing Pt levels recorded even in very remote areas (Sen and Peucker-Ehrenbrink, 2012; Soyol-Erdene et al., 2011). Emissions are expected to increase in the near future owing to the rise of PGE loading in vehicle exhaust purification systems due to

increasingly stringent emission regulations in developed countries and the introduction of catalysts in developing countries.

The present study responds to the recommendations made by the **European COST action TD1407: Network on Technology-Critical Elements (NOTICE)—from environmental processes to human health threats**. The TCEs are trace elements of critical economic importance that are used in an expanding list of emerging technologies. Platinum Group Elements and more specifically Pt are part of the TCE group. NOTICE aims at creating a network of scientists working on environmental cycles and potential impacts of trace elements critical for the development of new technologies. Final objectives of NOTICE are the definition of (i) current state of knowledge and (ii) priority research lines in the fields of analysis and inter-calibration, environmental impact and cycling, as well as human exposure and (eco)toxicology. As reported by Cobelo-García et al. (2015), the current significant gaps in our knowledge and understanding of TCEs, from their environmental levels and fate to their potential (eco)toxicological impact, are mainly explained by two factors: (i) their typical ultra-trace concentrations, making their analytical determination extremely difficult and/or time-consuming, and (ii) the absence of any significant industrial role (apart from some biomedical applications) prior to their current massive use following the increasing demand by new technological applications. Analytical challenges in Pt determination in environmental matrices are major, particularly in samples from marine environments. The few existing publications report extremely low natural Pt levels, yet, detect potential anomalous enrichments in urbanized areas. The European COST Action recommends the study of environmental impacts of TCEs that include Pt.

Furthermore, the present work is performed in the frame of the European Union 7th Framework Program: **EU-FP7 SCHeMA Project: Integrated in situ Chemical Mapping Probe**, which funded the PhD thesis. This project follows the recommendation of the EU, highlighting the need for accurate regular monitoring of European coastal water quality. Indeed, identification of relevant types of hazards at the appropriate temporal and spatial scales is crucial to detect their sources and origin, to understand the processes governing their magnitude and distribution, and to ultimately evaluate and manage their risks and consequences preventing economic losses (Tercier-Waeber et al., 2015).

Overall, the specific aim of the SCHeMA Project is structured around a fourfold objective:

- The development of an array of novel chemical sensors taking advantage of various innovative analytical solutions.
- The incorporation of the novel sensors into miniature, low power consumption, multichannel probes.
- The development of dedicated wired/wireless communication network and web-based front-end system compatible with EU standard requirements.
- The evaluation, optimization, validation and demonstration of the SCHeMA sensing tools and integrated system via short and long-term field applications in Atlantic and Mediterranean coastal areas.

The SCHeMA system should be designed for deployment from different facilities, i.e.: platform, moored buoy, boat, unmanned surface or submersible vehicles, and landers. It should acquire a wealth of information, at high spatial and temporal resolutions, on a range of chemical hazardous compounds coupled to master variables. Such a rich database offers the possibility to gain insights into phenomena that are currently poorly understood but are significant for the understanding of aquatic ecosystems functioning. It is of major importance for predictions of toxicological impact and, ultimately, for sustainable management based on scientific knowledge. With this valuable original dataset, SCHeMA will contribute to enhance ocean observing capabilities and support policies of several EU directives.

The European Consortium is constituted of five universities, one federal school, and two small/medium enterprises. The University of Bordeaux and more precisely the TGM team (EPOC laboratory) is Work Package Leader of the *WP8: Prototype field evaluation and application in coastal sites*. The objectives of this Work Package are (i) to choose representative sites providing optimum working conditions for the field tests and application campaigns; (ii) to validate the sensor performance and ruggedness under contrasting real-life environmental conditions; (iii) to apply the SCHeMA system in the Atlantic and Mediterranean coastal zones and to acquire excellent new data and understanding on biogeochemical processes involving nutrients, metallic and organic contaminants. Although Pt, as an emerging contaminant, has not been intended to be integrated in the SCHeMA probe, the project also aimed at providing first data on Pt in the different European study sites, as a first step towards improved monitoring systems for this metal in the marine environment. Participation in various field campaigns allowed for sample collection at high spatial and temporal resolution in contrasting sampling sites, with subsequent analysis, as for the validation measurements of more ‘traditional’ contaminants (Cu, Cd, Zn, Pb). The present work therefore greatly benefited from the SCHeMA

Project which allowed performing multidisciplinary field work towards the understanding of trace element cycles, including Pt behavior and distribution in marine environments.

Environmental pollution monitoring may also consist in measurements of chemical concentrations in living organisms, also called “biomonitoring”. By bioconcentrating elements, marine bivalves for instance have proved to be good bioindicators of trace metal levels in seawater (Phillips, 1977). Biomonitoring programs such as the **French National Network for the Observation of Marine Environment Quality RNO/ROCCH** (Ifremer) consider bivalves from the French coastal environments in the Atlantic and Mediterranean coasts. Based on annual, bi-annual or even monthly sampling at a huge number of coastal sites since the late 1970’s the RNO/ROCCH has constituted a valuable historical sample bank (mainly oysters and mussels, depending on the sites). This study also greatly benefited from samples originating from this national sample bank, as chemical analyses of such unique environmental archives is an excellent approach to assess past and/or current potential metal contamination. In addition, time-resolved sediment cores from the CARTOCHIM project and the TGM EPOC sample bank were part of the samples studied in this work, also representing archives of past environmental conditions.

The main objectives of the present work are:

- To determine, under controlled laboratory conditions, the **rate and kinetics of Pt accumulation in marine bivalves** and **related physiological effects** at different levels of biological organization.
- To **evaluate the potentiality of using marine bivalves as reliable sentinels of Pt contamination in coastal environments**.
- To **identify and document past and present Pt contamination and sources** by combining information from **historical records and (bio)monitoring campaigns in contrasting** (from relatively pristine to strongly impacted) **coastal sites** along the **Atlantic and northwestern Mediterranean coasts**.
- To **characterize and understand the factors controlling short-term Pt dynamics and partitioning** between seawater and suspended particles including **phytoplankton**.

The present work includes two publications in peer-reviewed journals and four manuscripts ready for submission. The manuscript is divided in four chapters:

Chapter 1: Scientific context

This first chapter introduces the state-of-the-art knowledge on Pt element including its physical and chemical properties. Anthropogenic applications of this TCE are reported as well as the related anthropogenic dispersion in the aquatic environment. This chapter also provides a concise overview of the existing literature reporting Pt occurrence, distribution and behavior in aquatic systems going from rivers to open oceans. Finally, literature on field-derived observations and laboratory investigations on Pt bioavailability and bioaccumulation in aquatic organisms will be introduced.

Chapter 2: Material and methods

This second chapter aims at introducing the study areas and sampling strategy. It describes sample collection in the frame of environmental monitoring programs including National Biomonitoring Programs and the different sampling campaigns performed within the SCHeMA project. This chapter also explains the experimental design of a laboratory exposure experiment and documents analytical procedures available for Pt detection in the studied environmental matrices, and more specifically the two main techniques applied to our samples being Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Successful inter-comparison of results obtained using these two methods, including different acid-digestion and detection protocols allowed for validation of analytical procedures for biological matrices is documented and observed limitations are discussed.

Chapter 3: Platinum accumulation and physiological response in oyster Crassostrea gigas

This chapter reports on the results of a laboratory experiment exposing oysters (*Crassostrea gigas*) to a wide range of concentrations of isotopically-labelled Pt in seawater over 35 days. Different endpoints were investigated through this exposure study.

The first part addresses Pt accumulation kinetics in oyster, revealing the evolution of Pt concentrations in soft tissues of whole organisms through time. The main objective of this experiment was to determine if oysters, and more generally marine bivalves, are suitable sentinel organisms for Pt biomonitoring in coastal environments. Outcomes from this study are published in the peer-reviewed scientific journal *Science of the Total Environment*, under the title **“Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species in coastal marine environments”**.

The second part of the chapter focuses on the physiological responses of oysters following similar Pt exposure and this at different biological organization levels. Accordingly the objectives are to determine Pt effects at (i) cellular level, with the measurement of lipofuscin and lipid contents; (ii) tissue level, including quantification of histopathological alterations and digestive gland integrity; and (iii) organism level, determining Pt concentration distribution in the different organs (organotropism) and possible excretion through faeces. Outcomes from this study are ready for submission to the peer-reviewed scientific journal *Environmental Science and Pollution Research*, under the title **“Organotropism and biomarker response in oyster *Crassostrea gigas* exposed to platinum in seawater”**.

Chapter 4: Temporal and spatial aspects of biogeochemical platinum cycles in coastal environments

This chapter is divided in four parts reporting on outcomes from field studies performed in contrasting study sites and various environmental matrices.

The first part entitled **“Past and present platinum contamination of a major European fluvial–estuarine system: Insights from river sediments and estuarine oysters”** published in the peer-reviewed scientific journal *Marine Chemistry* addresses historical records of Pt in river sediments and estuarine oysters from the Gironde fluvial–estuarine continuum (SW France), comprising the Lot River. Sediment cores from the Lot River dating from 1952 to 2001, and wild oyster samples from the Gironde Estuary mouth collected from 1981 to 2013, allowed documenting past and present Pt contamination of this major European aquatic system.

The second part of the chapter presents historical records of Pt contamination in another major coastal environment: the northwestern Mediterranean coastal area. Platinum concentrations were determined in (i) sediment cores and farmed mussels from the severely metal-polluted Toulon Bay in order to assess historical Pt contamination in a highly urbanized harbor, and (ii) mussels sampled along the coastline in order to document Pt contamination levels in mussels from contrasting sites. Historical records of both sediment cores and mussels collected in the Toulon Bay provide knowledge on former and recent Pt sources and on the extent of Pt contamination. Seasonality of Pt bioaccumulation in mussels is also investigated. Results from this field study are ready for submission to the peer-reviewed scientific journal *Chemosphere*, under the title **“Platinum in sediments and mussels from the northwestern Mediterranean coast: temporal and spatial aspects”**.

The third part reports and discusses occurrence and distribution of Pt concentrations in both the dissolved and particulate phases together with physical/chemical key variables along two contamination gradients in a severely polluted harbor, the Genoa Harbor (NW Italy). Different Pt concentration gradients occurring in this system reflecting multiple potential Pt sources. Results from this study are ready for submission to the peer-reviewed scientific journal *Estuarine, Coastal and Shelf Science*, under the title **“Spatial variability and sources of platinum in the Genoa Harbor – from geochemical background to urban levels”**.

The last part of the chapter reports on Pt levels in the planktonic phase. Determination of Pt levels in such samples is of major importance in order to gain some knowledge on primary producers influence on biogeochemical cycles in coastal seawaters. For this high temporal monitoring studies were performed in three European coastal key areas. Accordingly, diurnal/tidal cycles, performed on an hourly basis, from the Gironde Estuary, the Arcachon Bay, and the Genoa Harbor, help to increase the knowledge on Pt behavior and dynamics between particulate, including planktonic, and dissolved phases. Outcomes from this study are ready for submission in the peer-reviewed scientific journal *Marine Pollution Bulletin*, under the title **“Short-term variations in platinum partitioning in contrasting European coastal environments: the role of primary production and local point sources”**.

Chapter 1

Scientific context

Chapter 1: Scientific context

1.1. The platinum element

Precious metals have a long and rich history of use dating from the Egyptian First Dynasty. In Egypt, mineralogists soon learned to purify gold that was thereafter used to create objects and offered as gifts (Arvizo et al., 2012). The early fascination with metals is also illustrated by the keen interest of early alchemy philosophers who believed in the spiritual connection between metals including gold and silver, with their respective heavenly bodies: the Sun and the Moon. Alchemists believed platinum to be an amalgamation of gold and silver (Figure 1).

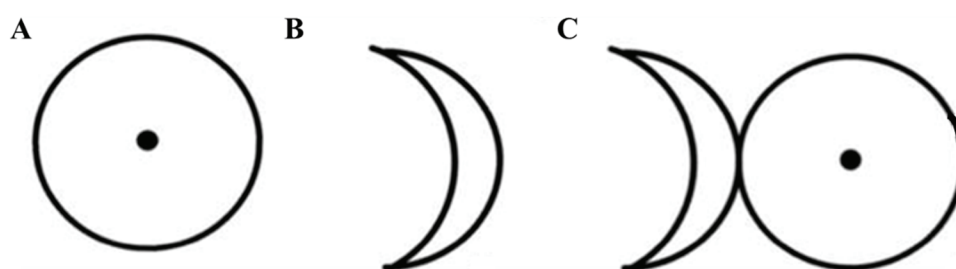


Figure 1: Alchemy symbols of (A) Gold, (B) Silver, (C) Platinum (Arvizo et al., 2012).

The first reported platinum (Pt) use was by South Americans ~ 2000 years ago (Arvizo et al., 2012). The Europeans discovered Pt only during the 16th century, when the Spanish came across the element following conquest of the lands of South America. When mining for gold in Columbia, they found lumps of platinum on the alluvial sands of the Pinto River, which they called platina del Pinto meaning “little silver of the Pinto River” (Arvizo et al., 2012). Some of the samples were brought back to Europe in 1557 and studied by Julius Caesar Scaliger, an Italian-French scientist, who concluded that the metal was not silver and in fact a new element: platinum. Thus J.C. Scaliger is widely reported to have discovered the metal even though, in 1735, the Spanish scientist Antonio de Ulloa rediscovered Pt in South America and in 1783 the French chemist Francois Chabaneau successfully purified it.

1.1.1. Physical and chemical properties of platinum

Platinum is a shiny silvery-grey-white transition metal (Figure 2). Platinum is symbolized Pt. With a density of 21.5 g.cm^{-3} (Table 1), this element is both malleable and ductile.

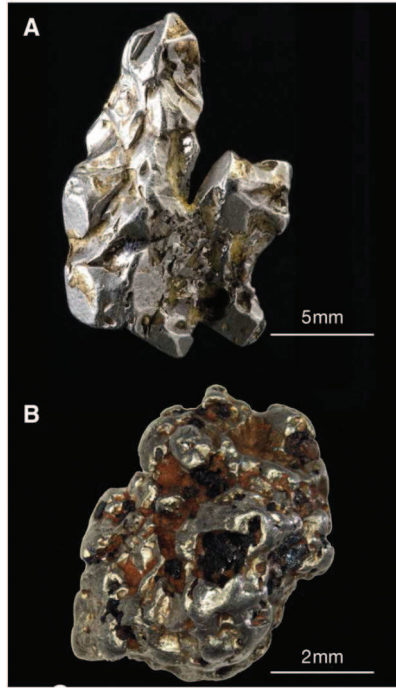


Table 1: Chemical and physical properties of Pt (after Reith et al., 2014).

<i>Chemical symbol</i>	Pt
<i>Atomic number</i>	78
<i>Group, period, block in periodic table</i>	10, 6, d
<i>Element category</i>	Transition metal
<i>Standard atomic weight</i>	195.084
<i>Physical state (25 °C, 1 bar)</i>	solid
<i>Density</i>	21.45 g.cm^{-3}
<i>Melting point</i>	$1,768 \text{ }^{\circ}\text{C}$
<i>Boiling point</i>	$3,825 \text{ }^{\circ}\text{C}$
<i>Oxidation states</i>	6, 5, 4, 3, 2, 1, -1, -2

Figure 2: Optical micrographs of Pt nuggets. (A) cubic primary nugget (B) subhedral surface transformed placer nugget (Reith et al., 2014).

Platinum is referred to as a noble metal due to its relative chemical inertia. In fact, Pt is the least reactive metal in the periodic table and exhibits a remarkable resistance to corrosion (Reith et al., 2014), due to a very high oxidation potential, which makes it stable in air, even at high temperatures (it does not tarnish nor corrode). Platinum does not dissolve in nitric acid, but forms chloroplatinic acid when treated with hot *aqua regia*. The most common forms of Pt are the +2 and +4 oxidation states (+1 and +3 are found less often, Pawlak et al., 2014). Other physical and chemical properties of Pt are summarized in Table 1.

Six natural isotopes of Pt exist: ^{190}Pt , ^{192}Pt , ^{194}Pt , ^{195}Pt , ^{196}Pt , and ^{198}Pt . The most abundant of those is ^{195}Pt , with a relative abundance of 33.83 % (Table 2).

Table 2: Natural isotopes of Pt and their relative abundance

<i>Isotope</i>	^{190}Pt	^{192}Pt	^{194}Pt	^{195}Pt	^{196}Pt	^{198}Pt
<i>Abundance</i>	0.014	0.782	32.97	33.83	25.24	7.16

1.1.2. Platinum mineralogy and deposits: natural sources

Due to its scarcity on the Earth surface with typical abundances in the continental crust of 0.51 ng.g^{-1} (Peucker-Ehrenbrink and Jahn, 2001), Pt is considered as a “precious” metal. It belongs to the Platinum Group Elements (PGE) comprising also iridium (Ir), osmium (Os), palladium (Pd), ruthenium (Ru), and rhodium (Rh). As reported in the review of Reith et al., (2014), Pt shares properties with both siderophilic and chalcophilic elements. Like the siderophilic elements iron (Fe) and nickel (Ni), Pt has the tendency to form metallic rather than ionic bonds. But like all PGEs and chalcophilic metals including copper (Cu), silver (Ag) and gold (Au), Pt shares a tendency to form covalent bonds with sulphur (S) in preference to ionized bonds with oxygen (O) and can therefore be considered as a chalcophile element. Accordingly, the fundamental controls on the behavior and distribution of Pt on Earth are determined by the presence/absence of metallic and sulfidic phases (Reith et al., 2014).

Platinum group metals are as aforementioned, amongst the rarest elements on Earth and consequently, significant enrichment is required to form a deposit with economic PGM grade (currently most mining operations exploit deposits with grades ranging from 2 to 20 g.t^{-1} PGM (Koek et al., 2010). Accordingly economically productive ores are rare and few large deposits are identified. In these deposits, chromite and sulfide minerals concentrate the PGEs (Cawthorn, 2010). Deposits where the PGE are the main products and Ni and Cu are the by-products are called PGE reef-type deposits (Figure 3). Important examples of reef-type deposit include the giant Bushveld Complex, located in South Africa. The first economically important discovery of Pt in the Bushveld Complex was found in a single layer, associated with the sulfides, that we now call the Merensky Reef after its discoverer, Dr Hans Merensky. To date it has supplied most of the world’s PGEs, and also yields important quantities of Cu, Ni, cobalt (Co) and Au as by-products (Cawthorn, 2010). Other examples comprise UG2 Chromitite Layer and Platreef which are also part of the Bushveld Complex, and the Stillwater Complex (USA), or the Main Sulfide Zone of the Great Dyke (Zimbabwe, Cawthorn, 2010).

Other important primary deposits are Ni–Cu deposits (Figure 3). In these deposits, Pt mineralization occurs as a by-product (together with other PGE and Au) during the production of Cu and Ni (Maier, 2005). In these sulfide-rich deposits, PGEs occur as sulfides (e.g. (Pt,Pd)S), tellurides (e.g. PtBiTe), antimonides (PdSb), arsenides (e.g. PtAs₂), or alloyed to Ni and Cu (Reith et al., 2014). Important examples of this type of deposit include the deposits of Norilsk (Russia) and Duluth (USA). Other (primary) deposits comprise magmatic (e.g. Sudbury Igneous Complex, Canada, or Kambalda, Australia) and hydrothermal (e.g. Lac des Iles, Canada) deposits (Reith et al., 2014). Platinum also occurs in laterite environments and in numerous placers, where the most important alloy compositions lie in the Pt–Fe– and Ru–Os–

Pt–Ir systems (Reith et al., 2014). Even though placer deposits currently contribute approximately only 1 % of the global production, recent discoveries of large placers in Russia may increase their economic importance in the future.



Figure 3: World map showing the location of the largest primary Pt deposits, placer and other secondary Pt deposits described in the text, and the countries with at least two known localities of Pt minerals (Reith et al., 2014).

There are less than ten significant PGE mining companies in the world and of the few known deposits, global production of PGEs is dominated by South Africa due to their large resources in the Bushveld Complex, followed by Russia's Noril'sk–Talnakh field. Accordingly, South Africa and Russia control more than 95.9 % of the 80,000 t known PGE reserves (Koek et al., 2010) with their large deposits accounting for more than 90 % of the annual global production (Mudd, 2012). It is therefore noteworthy that Pt is mainly exported from where it is mined and, Pt use and potential release in the environment typically occur further away from its production zone. Other producer countries include Canada, Zimbabwe, and the United States which play a minor but still important role (Mudd, 2012).

Economic geologists report that abundant PGE resources permit sustainable production for a long time, as there are enough PGE deposits in the Bushveld Complex to supply world demands for many decades or even a century using current mining techniques (Cawthorn, 2010). Considering the current exponentially increasing demand, concerns may arise about the long-term ability to supply PGEs to meet likely future demands (Mudd, 2012). Platinum is in fact used for many industrial and technical applications that are described in the following section.

1.1.3. Technical uses of platinum and anthropogenic sources

Due to its different physical and chemical properties described previously, Pt serves for several applications related to corrosion resistance even at high temperatures, a high melting point, a considerable mechanical strength, and good ductility. Principal use of Pt, and other PGEs, relates to their unique catalytic properties. Due to all these properties, Pt is used in the glass, petroleum, electric, and electronic industries, and the manufacture of jewelry (Pawlak et al., 2014). This last application is common since more than 2000 years, and most Pt- jewelry manufacturing countries, produce jewels made in a purity of at least 85 % Pt. This sector accounted for ~ 10 % of the European Pt demand in 2017 (Figure 4; Johnson Matthey, 2017). Since the early 1960's, Pt-based drugs serve for cancer treatment, as Pt has the ability, in certain chemical forms, to inhibit the division of living cells. Since then, various molecules with Pt as the active agent (e.g Cisplatin, Carboplatin, Oxaliplatin) have been developed for anti-cancer drugs to decrease their toxicity and increase their efficiency (Eckstein, 2011). The medical use in Pt-based drugs does not exceed 5 % of the total European Pt demand. Furthermore, due to its chemical inertia (it does not corrode inside the body) and its good electrical conductivity, Pt components serve in pacemakers and other heart treatments or in dental alloys. Platinum may also be used in the chemical processing sector (nitric acid, silicones...), in electronic components, as a sensor in engine control systems to measure oxygen and nitrous oxide levels and in home safety devices to detect carbon monoxide.

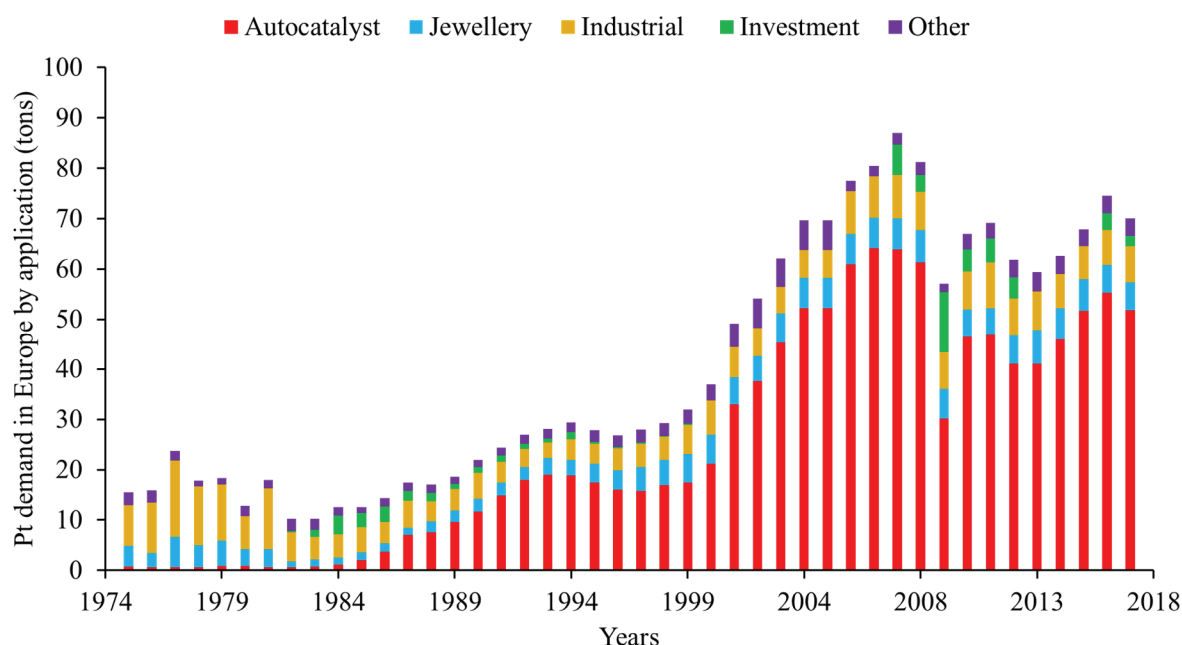


Figure 4: European Pt demand per application from 1975 to 2017 (forecast values for the year 2017). Values are expressed in tons (after Johnson Matthey, 2017).

A diversity of other Pt applications exist including glass, watches, fuel cells which correspond to devices for generating electrical power, spark plugs, and turbine blade coatings. Platinum catalysts are also used to produce petroleum and petrochemical feedstocks for plastics, synthetic rubber and polyester fibers. The most important application sector, representing more than 70 % of the total European demand for Pt in 2017, are the car catalytic converters, introduced in Europe in the late 1980's.

A car catalytic converter is a unit that fits into the front part of motor exhaust systems, close to the engine, and which aims at reducing gaseous pollutant emissions, mainly carbon monoxide (CO), nitrogen oxides (NO_x), and hydrocarbons (HC). It is usually fitted with a heat shield to limit heat losses (Figure 5). Removal of the pollutant gases from the exhaust occurs either after reduction, or oxidation (Ravindra et al., 2004).

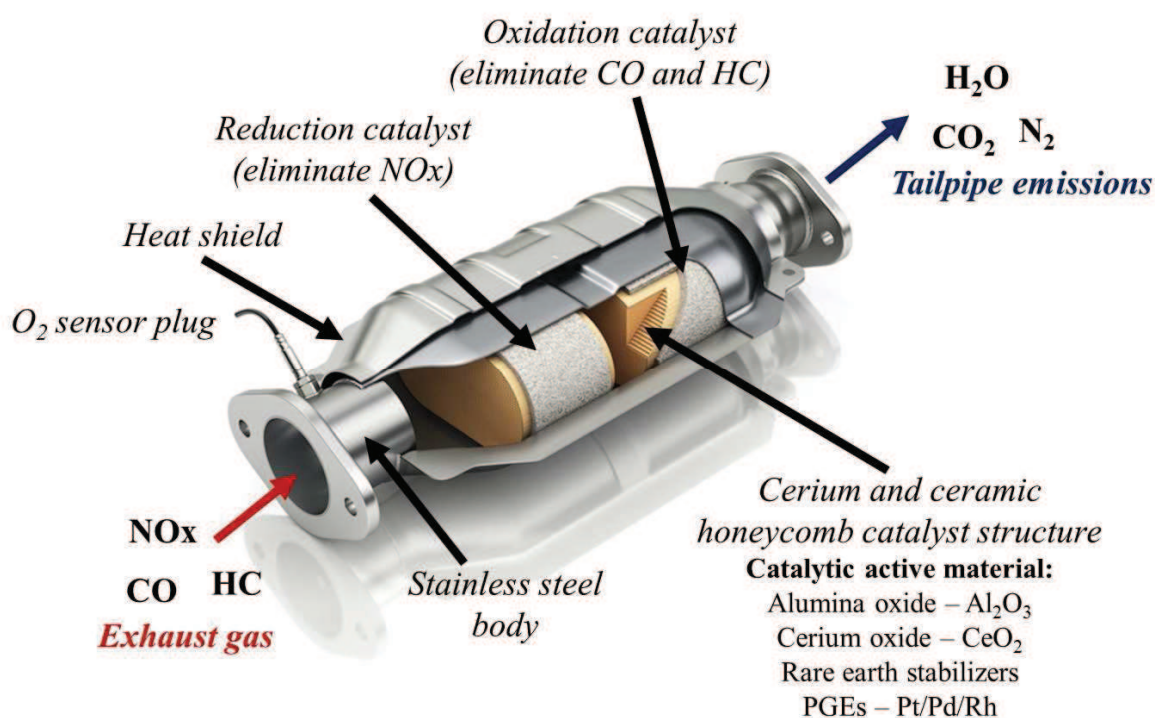


Figure 5: Car catalytic converter functioning (modified from oxyggroup.fr).

Car catalytic converters convert more than 90 % of CO, HC and NO_x into carbon dioxide (CO₂), water (H₂O), and nitrogen (N₂) respectively. Both CO and HC are eliminated together by oxidation reactions while NO_x are reduced to harmless N₂. The regulation system (lambda-probe and fuel injection management) controls the quantity of O₂ that enters into the engine ensuring that O₂ and fuel compounds are in optimum stoichiometric proportions to optimize converter efficiency (Ravindra et al., 2004).

Modern catalytic converters consist of a monolithic honeycomb support made of alumina-coated cordierite (a phase of $2\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2$; Mg: magnesium, Si: silicon) housed in a stainless steel box (Figure 5). This activated, high surface alumina layer is called “washcoat” and consists of $\sim 90\%$ $\gamma\text{-Al}_2\text{O}_3$ and a mixture of base metal additives, mainly oxides of cerium (Ce), zirconium (Zr), lanthanum (La), Ni, Fe, and some alkaline earth metals improving the catalyst performance (Ravindra et al., 2004). The PGEs (Pt, Rh and Pd) are fixed on the washcoat surface usually by impregnation, or coating from a solution of hexachloroplatinic (IV) acid ($\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$), palladium chloride (PdCl_2), and rhodium chloride (RhCl_3) salts. After evaporation of the solvent, decomposition and reduction of PGE salts are performed to obtain the highly dispersed catalytically “active” metallic forms (Ravindra et al., 2004).

There is a wide range of PGE combinations and concentrations in converters. Accordingly, Pt, Pd/Rh, Pt/Rh, Pt/Pd or Pt/Pd/Rh catalysts exist, yet, with overall percentage regarding the whole converter mass of less than 0.1 %. The combination and amount of Pt, Rh and Pd in catalytic converters have been influenced by technological developments and by changes in clean air legislation. First, from 1976 to 1979, the two-way catalysts, containing Pt and Pd, allowed the oxidation of HC and CO while the second generation (from 1979 to 1986) are the “three-way catalysts” including Pt, Pd, and Rh which serve also to reduce NO_x (Ravindra et al., 2004). The third generation of three-way catalytic converters comprises the same composition but is better adapted to high temperature working conditions in relation with new fuel efficient engines. Finally, from 1992 to the mid-1990s, another generation of three way Pd-rich catalysts was extensively applied by car manufacturers both in US and in Europe, in the new models to meet the even stricter emission legislation (Ravindra et al., 2004).

Since the beginning of 1993, all new cars sold within the E.U. must be fitted with catalytic converter (Jarvis et al., 2001). A catalytic converter designed for a family car normally contains about 1.75 g of PGE and a typical ratio of Pt/Rh of 5 (Barefoot, 1997). Catalytic converters generally have a service life of 80,000 - 160,000 km, although several factors may reduce this lifetime. Factor influencing its correct functioning include Pb pollution or short journey use that prevents the converter from reaching the optimal working temperature (around 400°C , Ravindra et al., 2004). However, the principal cause of converter failures is carbon pollution, leading to a partial, or sometimes, a total blockage of the catalyst, and the internal fracture of the catalyst surface, usually induced by external/internal physical damage.

Although catalytic converters are recycled, typical recovery is only around 20 - 30 %. Therefore over the 5 - year (or 80,000 km) average lifetime of a catalyst, up to 80 % of the Pt used in production will be released into the environment in one form or another (Jarvis et al., 2001).

However Pt emissions are not the only source of anthropogenic Pt dispersion in the environment.

1.2 Platinum dispersion and behavior in aquatic environments

The diversity of applications of PGEs in modern technologies is accompanied with anthropogenic dispersion of these elements in the natural environment. Consequently, PGEs are considered as emerging inorganic contaminants in the environment for which it is required to make reasonable quantitative estimates of not only environmental pathways, loads, and concentrations but also the socioeconomic drivers and "upstream" control measures (control, reduction, or elimination of emissions, Cobelo-García et al., 2015; Rodrigues et al., 2009).

Mitra and Sen (2017) established a quantification of natural and anthropogenic Pt fluxes across the different spheres: geo-, atmo-, bio-, hydro-, pedo-, and anthropo-spheres. Human contributions to the total anthrobiogeochemical Pt cycles are 5 %. When the Earth's surficial processes only are considered, soil erosion is the dominant flow for Pt mobilization, comprising 13 % of the total mobilization on the Earth's surface. On the other hand, mining activities, fossil fuel burning and automobile emissions are the most important anthropogenic flows and human contributions to the anthrobiogeochemical Pt cycles, considering only surficial processes, are greater than 70 %.

First works on Pt contamination of natural environments considered the exponentially increasing use of Pt in relation with car catalytic converters. Considering thermal and mechanical abrasion from the surface of the device, Pt, and other PGEs, particles are released into the environment and deposited in roadside soils. Consequently, first monitoring studies of Pt pollution in urban areas considered terrestrial environments with the analysis of soils and/or different types of plants (Morton et al., 2001; Schäfer et al., 1998). Anthropogenic PGE signals have been detected in all Earth compartments (Mitra and Sen, 2017). In addition, in urban areas, potential sources include not only automobile catalysts but also industrial processing, fossil fuel combustion and medical centers (Rauch and Peucker-Ehrenbrink, 2015). After emissions, this different Pt inputs may be dispersed in aquatic systems as described in the following section.

1.2.1. Anthropogenic dispersion of platinum in aquatic systems

Platinum Group Elements pollution is monitored in aquatic systems near mining zones in order to prevent potential environmental risks. Accordingly, in a recent study Almécija et al. (2017) evaluated the concentrations of PGE in stream sediments of the Hex River, which drains the mining area of the Bushveld Igneous Complex (South Africa). The highest concentrations were observed closer to the mining area, decreasing with distance and in the $< 63 \mu\text{m}$ fraction, probably derived from atmospheric deposition and surface runoff of PGE-rich particles released from mining activities. Thus, mining activities are causing some disturbance of the surface PGE geochemical cycle, increasing the presence of PGE in the fine fraction of river sediments (Almécija et al., 2017).

Distant from mining zones, Pt and other PGEs anthropogenic dispersion occurs especially in urbanized environments. In such systems anthropogenic emissions have largely been attributed to car catalytic converters. Accordingly, during the release of the exhaust gases from the engine, the surface of the washcoat is chemically and physically stressed by the fast change of oxidative/reductive conditions, high temperature and mechanical abrasion. This produces the emission of Pt, and other PGEs, containing particles into the environment. It is generally believed that mechanical erosion of the catalysts surface is the major cause, although thermal and chemical processes may also contribute to the emission (Rauch and Morrison, 2008). The amount and rate of PGE emissions from the catalytic converter are affected by the speed of the automobile, the type of the engine, the type and age of the catalyst, and the type of fuel additives (Artelt et al., 1999b). Emission can be intensified by unfavorable operating conditions (misfiring, excessive heating), which may even destroy the converter (Schäfer and Puchelt, 1998 and references herein). Measurements under laboratory conditions indicate that emission rates are in the low ng.km^{-1} range (Artelt et al., 1999b; König et al., 1992). Several studies suggest that emission rates are significantly higher for diesel catalyst than for three-way catalysts used in gasoline engines (Moldovan et al., 2002), and at higher speeds (König et al., 1992). Using the average emission rate of automobile catalytic converters and different deposition rates at different environments, the total deposition of Pt, Pd and Rh in the northern hemisphere is calculated. Total deposition from automobiles lies between 9 – 20 metric tons Pt.year^{-1} , 20 – 50 metric tons Pd.year^{-1} and 2 – 4 metric tons Rh.year^{-1} (Rauch et al., 2005). However, accurate estimation of PGE emissions from car catalytic converters remains uncertain despite nearly 30 years of research (Rauch and Peucker-Ehrenbrink, 2015).

Overall, calculated flows of Pt from automobile emissions represent the majority of total anthropogenic flows (Mitra and Sen, 2017). Following their emission, traffic related heavy metals are either subjected to atmospheric transport or are deposited on the road (Haus et al., 2007). Automobile atmospheric Pt dispersion is expected to have a relatively limited extent because PGEs are bound to fine particles (Rauch and Peucker-Ehrenbrink, 2015). A sharp decrease in PGE concentrations is observed within a few meters from automobile traffic (Jarvis et al., 2001; Schäfer and Puchelt, 1998). Therefore, when deposited on the road, Pt is washed into the drainage system during a later precipitation event, from where it may enter freshwater systems or sewage treatment systems (Haus et al., 2007).

Studies report that most of the PGEs released from catalytic converters are in particulate form (Ravindra et al., 2004). These elements might be emitted in the form of abraded washcoat particles onto which the PGEs are bound, and Ce is a major component of the washcoat. Platinum Group Elements are closely associated with Ce in road sediments, suggesting that they remain bound to autocatalyst particles and have a limited mobility (Rauch et al., 2000). Except for the association Pt-Rh, PGEs show a low degree of association with each other suggesting that they are emitted separately. The association of Pt and Rh might be explained by the formation of a Pt-Rh alloy in the autocatalyst (Rauch et al., 2000). Emitted particle sizes range from the sub-micron to $> 63 \mu\text{m}$ in automobile exhaust and in the urban environment (Rauch and Morrison, 2008). Difference in particle sizes is related to the fact that the morphology of PGEs in the converter is subject to physical and chemical alteration during vehicle operation (Rauch et al., 2001). Thus, while fresh catalysts present a smooth dispersion of noble metal particles in the range of 1 - 10 nm, the size of Pt in the aged catalyst can range from 20 nm to several microns due to sintering (Rauch et al., 2001). As aforementioned, typical Pt/Rh ratio for car catalyst are of 5 (Barefoot, 1997), but ageing of these catalyst through vehicle operation may lead to increasing ratio from 5.5 to 12 (Rauch et al., 2001).

Previous publications state that examination of these particles showed that around 99 % of Pt is in the metallic state Pt(0) sorbed to small particles of alumina matrix that is considered to be extremely inert (Artelt et al., 1999b). If at all, soluble platinum is emitted in only very small quantities i.e. $\leq 1 \%$. However, another study confirmed the particulate form of catalyst-derived Pt but showed that soluble Pt amount in exhaust fume samples of fresh gasoline and diesel catalysts is important even though it represents less than 10 % (Hill and Mayer, 1977; Moldovan et al., 2002). Because soluble fraction is defined as filter passing ($< 0.45 \mu\text{m}$), it may also include PGE nanoparticles (Rauch and Morrison, 2008). Accordingly, recent research performing road dust sample characterization through single particle Inductively Coupled Plasma-Mass Spectrometry (spICP-MS) revealed that Pt in the extracted leachate (0.2 to 18 %

of the road dust material) is entirely present as nanoparticles of sizes between 9 and 21 nm. Although representing only a minor fraction of the total content in road dust, the nanoparticulate Pt leachate is most susceptible to biological uptake and hence most relevant in terms of bioavailability (Folens et al., 2018).

Furthermore, although weathering by oxidation of PGE in natural system is low, moderately strong oxidizing solutions containing complexing agents such as chlorides or humic matter could lead to PGE dissolution processes (Jarvis et al., 2001). Accordingly, PGEs emitted by the catalytic converters, may not be in metallic form in exhaust fumes, or at least they could be rapidly altered, once they are deposited in the environment (Jarvis et al., 2001). Rauch et al. (2000) confirm that transformation of PGEs into a more mobile form might occur in the roadside environment, during transport through the storm water system or in urban rivers. Rainwater usually provides an important medium for the transportation of PGE particles until deposition. In run-off water up to $1 \mu\text{g.L}^{-1}$ Pt was found (Laschka and Nachtwey, 2000). Thus, measurements conducted in municipal wastewater treatment plants show an increase in average Pt loads in periods of rainy weather as compared to dry weather conditions, attributed to car traffic that causes increased Pt inputs into sewage treatment plants after rainfall (Laschka and Nachtwey, 2000).

Recently, Rauch and Peucker-Ehrenbrink (2015) addressed in a review the question: Are automobiles the main source of PGE in urban areas? While many studies support this source, other present discrepancies such as relatively small differences reported between cities with contrasting population sizes, vehicle numbers and catalyst introduction dates. Other divergences include traffic patterns and intensities at specific sampling locations. Discrepancies between expected and observed PGE concentrations or abundance ratios (used as a tracer of catalytic converter pollution) suggest that a number of sources contribute to PGE fluxes in urban areas. Non-automobile sources may contribute to both local and global PGE cycles (Figure 6).

Industrial sources emitting Pt into the atmosphere are likely but difficult to quantify according to Helmers and Kümmerer (1999), because few data are available for industrial PGE emissions. Moreover, Pt pollution by hospitals is also to consider in urbanized areas. As aforementioned, for the past 25 years Pt compounds have been used to treat numerous types of tumors (e.g. testicular, ovarian, bladder). Given the number of cancer cases, another important source of environmental pollution with Pt are hospitals, particularly those with chemotherapy departments (Pawlak et al., 2014). After treatment, chemotherapeutic agents are excreted by patients. For instance, carboplatin is mainly excreted via patient urine in its intact form. Hence, it can be deduced that aquatic systems most of the emitted compound is present as parent drug, while oxaliplatin degradation in aqueous media depends on chloride concentration (chloro-

aqua-complexes, (Hann et al., 2005). Since effluents from hospitals are not treated in any special way, these compounds are released directly to municipal wastewater systems (Pawlak et al., 2014). The concentration of Pt in the sewage of various hospitals was determined and the enriched Pt concentrations were detected with diurnal variations (higher excretion during daytime, Kümmerer et al., 1999). Variability of Pt discharges was also observed over a three-week period of time monitoring effluents from a major UK hospital (Vyas et al., 2014). About 70 % of the Pt, administered in the form of either cisplatin, or carboplatin, is rapidly excreted, and, therefore, would end up in hospital effluents (Ravindra et al., 2004). Such excretion also continues outside the hospital, as more and more patients leave for home directly after the treatment (Vyas et al., 2014). Therefore, the lack of appropriate treatment methods for the purification of such active compounds contributes to Pt contamination of the aquatic environment. Although predicted concentrations are below European Medicines Agency (EMA) guidelines warranting further risk assessment, the presence of substances in surface waters that are potentially carcinogenic, mutagenic and teratogenic and whose environmental effects are not understood is cause for concern (Vyas et al., 2014). Platinum emitted by hospitals represent between 3 and 12 % of the amount of Pt emitted by car catalytic converters (Reith et al., 2014). Elevated PGE concentrations reported in urban sewage and waste originating from various aforementioned sources, may potentially become secondary PGE sources. They may lead to PGE emissions during sewage treatment, reuse, or disposal (Rauch and Peucker-Ehrenbrink, 2015). Thus, depending on characteristics of the sewage network, sewage discharges as secondary sources can result in the release of PGE into both the agricultural and the aquatic environments.

Today PGE are used in a wide range of applications and emissions might occur during PGE production, manufacture of PGE-containing products and use and disposal of these products. Although emissions from PGE production and manufacture are expected to be limited or relevant to specific sites, the use and disposal of PGE-containing items are of concern because of the potential leaching of PGE (Rauch and Morrison, 2008). Same authors also reported that the contribution of natural sources, including erosion and volcanic emissions, and the potential impact of human activities on some natural sources also need to be investigated since increased erosion resulting from agriculture or deforestation may for instance contribute to elevated concentrations at remote sites where no direct anthropogenic sources are present. Mitra and Sen (2017) have addressed such Pt flows revealing that mining activities, fossil fuel burning and automobile emissions are the most important anthropogenic flows (Figure 6). In addition, PGEs are of great relevance in the development of emerging key technologies and are in this context considered as Technology-Critical Elements (TCEs). Associated environmental impacts of

such elements (from mining to end-of-life waste products) is not restricted to a national level but covers most likely a global scale (Cobelo-García et al., 2015).

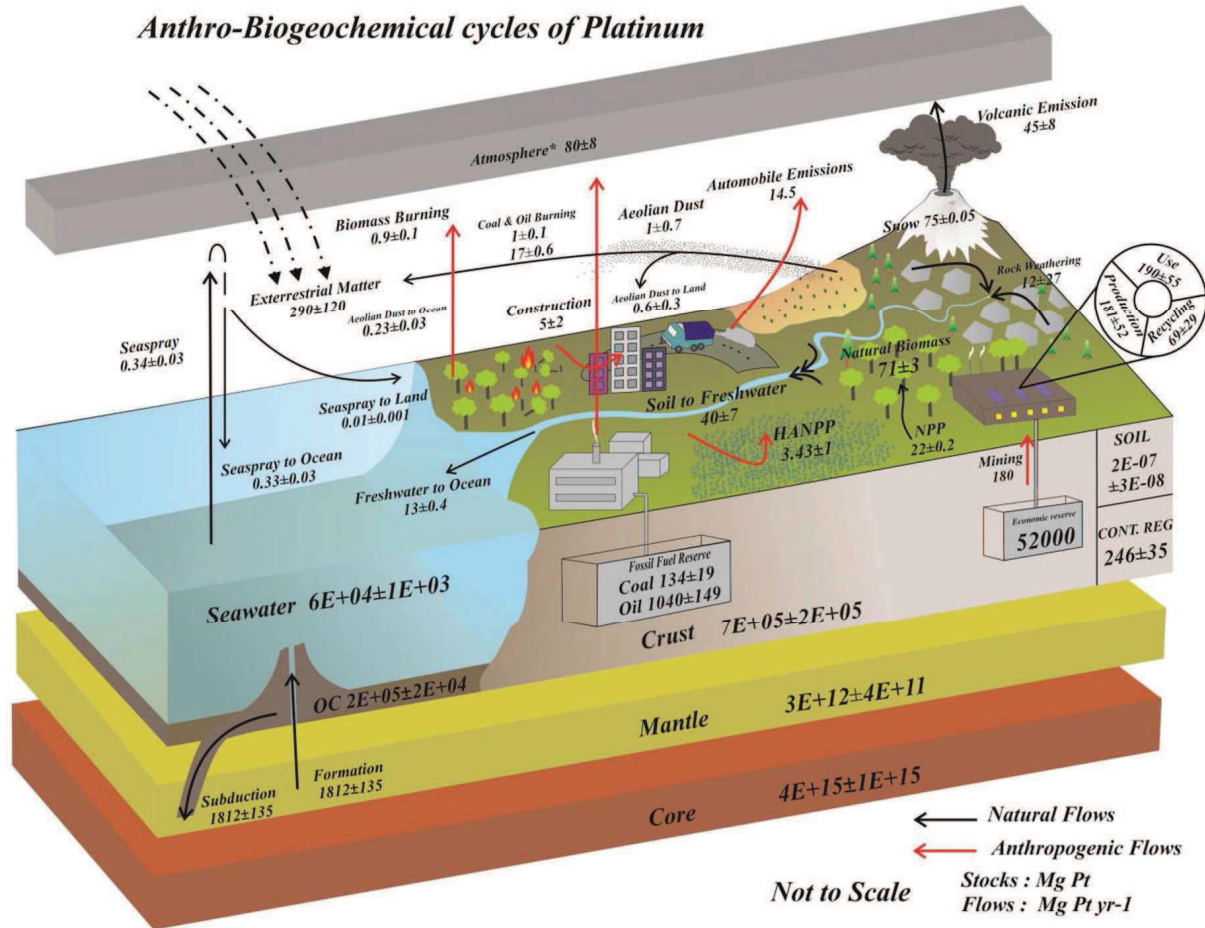


Figure 6: Stocks and flows of the platinum cycle. Natural flows are marked by black lines, whereas anthropogenic flows are marked by red lines. Atmospheric stock of Pt is calculated by adding the natural and anthropogenic flows that are moving elements from different spheres to the atmosphere. Authors have assumed that the atmosphere is not a proper repository and PGEs have a short residence time, like other industrial metals (Mitra and Sen, 2017).

Overall, most of the PGEs were thought to behave in an inert manner, and to be immobile (Zereini et al., 1997). However, other studies proving their solubility and occurrence in various environmental compartments proved the importance of studying how these metals may become chemically/biochemically active, and mobile in interactions with different environmental matrices and especially in aquatic systems. Despite uncertainties in emission and release estimates, available data are consistent with the anthropogenic dispersion of PGE and more particularly Pt into the natural aquatic environment. According to the source of emission and to the recipient compartment, Pt present different speciation and chemical behavior.

1.2.2. Platinum geochemical behavior in aquatic systems: river, estuary, coastal systems, open oceans

Aquatic ecosystems can be considered as an important sink of PGEs. As aforementioned, different sources, like road runoff or industrial effluents are directly discharged into these ecosystems (Ruchter et al., 2015).

Freshwater

As reported in Ruchter et al. (2015), freshwater lakes (lentic water systems) are a good starting point to study anthropogenic inputs of PGEs into aquatic ecosystems. As metals accumulate in lake sediments, sediment cores may be used as pollutant archives to trace back historical evolution of Pt inputs in a system (Von Gunten et al., 1997). Accordingly, analysis of dated sediments from an urban lake near Boston, Massachusetts reflected increasing PGE concentrations following the introduction of catalysts with accumulation rates 6 to 16 times greater in the period 1992 – 2002 than before their introduction (Rauch et al., 2004; Rauch and Hemond, 2003). Ratios of these elements closely match their ratios in catalysts, providing further evidence of an automobile source and introduction through road runoff (Rauch et al., 2004).

Among all investigated aquatic systems, most information originates from rivers (flowing water systems) for which several Pt sources are identified. Rivers are non-tidal zones where the salinity does not exceed $S = 0.5$ (Elliott and McLusky, 2002). These studies include direct discharges due to industry and waste water treatment plants which often combine PGEs from road runoff, hospitals, dental clinics, domestic wastewater, etc., and diffuse discharges through highway runoff (Ruchter et al., 2015). Despite the relatively large (compared to field studies on other aquatic system) number of studies existing on PGE distribution in riverine systems, limitations in comparison occur since in some of the studies cited in Figure 7, sampling points are barely described. It is therefore often unclear if there is a source of PGE near the sampling point or if samples were collected in urban or rural areas (Ruchter et al., 2015).

Water Pt concentrations in rivers often range at levels near or below the detection limits. According to literature urban rivers show levels between 0.006 and 2.6 ng.L⁻¹ Pt (Figure 7). Dissolved Pt (Pt_D) concentrations in the Leréz River flowing through the city of Pontevedra, a small urban system in Spain, are 0.006 – 0.04 ng.L⁻¹ in the freshwater end member (Cobelo-García et al., 2013). Dissolved Pt concentrations in the Garonne River, France, are of ~ 0.1 ng.L⁻¹ (Cobelo-García et al., 2014a). Mashio et al. (2016) reported Pt_D concentrations between 0.014 and 1.3 ng.L⁻¹ in two urban rivers flowing through densely populated areas of

Tokyo, while maximum concentration of 6.9 ng.L^{-1} were already observed in the same area (Obata et al., 2006). This result proves the spatial and temporal variation of Pt_D concentrations even in the same river. The high Pt_D concentrations in these rivers could not be explained from rainwater inputs directly since maximum values in rain were of $\sim 0.6 \text{ ng.L}^{-1}$, but indicate additional sources such as aerosols, river sediments, and sewage treatment plants (Mashio et al., 2016).

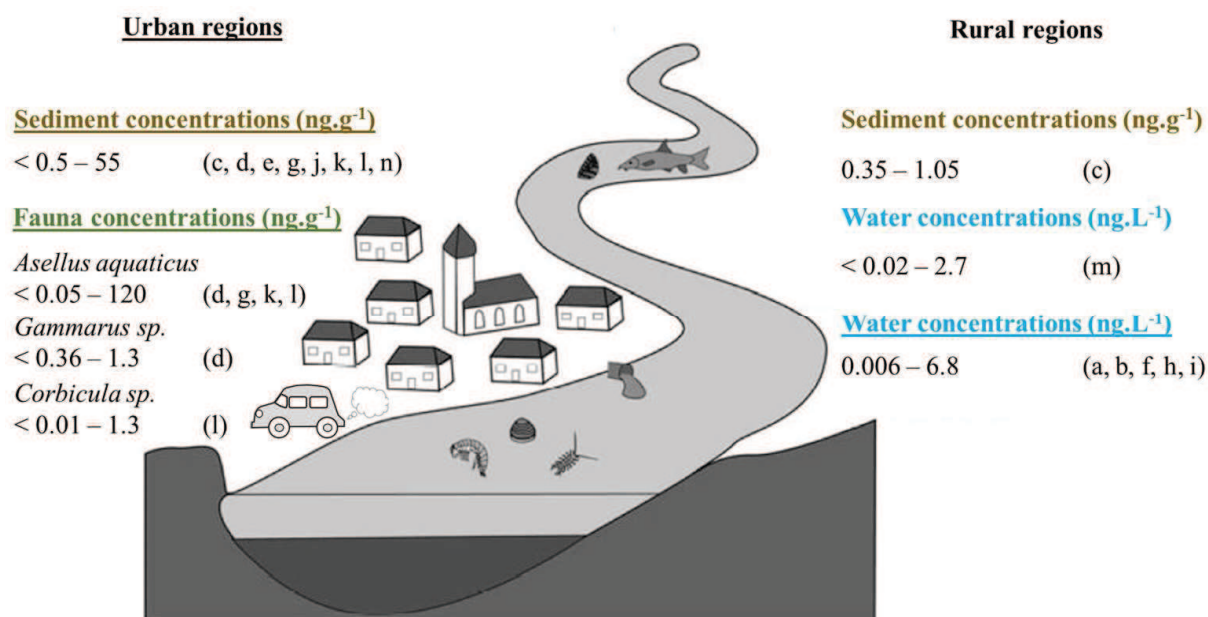


Figure 7: Literature data of PGE concentrations in river environments. References: a, b: Cobelo-García et al., 2014a, 2013; c: de Vos et al., 2002; d: Haus et al., 2007; e: Jackson et al., 2007; f: Mashio et al., 2016; g: Moldovan et al., 2001; h: Monticelli et al., 2010; i: Obata et al., 2006; j: Prichard et al., 2008; k: Rauch and Morrison, 1999; l: Ruchter and Sures, 2015; m: Soyol-Erdene and Huh, 2012; n: Wei and Morrison, 1994 (diagram modified after Ruchter et al., 2015).

Concerning particulate Pt (Pt_P) in sediments and SPM, inputs mainly originate from soil erosion and runoff from urban surfaces (Cobelo-García et al., 2014a). Therefore riverine sediments are assumed to be the first sink of Pt and other traffic-related metals (Haus et al., 2007). Accordingly, Pt concentrations in sediments from urban regions may reach much greater levels than maximum values in rural environments (Figure 7). In another urban environment, Ruchter and Sures (2015) studied the importance of road runoff inlets on the distribution of Pt in natural compartments including riverine sediments. They concluded that in contrast to road runoff, point sources usually discharge metals continuously. In fact, road runoff water discharges are often short time events (some minutes to a maximum of several hours) and the main metal load is discharged immediately at the beginning of the event (Ruchter and Sures, 2015). Sediments

in rivers may serve as archives, and traffic-affected habitats can be identified through Pt enrichment as already seen in urbanized aquatic systems (Haus et al., 2007). The distribution and variation in PGE concentrations and element ratios in the contemporary fluvial sediments of the Kentish Stour correspond strongly to land-use changes (urban versus rural) and with points of discharge from sewage works (de Vos et al., 2002).

The absence of a typical catalytic converter signature in the river sediments indicates that source signatures may be mixed in sewage works or that PGE may chemically fractionate in the fluvial environment (de Vos et al., 2002). In fact, once dispersed in the environment, Pt may undergo chemical transformation leading to the presence of different Pt forms and species according to the characteristics of the recipient system. Consequently, another important information to include in biogeochemical cycles of elements is metal speciation as the physical and chemical form of contaminants may considerably influence their biological availability. There is only limited thermodynamic information relevant to the speciation of Pt in aquatic environments. Available data suggest that both Pt(II) and Pt(IV) complexes exist in nature i.e. mainly tetrachloroplatinates and hexachloroplatinates (Artelt et al., 1999a). In freshwater, speciation of Pt(II) and Pt(IV) is dominated by $\text{Pt}(\text{OH})_2$ and $\text{Pt}(\text{OH})_5$ respectively. Moreover, calculations undertaken by Cobelo-García et al. (2013) suggest that Pt(IV) is the most important oxidation state in seawater while Pt(II) dominates in freshwater. If today there is clearly more information on contamination by Pt and other PGEs in freshwater systems, information on marine systems remains extremely scarce although some of the earliest experiments for the establishment of analytical procedures for Pt determination were conducted with seawater (Hodge et al., 1986; van den Berg and Jacinto, 1988). Understanding the transfer processes of anthropogenic Pt contamination from the continent to the ocean requires information on Pt behavior in estuaries, for which the first comprehensive data were obtained only recently.

Estuaries

Estuaries are transition zones between riverine (freshwater) and marine waters. They correspond to the semi-enclosed downstream part of a river, subject to the tide and containing from the limit of brackish waters. River estuaries are coastal inlets where, unlike “large shallow inlets and bays”, there is generally a substantial freshwater influence (Elliott and McLusky, 2002). Upstream, estuaries extend landward as far as the limit of the highest tide. Even though some discrepancies may exist in the accurate definition of estuaries, common features comprise a set of gradients in physical and chemical conditions from the open sea to the sheltered estuary. Along these gradients, there are clear changes in salinity ranging from full strength seawater

decreasing to freshwater as well as the often-associated sedimentary changes from coarse sediment outside the estuaries to fine sediments within them. Other changes relate to alterations in the turbidity of the water column, or in chemical composition including pH, changes in nutrients, dissolved gases and trace metals (Elliott and McLusky, 2002).

Obata et al. (2006) were among the first to report Pt_D values in estuarine waters. Mean Pt_D concentrations above 1 ng.L^{-1} occurred in the Tama and Ara estuaries (Tokyo, Japan). Platinum enrichment in the dissolved phase in the middle of the Tokyo Bay estuaries was mainly attributed to enhanced anthropogenic emissions of PGEs in the densely populated Tokyo area which receives huge deposits of anthropogenic materials (Obata et al., 2006). Similarly, Pt enrichment in estuarine sediments in Avondale Creek (Australia) is likely caused by the transport of road sediments by the local drainage system and their dispersion into local creek and estuary sediments (Pratt and Lottermoser, 2007). Investigations were conducted in order to gain some knowledge on Pt behavior during estuarine mixing including particle–water interactions observed in laboratory experiments (Couceiro et al., 2007; Turner, 2007), and field observations (Cobelo-García et al., 2013; Obata et al., 2006). Laboratory works suggest that, regarding Pt speciation in seawater, the inorganic equilibrium speciation of Pt(II) and Pt(IV) is dominated by $PtCl_4^{2-}$ and $PtCl_5(OH)^{2-}$, respectively (Gammons, 1996). Platinum is believed to form a stable chloro-complex in seawater. Therefore, increased concentrations of chloride along the estuarine gradient might inhibit the precipitation of Pt (Obata et al., 2006). Furthermore, along estuarine salinity gradients, relatively low particle affinity occurs in relation with decreasing electrostatic attraction between inorganic complexes and the negatively charged surface of estuarine particles (Turner, 2007). Laboratory observations reporting decreasing particulate-aqueous distribution coefficient with increasing salinity (Cobelo-García et al., 2008; Turner, 2007), suggesting the occurrence of dissolved Pt release, are consistent with mid-estuarine Pt_D enrichment observed in nature (Obata et al., 2006).

Cobelo-García et al. (2013) investigated the factors controlling the behavior of Pt during estuarine mixing from two estuarine transects performed in the Lérez Estuary (Pontevedra Ria, NW Iberian Peninsula). Non-conservative behavior of Pt during estuarine mixing was observed for both transects. Concentrations in the seawater-end members (0.12 ng.L^{-1}) exceeded those of typical North Atlantic waters (0.05 ng.L^{-1} , Colodner, 1991), suggesting either addition by biogeochemical transformation and/or inputs of this element within the estuary. The decrease in Pt particle-reactivity during estuarine mixing observed in both transects requires an increase in the proportion of negatively charged Pt species that have relatively little affinity for the negatively-charged particle surfaces as previously observed in laboratory experiments. Similar to other elements such as Cd, Pt in estuaries might desorb from suspended particles as it crosses

the estuarine interface, especially in those fed by anthropogenically-impacted freshwater inputs (Cobelo-García et al., 2013).

In the macrotidal Gironde Estuary, Cobelo-García et al. (2014a) also reported dissolved Pt mobilization from suspended particles crossing the estuary, even though some discrepancies occurred. This has been related to the highly turbid conditions and high particulate matter stock in the Gironde Estuary. In fact, minimum Pt_D concentration in the low salinity range observed under high discharge conditions suggests that Pt removal may occur in the maximum turbidity zone (MTZ) of the Gironde Estuary. Particulate Pt also exhibited mid-estuarine maxima, attributed to the presence of anthropogenic Pt in this region, but no clear trend for particulate/dissolved Pt distribution coefficients was evident (Cobelo-García et al., 2014a). Platinum estuarine partitioning appears to be kinetically constrained and sensitive to discharge conditions, and also, to the different forms of Pt adsorbed on SPM with different labilities. In addition, specificities of the suspended particulate matter may also influence Pt distribution.

Accordingly, discrepancies observed in Pt behavior between the Lérez and Gironde Estuaries could originate from higher estuarine turbidities implying the presence of SPM with larger mean diameters and lower surface areas (Turner and Millward, 2002), leading to lower propensity for aqueous metal adsorption (Cobelo-García et al., 2014a) and therefore lower Pt_P concentrations. More recently, Mashio et al. (2016) investigated Pt behavior in urban estuaries around Tokyo Bay and Otsuchi Bay (Japan) and confirmed the adsorption-desorption mechanism of Pt in such systems. Platinum levels in the different compartments from the upstream estuary, to the coastal area and finally the open ocean are reported in Figure 8. Organic forms of Pt (anticancer drugs) also react through estuarine salinity gradient confirming the aforementioned behavior, since there is a decreasing adsorption on SPM with increasing salinity (Turner and Mascorda, 2015). For estuarine sediment samples, Pt concentrations are generally lower compared to those of riverine systems (including lower maxima). In analogy to lake sediments, where higher Pt concentrations at the sediment surface compared to deeper layers suggest a relatively recent Pt contamination, one may expect similar distribution patterns in estuarine and marine sediments (Cobelo-García et al., 2011). Geochemical signals in sediments may also be modified by early diagenetic processes (see Chapter 4), although the range of Pt values in Suspended Particulate Matter (SPM) is rather similar to sediment concentrations (Cobelo-García et al., 2014a).

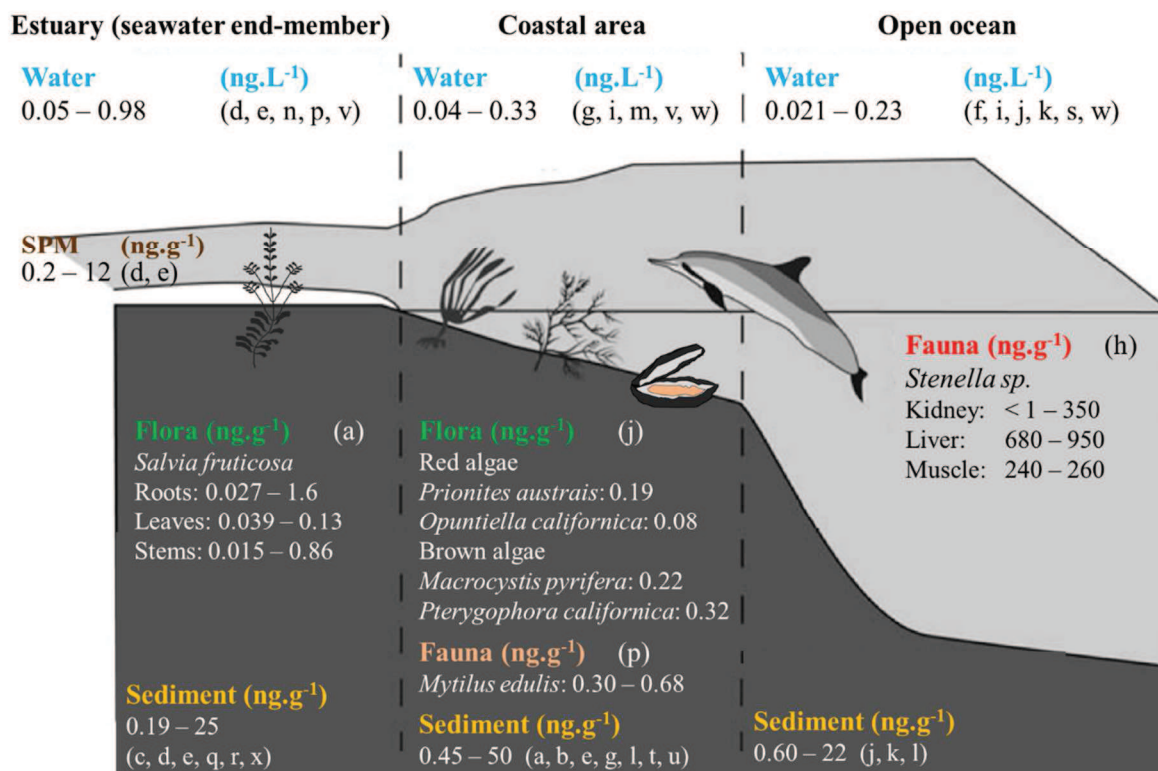


Figure 8: Platinum concentrations in estuaries, marine coastal zones, and open oceans. References: a,b: Almécija et al., 2015, 2016; c: Cobelo-García et al., 2011; d,e: Cobelo-García et al., 2013, 2014a; f: Colodner et al., 1993; g, h: Essumang, 2008, 2011; i: Goldberg et al., 1986; j, k: Hodge et al., 1986, 1985; l: Koide et al., 1991; m, n: Mashio et al., 2016, 2017; o: Neira et al., 2015; p: Obata et al., 2006; q: Pratt and Lottermoser, 2007; r: Prichard et al., 2008; s: Suzuki et al., 2014; t: Terashima et al., 1993; u: Tuit et al., 2000; v: Turetta et al., 2003; w: van den Berg and Jacinto, 1988; x: Zhong et al., 2012 (diagram modified after Ruchter et al., 2015). SPM: Suspended Particulate Matter.

Coastal area

Along the transition zone at the continent-ocean interface, coastal areas range from the outer edge of the continental shelf to the upper limits of the tidal zone. In this area comprising lagoons, bays and outer estuaries, natural Pt geochemical behavior remains widely unknown. Average Pt contents in coastal sediments from the southeastern margin of the Japan Sea are generally similar to crustal abundance and Pt distribution is controlled by the geological characteristics of the studied area, grain size and redox potential of sediments, water depths, and dissolved O₂ concentration in seawater (Terashima et al., 1993). However, Pt concentrations observed in coastal sediments from Chile reached ~ 9 ng.g⁻¹ (Koide et al., 1991). Such relatively high levels may reflect either natural processes as observed for deep sea sediments (see below, Hodge et al., 1985) or anthropogenic inputs.

According to Tuit et al. (2000), there are two possible patterns of chemical behavior of anthropogenic Pt in the coastal environment. If chemically inert, Pt may serve as tracer of recent anthropogenic input, particularly from road runoff. Alternatively, if Pt is chemically labile, Pt could undergo speciation changes and potentially be available to the food web (Tuit et al., 2000).

A study conducted in Boston Harbor sediments has shown that anthropogenic concentrations of Pt and Pd exceed those in pristine sediment by at least 5-fold, suggesting that anthropogenic enrichments can significantly influence coastal marine inventories of PGEs (Tuit et al., 2000). While no maximum Pt_p values occurred in topmost sediment layers from the Tagus Prodelta (Spain), maximum concentrations observed at the base of the surface mixed layer is probably driven by the scavenging of Pt at the oxic/anoxic boundary (Cobelo-García et al., 2011). Such chemical behavior was also observed in sediment cores collected in the Boston Harbor revealing Pt post-depositional mobility (Tuit et al., 2000).

Platinum concentrations in saltmarsh sediments at a site in the Tagus Estuary impacted by high traffic showed a surface peak value exceeding 40 ng.g^{-1} , i.e. 15 times greater than those recorded at a “low traffic” site in the same system (Almécija et al., 2015). In the contaminated site, Pt concentration decreases with depth, reaching average background values of $\sim 0.7 \text{ ng.g}^{-1}$; below 5 cm. This study also evidenced decoupled Os and Pt behavior in intertidal sediments resulting from differences in geochemical reactivity and range of dispersion from their sources (Almécija et al., 2015). Relatively high values were also reported in the coastal belt of Ghana with Pt concentrations in sediments exceeding 50 ng.g^{-1} (Essumang, 2011). In this country as in many other developing countries, examination of car fleet records shows an increasing proportion of relatively old catalytic converter-equipped cars imported from developed countries. The unregulated use of catalytic converters, added to the fact that Pb is still used as a petrol additive, may lead to increasing Pt emissions (Kylander et al., 2003). Other reported Pt concentrations in coastal sediments ranged between 0.51 and $\sim 7.5 \text{ ng.g}^{-1}$ (Cobelo-García et al., 2011; Terashima et al., 1993; Tuit et al., 2000).

Only a very limited number of studies reports on Pt_D concentrations in coastal waters (Figure 8). They include the studies of (i) van den Berg and Jacinto (1988) stating a concentration of 0.33 ng.L^{-1} in waters from the Menai Strait (UK), (ii) Turetta et al. (2003) revealing average value of 0.2 ng.L^{-1} in the Venice Lagoon (Italy), and (iii) Essumang (2011) reporting relatively high values between 4 and 12 ng.L^{-1} in Ghana.

Very recently, Mashio et al. (2017) reported Pt_D concentrations ranging from 0.04 to 0.29 $ng.L^{-1}$ in coastal seawater from Boso to Sanriki, Japan with the highest concentration in bottom water of the Boso coastal area, and at stations close to Tokyo Bay. Vertical profiles in the water column showed increasing Pt levels toward seafloors near coastal sampling sites and this enrichment was attributed to release from coastal sediments. In 2011, coastal areas in northern Japan were affected by a huge tsunami which has probably transferred many materials from inland to coastal areas, depositing them offshore along the eastern coastal Japanese area (Mashio et al., 2017). Variations in Pt distributions over time should be carefully monitored in the future in coastal areas, to evaluate the long-term response to the huge 2011 tsunami. Similarly, this need is obvious at the global scale, when considering the scarcity of field studies reporting Pt levels in marine coastal compartments.

Open Ocean

Hodge et al. (1986) and Goldberg et al. (1986) were the first to report Pt_D concentrations in the Pacific Ocean at different depths. Surface samples showed levels of $\sim 0.09 ng.L^{-1}$, while at $\sim 3,500$ m depth Pt_D was close to $0.2 ng.L^{-1}$. In the ocean water column, Pt similar to Pd, shows a nutrient-type profile in seawater (Hodge et al., 1986). The range of Pt_D in the Indian Ocean ($0.04 - 0.31 ng.L^{-1}$) is similar to that in the Pacific Ocean (Jacinto and van den Berg, 1989). However, unlike previous investigations reporting a nutrient-like behavior for Pt, data from the Indian Ocean suggest that Pt is scavenged down the water column and is depleted with depth (Jacinto and van den Berg, 1989). Such results are consistent with the predominant oxidation state of Pt in oxygenated seawater being $Pt(IV)$ and the association of Pt with manganese nodules in sediments. In fact, exceptionally high Pt values exceeding $900 ng.g^{-1}$ occur in the ferromanganese nodules, the so-called "platinum anomaly" (Hodge et al., 1986, 1985). In ocean sediments, Pt concentrations range from 0.7 to $22 ng.g^{-1}$ (Hodge et al., 1986). In marine pelagic sediment samples most of the Pt concentrations range around $0.4 ng.g^{-1}$. However, pelagic clays are often enriched, relative to other sediments, in Pt and other PGEs that are associated with ferromanganese phases and thus display relatively high Pt_P levels (Koide et al., 1991).

While Pt depletion occurs in surface waters from the Pacific Ocean and surface enrichment is recorded in the Indian Ocean, Colodner et al. (1993) reported low, invariant Pt concentrations along the depth profiles performed in the Atlantic Ocean.

Similarly, recent vertical profiles of Pt_D concentrations in the western North Pacific Ocean, the Sea of Okhotsk, and the Japan Sea confirmed conservative behavior of Pt distribution, implying rather limited interactions with biota and affinity with particles in seawater (Suzuki et al., 2014).

Such observations need to be completed by additional studies in order to understand biogeochemical cycles of Pt and relative speciation in oceans. Accordingly, vertical profiles of Pt_D concentrations in marine water columns measured 20-years ago by Hodge et al. (1986) and in 2010 by Suzuki et al. (2014) suggest that open oceans are not measurably influenced by anthropogenically-derived Pt. The range of Pt concentrations in dissolved phase and in sediments from the open ocean is reported in Figure 8.

1.3. Platinum bioavailability and bioconcentration in aquatic organisms

1.3.1. Field studies on aquatic organisms

There is a lack of field studies on the behavior of Pt in the aquatic biosphere. Platinum concentrations measured in wild living aquatic organisms are presented in both the Figure 7 and Figure 8. The relatively few publications report mainly on Pt uptake by freshwater organisms. After being introduced into aquatic habitats, Pt may accumulate in the sediments and get in contact with biota. Accordingly, relatively high Pt concentrations (up to 12 ng.g^{-1}) occurred in the freshwater isopod *Asellus aquaticus* sampled in urban rivers and in a storm water detention pond (Rauch and Morrison, 1999). Additionally, authors found that Pt concentrations in asellids depended on the molting status of the organisms. As they store ~ 50 - 75 % of the accumulated Pt in their exoskeleton, asellids can lose huge amounts of Pt just by molting (Rauch and Morrison, 1999).

Moldovan et al. (2001) measured Pt concentrations in *Asellus aquaticus* exceeding 100 ng.g^{-1} in some samples. Platinum uptake by *Asellus aquaticus* and *Gammarus pulex* in another urban river is relatively high compared to other traffic related heavy metals and the uptake rates may be similar to those of essential metals like Zn (Haus et al., 2007). In the freshwater mussel *Corbicula sp.*, soft tissue Pt concentrations remain relatively low (max Pt concentration: 1.3 ng.g^{-1}), despite high Pt enrichment in surrounding sediments from an urban river. Such low levels of Pt and other traffic-related metals in the clam tissues can possibly be explained by the active avoidance of the filtration of soluble metal forms, as well as particles in the water column (Ruchter and Sures, 2015). Although all studies conducted so far clearly indicate that Pt, and other PGEs introduced into natural river/estuary systems are potentially available for living organisms under field conditions, none revealed a correlation between sediment and biota concentrations (Ruchter et al., 2015).

Similarly, very few studies report on Pt concentrations in biota from marine environments. Almécija et al. (2016) reported the importance of vegetation on the geochemical behavior of Pt

in sediments due to roots controlling the redox conditions in the surrounding sediments (between the O₂ release and the organic matter degradation), implying potentially changing Pt species of varying bioavailability. In vegetated sediments Pt annual turnover is controlled by plants' activity and linked to Mn cycle (as already mentioned for Fe-Mn crusts and -nodules), reflecting dynamics of Pt co-precipitation/adsorbing/scavenging processes in the Mn-oxides formation (Almécija et al., 2016). Several marine/coastal algae species sampled in La Jolla (California, USA) showed Pt concentrations ranging from 0.08 to 0.32 ng.g⁻¹. The first study reporting on Pt levels in marine fauna from a coastal environment describes the temporal variation of Pt concentrations in marine bivalves (*Mytilus galloprovincialis*) sampled over several decades and provides evidence of the link between recently increasing Pt levels in animal tissues and the use of this metal in anthropogenic activities (i.e. catalytic converters in motor vehicles; (Neira et al., 2015). More recently, the temporal evolution of Pt in wild oysters from the Gironde Estuary supported similar conclusions.

Platinum concentrations in animal tissues (dolphins) collected along the Ghanaian Coast reached ~ 1 µg.g⁻¹ (Essumang, 2008). Such high levels might reveal intense anthropogenic pressure exerted by human activities and especially vehicle emissions in this country. Due to discrepancies in the described preparation methods (taxa remain unclear; although different tissues were analyzed, whole organism PGE levels are presented), Pt levels for other marine animals studied by Essumang (2008) are not referred to, as previously argued in Ruchter et al. (2015).

1.3.2. Laboratory studies on Pt uptake and biological response in aquatic organisms

Transformation of PGE and the occurrence of soluble species in the environment and the observed accumulation in wild organisms clearly suggest that Pt is present in bioavailable forms, and that exposure to PGE may result in bio-uptake and related toxic effects (Rauch and Morrison, 2008). Laboratory exposure studies, mostly on freshwater organisms, support that PGE are bioavailable in aquatic environment and can be taken up by aquatic organisms.

Veltz et al. (1996) studied the acute toxicity and bioaccumulation of Pt in *Lumbriscus variegatus* and proved that this metal is accumulated at constant rates, depending on exposure concentration, temperature, and time. Rauch and Morrison (1999) report on Pt uptake in the freshwater isopod *Asellus aquaticus*, highlighting the importance of Pt speciation for uptake. Works conducted by Zimmermann and co-workers contribute largely to increase the knowledge on Pt accumulation mechanisms, especially by exposing the freshwater zebra mussel *Dreissena polymorpha* to different Pt sources and -species (Zimmermann et al., 2002).

Speciation (dissolved, as precipitate, bound to organic substances, etc.) of Pt uptake by aquatic organisms remains unclear. So far, speciation analyses of the PGE in the exposure media are totally missing due to analytical restrictions. Furthermore, when exposed to particulate PGEs, particle size may have an important influence on metal uptake as the particle diameter affects the capture efficiency of bivalves. In addition to inorganic Pt species, ecotoxicological potential of cisplatin (originating from anticancer treatment) to the polychaete *Nereis diversicolor* was studied (Fonseca et al., 2017). Important burrowing impairment occurred in worms exposed to the highest cisplatin concentration (100 ng.L⁻¹) along with neurotoxic effects.

Exposure period is also an important parameter (Figure 9, Zimmermann and Sures, 2018) since exposure studies must last long enough to reach a steady state i.e. when tissue concentrations no longer increase with exposure time. Steady state also named accumulation plateau may be reached after some days or several weeks depending on different parameters such as metal source and test system. Due to these differences it is recommended to record the time-dependent course of PGE concentrations in the test organisms to ensure that the accumulation plateau is reached (Zimmermann et al., 2015). In most mussel exposure studies, a static test system with one initial metal addition to the tank water and no water replacement was used. In contrast, a flow-through system could provide a more constant exposure concentration but requires high amounts of PGE and produces high volumes of waste water (Zimmermann and Sures, 2018).

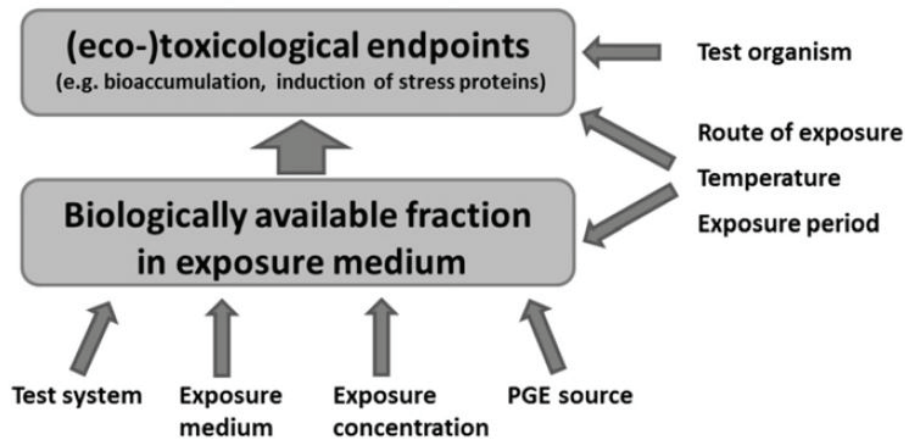


Figure 9: Possible experimental factors affecting the biologically available fraction and (eco-)toxicological endpoints of PGE in aquatic test systems (Zimmermann and Sures, 2018).

Ecotoxicological endpoints of exposure studies comprise measurement of tissue concentrations in order to determine bioaccumulation or bioconcentration factors (BAF and BCF respectively), or toxicological effects such as induction of stress proteins or tissue damage (histopathology). In addition to Pt bioavailability, different studies report a couple of adverse effects in aquatic organisms following Pt exposure under laboratory conditions. Biological adverse effects of Pt exposure can be expressed using different biomarkers at molecular, organelle, and tissue/organ levels. Molecular effects can be detected on DNA-, RNA- and protein-level, but few studies report on Pt effects at these level of organization.

Osterauer et al. (2011) investigated Pt toxic effects in zebrafish and ramshorn snail revealing no important genotoxicity in the first species, while significant DNA damages were observed in the second one. However, since Ruchter (2012) did not observe any Pt genotoxicity in clams, no general pattern of Pt genotoxic effects on mollusks can be drawn. Other physiological adverse effects following Pt exposure include the induction of heat shock proteins (hsp70, stress conditions; Singer et al. 2005), or tissue alteration (e.g. cytoplasm vacuolization; Osterauer et al., 2010).

Most of the above presented exposure-effect studies focus on freshwater and use soluble inorganic chlorine Pt compounds. Few experiments dealt with particle-bound PGE and/or environmental materials such as ground catalyst material, river sediment, and tunnel- or road-dust from heavily frequented roads (Zimmermann et al., 2015). Sures and Zimmermann (2007) explained the different behaviors of the three PGE in the tank water in the presence of humic matter by varying interactions of the metals with different fractions of humic substances, i.e. fulvic acids, humic acids and humins. Highest Pt uptake rate occurred in mussels exposed to suspending Pt-bearing particles (catalyst material or road dust) in humic water compared to tap water.

Only a limited number of publications report on Pt exposure in seawater media. They are summarized in Table 3 (reviewed from Zimmermann et al., 2015).

Table 3: Laboratory studies on the uptake and bioaccumulation of Pt by marine organisms (reviewed from Zimmermann et al., 2015).

Test organism	Exposure medium	Metal source	Exposure concentration	Exposure period	Reference
<i>Chlorella stigmatophora</i>	Seawater enriched with nutrients, trace metals, EDTA, and vitamins	Pt(IV), Pd(II), Rh(III) (standards)	20 µg.L ⁻¹ for each	24	Shams et al., 2014
<i>Ulva lactuca</i>	Natural seawater (filtered < 0.2 µm)	Pt(IV), Pd(II) (standards)	0.5 µM Pt + 1.0 µM Pd	≤ 180 min	Cosden et al., 2003
		K ₂ Pt(II)Cl ₄ , Pt(IV)Cl ₂ , Pd(II)Cl ₂	5.0 µM for each		
<i>Ulva lactuca</i>	Natural seawater (filtered < 5 µm)	Pt(IV), Pd(II), Rh(III) (standards)	10 µg.L ⁻¹ for each	≤ 100 h	Turner et al., 2007
<i>Ulva lactuca</i>	Natural seawater (filtered < 0.6 µm), “estuarine” water	Cis-PtCl ₂ (NH ₃) ₂	10, 25, 50, 100, 150 nM	≤ 48 h	Easton et al., 2011
<i>Arenicola marina</i>	Estuarine water (made of filtered seawater and MilliQ water)	Pt(IV), Pd(II) (standards) Ground catalyst material (150 µg.g ⁻¹ Pt, 0.55 µg.g ⁻¹ Pd)	40 ng.g ⁻¹ 8 g catalyst.L ⁻¹	10 days	French and Turner, 2008
<i>Littorina littorea</i>	Natural seawater (filtered < 0.6 µm)	Pt(IV), Pd(II), Rh(III) (multi-standard)	20 mg.L ⁻¹ (each)	5 days	Mulholland and Turner, 2011

Exposure route may also be important for Pt bioaccumulation in marine organism considering both direct (via dissolved phase) and dietary (via particulate phase) pathways. Mulholland and Turner (2011) showed that Pt accumulation by the marine snail *Littorina littorea* appears to mainly proceed through the aqueous phase, whereas French and Turner (2008) suggest that for the polychaete *Arenicola marina*, both aqueous and dietary sources are important for Pt bioaccessibility in sediment.

Despite the valuable knowledge obtained from exposure studies, most of them were performed with environmentally non-relevant exposure concentrations. As reported in Figure 8, natural seawater Pt concentrations generally do not exceed 0.3 ng.L⁻¹. Table 3 shows that exposure experiments typically apply high Pt concentrations exceeding by far environmental levels. This might be related to two main factors being i) the lack of knowledge of Pt ecotoxicological responses in the different test organisms, therefore leading to the application of high concentrations in order to ensure the observation of important effects at the different biomarker levels (Sures et al., 2015), ii) the analytical limitations in detecting Pt at environmental levels, especially in marine matrices. As stated by Zimmermann and Sures (2018), methods with low detection limits combined with a high sample throughput are needed for future exposure studies investigating the uptake and bioaccumulation of PGE at environmentally relevant concentrations.

In their review, the same authors made some recommendations for future PGE exposure studies with bivalves including the need for:

- Determination of the real exposure concentration during the exposure (that often differs from the nominal exposure concentration).
 - Monitoring of the real aqueous exposure concentration over the whole exposure period (especially regarding static or semi-static experiments).
 - Sufficiently long exposure period to reach the accumulation plateau.
 - Determination of PGE effects at subcellular levels including molecular biomarker responses.
 - Comprehensive description of exposure conditions (including PGE source, organism characteristics...) for reasons of reproducibility and comparability with other studies.
- In this sense, the use of standardized water as exposure medium is recommended.

Among these recommendations, one of the most important is the application of environmentally relevant exposure concentrations. Furthermore, important criteria for choosing a test species include availability in sufficient quantities, easy maintenance under laboratory conditions, and in respect of environmental aspects, also their representative role in the structure and function of the ecosystem (Zimmermann et al., 2015). In aquatic ecosystem, bivalves match all these criteria and therefore represent excellent test organisms that are, additionally, good biomonitoring species for a variety of pollutants.

Primary producers, plants constitute the first link in the food chain (Pawlak et al., 2014). Thus, in aquatic environments, algae should also be considered since they are grazed by higher trophic organisms including zooplankton and are also filtered by bivalves. In order to observe possible biomagnification through the food chains, those living organisms would be interesting targets for Pt cycle studies. Finally, long-term (chronic) toxicity studies at low exposure concentrations are needed for a reasonable assessment of the risk of PGE to aquatic organisms (Rauch and Morrison, 2008; Zimmermann and Sures, 2018).

Despite increasing concentrations, current knowledge on Pt levels in aquatic environments, and especially in marine systems, remains low and the presently available data are not sufficient for an accurate assessment of potential risks (Rauch and Morrison, 2008). Studies on the effects of Pt and other PGEs are sparse and do not provide enough environmentally relevant information. In this sense, and considering all the aforementioned recommendations, ecotoxicological studies exposing marine bivalve to environmentally relevant Pt concentrations may allow i) to use bivalves as a geochemical tool, enhancing natural signal and permitting to overpass some analytical challenges inherent to natural matrices, ii) accurately assess potential adverse effects of Pt to living organisms.

Chapter 2

Material and methods

Chapter 2: Material and methods

2.1. Introduction

In the scope of this study an original set of samples was constituted consisting in: (i) sediment cores collected through different environmental monitoring programs, (ii) samples from biomonitoring studies led by the French National Monitoring Program RNO/ROCCH, (iii) samples collected on-field during five sampling campaigns performed in various contrasting coastal sites, and (iv) laboratory-derived samples from an exposure study conducted on marine bivalves. The following section aims at introducing sample collection in the frame of the French national databank and the different sampling campaigns performed those last three years. The following section also introduces analytical procedures applied for Pt detection.

2.2. Study area and sampling strategy

Figure 10 shows the different sampling sites (stars) where samples were collected in the scope of this study.

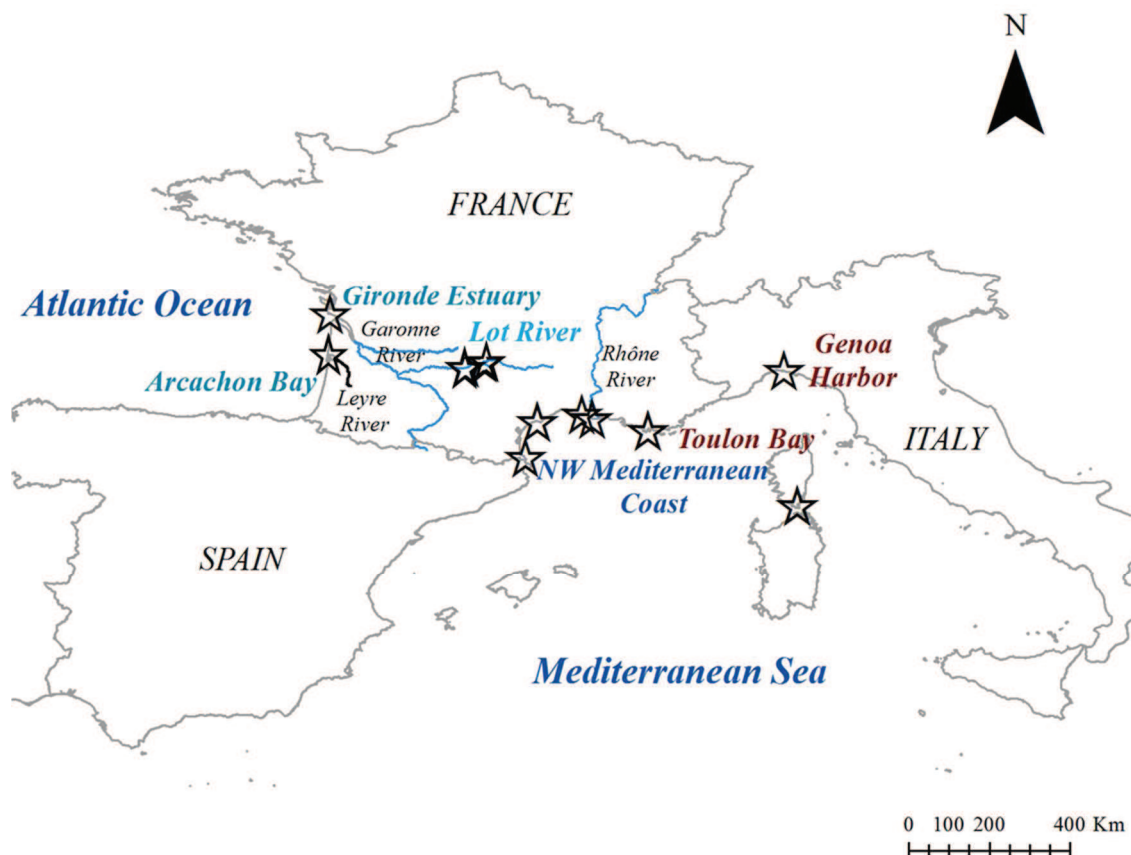


Figure 10: Sampling sites located in contrasting coastal environments. Sampling sites include riverine samples (light blue), estuarine and lagoon samples (darker blue) and coastal samples (dark blue) which include highly anthropogenically impacted sampling sites (red).

The following sections introduce sample characteristics as well as sampling campaigns performed for this study. In addition an exposure experiment was performed at the Marine Station of the University of the Basque Country (PiE-UPV/EHU, Spain) and experimental design are presented in the last section.

2.3.1. Sediment cores from the Lot River

The Lot–Garonne–Gironde fluvial–estuarine system is affected by multi-metal pollution originating from a common main point source being a former Zn-ore mining/metallurgic industry (e.g. 1842–1987: cadmium: Cd, zinc: Zn, copper: Cu, lead: Pb; Audry et al., 2004) on the Riou-Mort River near Decazeville (Figure 11).

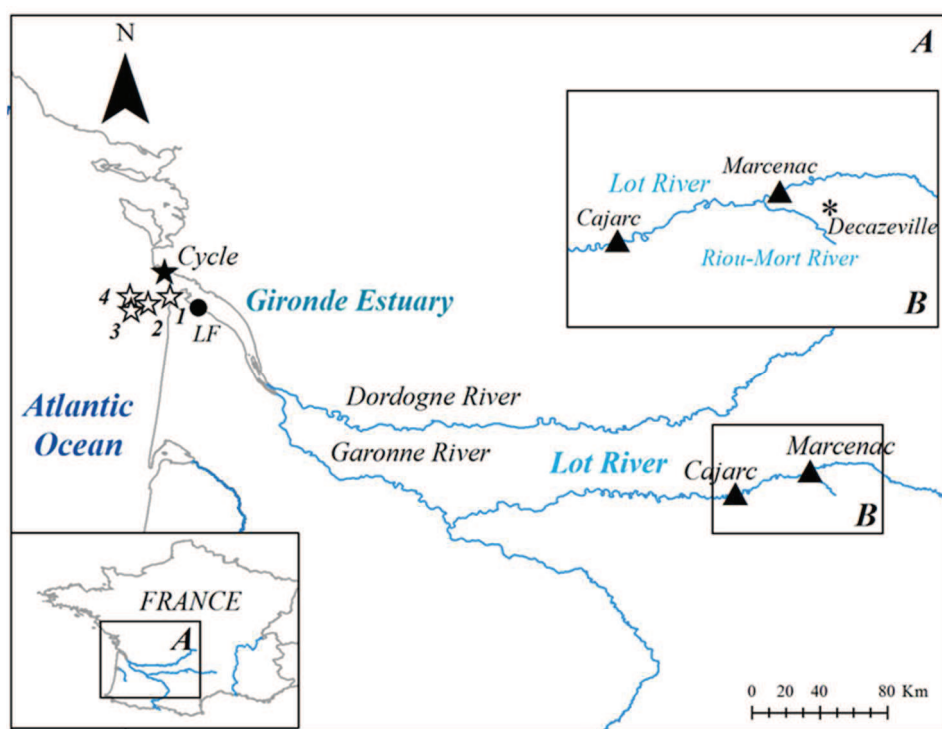


Figure 11: The Gironde fluvial–estuarine continuum (SW France) with sampling locations. Sediment cores were retrieved in the Lot River in Cajarc and Marcenac (triangles); oyster samples (*Crassostrea gigas*) were collected in the Gironde Estuary mouth at La Fosse (LF, circle); SCHEMA-VI-2017 sampling sites (stars) are located at the estuary mouth with diurnal cycle site (full star) and external sites (empty stars, noted from 1 to 4).

In 2001, two continuous sediment cores were collected (Audry et al., 2004; TGM EPOC sample bank). Sampling was carried out in two hydroelectric reservoirs of the Lot River: the reservoirs of Marcenac and Cajarc located respectively upstream and downstream the Riou-Mort River i.e. the hypothetical Pt pollution source (Figure 11). Sediment cores were dated based on ^{137}Cs (Audry et al., 2004) and allowed the observation of Pt signals in the Lot River since the 1950s.

2.3.2. Biomonitoring samples and field campaign in the Gironde Estuary

2.3.2.1. Estuarine wild oysters from the French national monitoring program RNO/ROCCH

The Gironde Estuary (about 170 km in length), draining an 80,000 km² watershed, is a major European estuary located in southwest France (Figure 10). It is considered a model for physical, hydrological and geochemical studies especially on trace element transport and reactivity in impacted watersheds (e.g. Lanceleur et al., 2011). The Gironde Estuary is characterized by a high turbidity gradient. In fact, ocean water fluxes at the estuary mouth are 30 – 40 times higher than fluvial inputs, and the asymmetrical progression of the tidal wave toward the upstream estuary induces a pronounced maximum turbidity zone (MTZ), where concentrations of suspended particulate matter (SPM) exceed 1 g.L⁻¹ in surface water (Sottolichio and Castaing, 1999). Other specificities of this study area include urban wastewater inputs by the Bordeaux Agglomeration (~ 1 million inhabitant equivalents) and related oxygenation problems in the MTZ during low discharge conditions.

Since 1979, a “Mussel Watch” program (Goldberg et al., 1978), the National Network for the Observation of Marine Environment Quality (RNO/ROCCH; i.e. the French Mussel-Watch, Ifremer; Ifremer.fr) has been carried out on bivalves from the French Shore. Initially, concentration levels of metal (mercury: Hg, Cd, Pb) and organic contaminants (e.g. Polycyclic Aromatic Hydrocarbon: PAH, Polychlorinated Biphenyl: PCB) have been determined in soft parts of mussels and oysters sampled on a quarterly basis (Claisse, 1989). Today a large number of chemicals are monitored with sampling performed on an annual basis (Winter oysters) Hot spots included particularly the Gironde Estuary for which the program pointed out a dramatic Cd contamination in oysters.

For this study, we benefited of samples from this national bank. Accordingly, a time series (1981 - 2013) of mature (2 years old) wild growing *Crassostrea gigas* Japanese oyster samples was extracted from the RNO/ROCCH specimen bank. Individuals selected for this study originated from the mid-salinity range of the Gironde Estuary, at the La Fosse sampling site (LF, salinity 15 - 20; Figure 11), where highest trace metal concentrations are recorded in the same set of oyster samples (e.g. Cd and Ag; Lanceleur et al., 2011).

2.3.2.2. SCHEMA-VI-2017 field campaign

The SCHEMA-VI-2017 sampling campaign took place from June 16th to 23rd 2017 on-board R/V Thalia (Figure 12). This field campaign was performed in the scope of the SCHeMA Project and included several probe testing and measurements especially along the turbidity gradient. For this study, samples were collected at more external sites being at the estuary mouth and further in the outer estuary (stars, Figure 11). A diurnal cycle of sampling was performed in order to observe dissolved and particulate (P_{tD} and P_{tP} respectively) concentration variations along a tidal cycle and possible day/night shifts in an area with lower turbidity and with oceanic influence (including higher phytoplankton biomass).



Figure 12: The SCHEMA-VI-2017 field campaign on the Gironde Estuary with the R/V Thalia loading and boarding (up), and SCHeMA system deployment (down).

2.3.3. Field campaigns in the Arcachon Bay

Located in southwest France, the Arcachon Bay is a 156 km² mesotidal lagoon, characterized by a semi-diurnal tide. This coastal system is a touristic place where numerous recreational activities (mainly boating) take place, especially during summer. This site is one of Europe's most important oyster spat production zones. However, the Arcachon Bay suffers from constantly growing pollution of its waters for several years. Since the early 1980s, tributyltin (TBT) had been used as an active biocide in antifouling paints. The first evidence of the harmful effects of TBT on oysters came from the Arcachon Bay where TBT contamination from boats was linked to high mortalities of oyster larvae and severe malformations of the adult shells (Evans et al., 1995). Since the stepwise ban of organotin compounds in antifouling paints, the Arcachon Bay water and shellfish suffer other contamination issues related in particular to trace metals and especially Cu pollution (Cu replacing TBT in the antifouling paints composition). Furthermore, abnormal proliferation of toxic green algae leads frequently to the cessation of oyster sales due to human health risk related to consumption.

Two sampling campaigns were performed on-board the R/V Planula IV (Figure 14), the SCHEMA-I-2015 and the SCHEMA-V-2017 respectively from April 19th to 24th 2015 and May 15th to 19th 2017. The crucial point of those two field campaigns consisted in a diurnal cycle of sampling held at the Comprian sampling site (Figure 13).

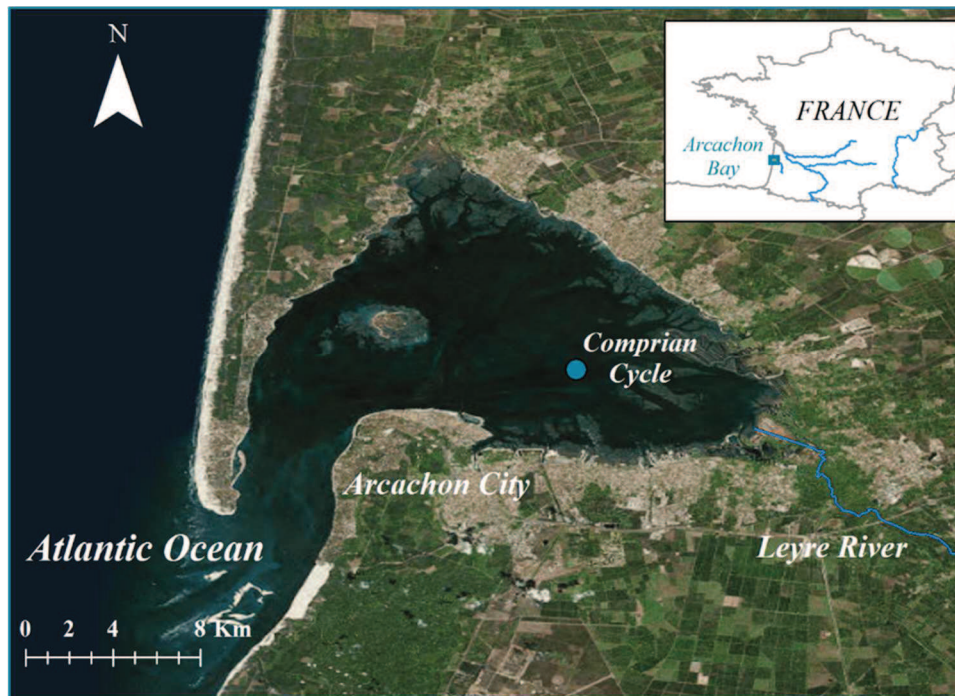


Figure 13: The Arcachon Bay (SW France) with Comprian sampling site during the field campaigns SCHEMA-I-2015 and SCHEMA-V-2017.

This site was selected as it is located in a part of the bay, receiving the influence of the Leyre riverine inputs, the Arcachon City, and seawater influence (Atlantic Ocean). Sampling was performed during spring in order to observe biogeochemical cycles of trace metals in relation with primary producers' activity.

During the first sampling campaign, wild oysters were collected near the Comprian sampling site at low tide (Figure 14). In addition phytoplankton net was deployed several times during the two sampling campaigns (Figure 14).

Contamination of Teflon bottles used for seawater collection for the second sampling campaign (SCHEMA-V-2017) impeded the determination of dissolved Pt concentrations.



Figure 14: Field campaigns in the Arcachon Bay on-board R/V Planula (IV). From left to right: loading and boarding, seawater filtration during nighttime of the diurnal cycle, seawater filtration for complementary measurements, wild oyster sampling, phytoplankton sampling.

2.3.4. Biomonitoring samples from the northwestern Mediterranean Coast

The Mediterranean Sea is a semi-enclosed system with ever growing anthropogenic pressure along its northwestern coast (Martín et al., 2009) that comprises several highly populated cities with extensively urbanized and industrialized areas. It is the place for various economical exchanges, industrial production, and tourist leisure activities including navigation. Despite progressive urbanization of this coastal area and the possible changes of seawater chemistry due to additional anthropogenic inputs, few studies report on the Mediterranean Coast environmental quality (Oursel et al., 2013).

Environmental pollution issues occurring in the Mediterranean coastal area have led to the implementation of several monitoring and conservation programs in the frame of the Mediterranean Action Plan (MAP, UNEP). Mussels have been employed worldwide (the “Mussel Watch” concept, Goldberg et al., 1978). As oysters, these sessile and filter-feeding organisms are very suitable as sentinels because they accumulate contaminants to a great extent and respond significantly to pollutant exposure. In the frame of the French monitoring program RNO/ROCCH, wild living mussels *Mytilus galloprovincialis* were collected seasonally at various contrasting sites of the northwestern Mediterranean Coast (asterisks, Figure 15).

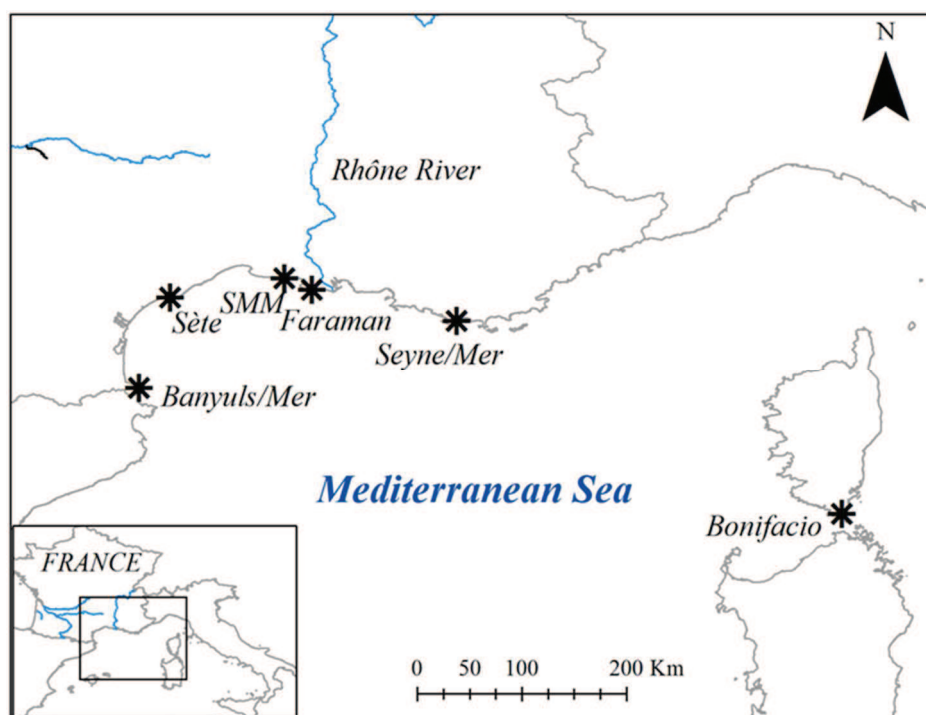


Figure 15: Sampling sites of wild mussels by the RNO/ROCCH along the northwestern Mediterranean Coast. SMM: Saintes Maries de la Mer.

2.3.5. Sampling from two highly anthropogenically impacted coastal sites: the Genoa Harbor and the Toulon Bay

2.3.4.1. SCHEMA-II-2016 and SCHEMA-IV-2017 field campaigns in the Genoa Harbor

The Genoa Harbor is an artificial harbor located on the northwest coast of Italy. It is one of the most important ports of the Mediterranean Sea, with a wide range of different activities. Port infrastructures extend over approximately 7 million m² along about 20 km of coastline, protected by wavebreakers. Industrial effluents have been the major cause of water pollution in the Genoa Harbor for many years, before the main heavy industries adjacent to the harbor closed during the 1990s. Nowadays, discharges from sewage screening plants and from creeks account for a general elevation of nutrients, organic matter and faecal pollution in the harbor waters (Ruggieri et al., 2011).

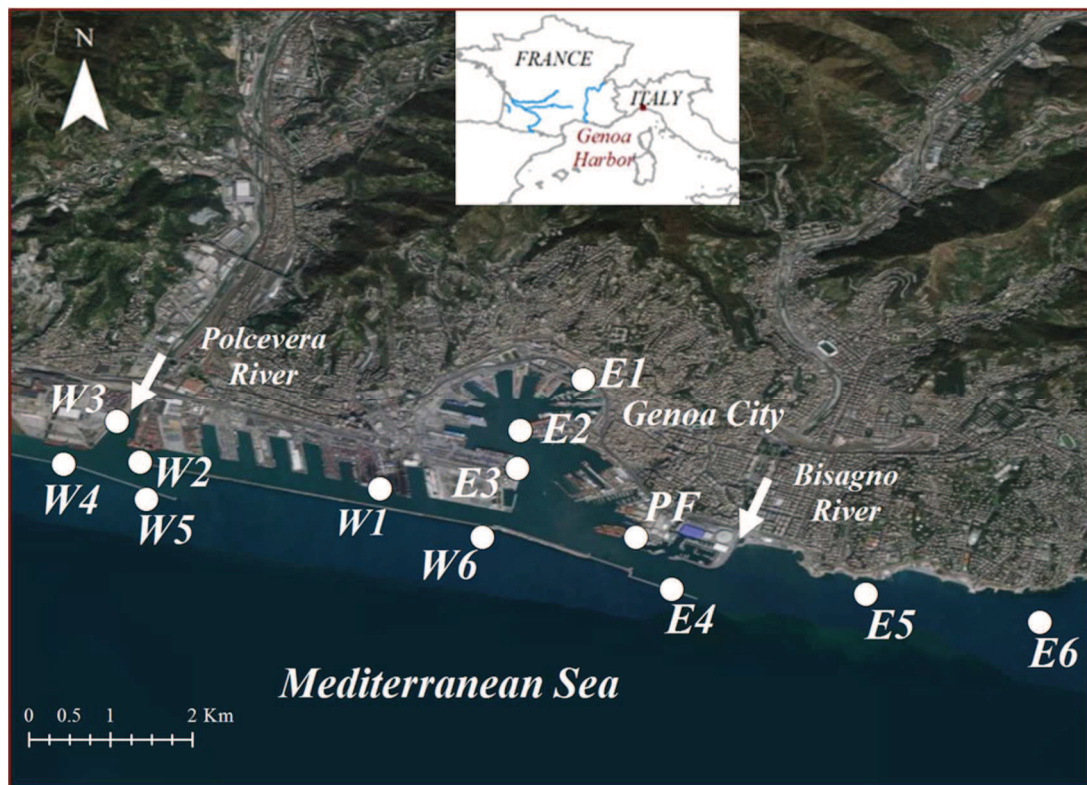


Figure 16: Sampling sites in the Genoa Harbor (NW Italy) corresponding to the field campaigns SCHEMA-II-2016 and SCHEMA-IV-2017. During the SCHEMA-II-2016 campaign, a diurnal cycle was performed at site PF (CNR Platform). During the SCHEMA-IV-2017 campaign, two longitudinal sampling profiles were performed with an eastward profile (sites E1 to E6) and a westward profile (sites W1 to W6). White arrows indicate river mouths.

Following the sampling scheme of the first sampling campaign held in the Arcachon Bay, a diurnal cycle of 25 hours was performed from the CNR (National Research Council) platform (site PF, Figure 16). During this cycle, several parameters were acquired and seawater samples were collected hourly.



Figure 17: Field campaigns in the Genoa Harbor at the CNR platform and on-board R/V MASO. From left to right: laboratory installation at the CNR platform (diurnal cycle), sampling on-board R/V MASO, wild mussel sampling next to the platform, phytoplankton sampling on-board in front of the Genoa City.

For the second field campaign held in this same site, the aim was to perform a mapping of different parameters and especially Pt levels, along two longitudinal profiles inside and outside the harbor on-board R/V MASO (Figure 17). The port mainly consists of three sub-basins, the Genoa Basin, the Multedo Oil Terminal and the Voltri Container Terminal. The inner part, the Old Port, is the oldest part of the port of Genoa and includes a ferry terminal, a small tourist port and a restructured zone, converted into an exhibition area (aquarium, congress center, and cinema). There are sewage treatment water discharges in this area, carrying large amounts of nutrients. The objectives of these two sampling campaigns were to observe diurnal variations and spatial differences in seawater and suspended particulate matter from the Genoa Harbor in order to investigate temporal and spatial Pt level variations within a highly contaminated area.

2.3.5.2. Sediment cores and farmed mussels (*RNO/ROCCH*) from the Toulon Bay

Another set of samples were collected in the Toulon Bay, which is also an industrial harbor. Toulon City with its relatively high population density (more than 600,000 inhabitants) is located on this bay. The Toulon Bay is divided into two parts by an artificial embankment, forming the Large Bay and the Small Bay (Figure 18). The Small Bay, comprising our sampling sites, is located at the west and is characterized by a semi-closed shape with a surface of $\sim 9.8 \text{ km}^2$. The Large Bay is located at the east and is characterized by a surface of 42.2 km^2 . Due to the morphology of the Small Bay, limited water circulation occur favoring sediment accumulation and associated contaminants in the bay (Tessier et al., 2011). Particularly, the Toulon Bay is highly influenced by human activities with various anthropogenic inputs originating from both historic (bombardments, fleet scuttling during the Second World War) and more recent activities (French Navy, commercial traffic, raw sewage of urban area, industry, tourism) in this area (Dang et al., 2015; Tessier et al., 2011). Sediment cores and farmed mussels were collected in this study site.



Figure 18: Sampling sites in the Toulon Bay. Sediment cores were sampled in sites TB1 and TB2 and farmed mussels (*M. galloprovincialis*, RNO/ROCCH) were sampled in site TB2.

The sediment sampling campaigns were performed by the laboratory PROTEE (University of Toulon) with the assistance of French National fleet research vessels (CARTOCHIM Project). Sediment cores were collected during sampling campaigns held from November 2008 to June 2009 in the context of a wider survey on sediment quality in the Toulon Bay (Tessier et al., 2011). The two sediment cores analyzed in this study were collected at sampling site TB1 located in the eastern part of the Small Bay, close to a former Navy submarine area and site TB2 located in the western part of the Small Bay, close to a fish/mussel farming area (Figure 18).

Furthermore, farmed mussels (35 - 65 mm, *Mytilus galloprovincialis*) were annually sampled by the RNO-ROCCH Mussel Watch Program since 1984 at the sampling site TB2. Dry soft tissues were stored in the national Mussel Watch sample bank. In the present work, mussel samples taken at 3 years intervals were analyzed covering a period from 1984 to 2014.

2.3.6. Laboratory exposure experiment – experimental design

In order to observe Pt ecotoxicological effects in marine organisms, wild oysters (*Crassostrea gigas*) were exposed to different Pt concentrations spiked in seawater. This experiment was held at the Plentzia Marine Station (PiE, UPV/EHU, Basque Country, Spain). Organisms were exposed to isotopically-labelled Pt (^{194}Pt) in seawater for 35 days (Figure 19).

Seawater was naturally filtered by sand in the uptake wells aided with a pump that sends the water to the Marine Station. The term “seawater” refers to this water. The different exposure tanks were filled with seawater, spiked to the respective nominal total Pt concentrations for 12 days with daily renewal. This step was carried out in order to equilibrate tank walls with the experimental seawater prior the exposure experiment, limiting adsorption processes. Oysters were also acclimatized for 12 days prior the beginning of the experiment (Figure 19).



Figure 19: Exposure experiment at the PiE Marine Station (NW Spain). From left to right: oyster acclimatization, control, exposure and feeding tanks, platinum spike to seawater.

Three exposure conditions were set by spiking ^{194}Pt to seawater: 50 ng.L^{-1} Pt (B condition); 100 ng.L^{-1} Pt (C condition); $10,000 \text{ ng.L}^{-1}$ Pt (D condition). Each condition was carried out in replicate (i.e. in separate tanks), as reflected by the tank numbers: B1 and B2, C1 and C2, D1 and D2. The tanks A1 and A2 corresponded to control conditions without Pt addition. Twenty-five oysters were isolated and dissected, representing the $T = 0$, i.e. the initial conditions for the different types of analysis. Then, 520 individuals were evenly (65 individuals per tank) distributed in 8 polypropylene experimental 45 L tanks filled with 40 L seawater, aerated by continuous air flow, in a temperature-controlled room (17°C) with an artificial photoperiod (12 h:12 h, light:dark cycle). Spikes were performed daily using the isotopically-labelled stock solution of ^{194}Pt (650 mg.L^{-1} total Pt) for the D tanks and a diluted solution (6.5 mg.L^{-1} total Pt; dilution with MilliQ water) for the B and C tanks (Figure 19). In this way, exposure to ^{194}Pt was maintained as constant as possible from day to day, minimizing experimental biases (e.g. due to adsorption to container walls). In order to observe early stage contamination processes, individuals were sampled in each tank and dissected after three days of exposure ($T = 3$). Then, weekly dissections were performed $T = 7$, $T = 14$, $T = 21$, $T = 28$ and $T = 35$ days after exposure. The experimental design accounted for three types of analyses: i) chemical analyses of Pt concentration in total soft tissue, ii) chemical analyses of Pt distribution between oyster organs (organotropism), and iii) histological impact of Pt exposure.

2.4. Analytical procedures for platinum determination in environmental and experimental samples

Platinum is among the least abundant element in the Upper Continental Crust (UCC, 0.5 ng.g⁻¹, Rudnick and Gao, 2003). This specific low abundance make the analytical methodologies for reliable quantification of Pt a challenge. In particular samples analyzed in this study mostly originate from the marine environment. Seawater samples are peculiar samples consisting in a challenging matrix since the salt may cause i) spectral interferences in the analytical detection of the targeted element, ii) analytical problems directly related to the presence of salt crystals. Wild-living organisms, depending on their environments, may show relatively low concentrations often very close to detection limits. Concerning sediments, environmental levels may vary according to the sampling sites including urbanized or supposedly pristine areas.

Several analytical techniques exist for the detection of Pt in environmental matrices. Yet, most available detection techniques do not allow direct measurement of solid samples or detection of PGE traces in digests of the samples, because of either insufficient detection limits or several interferences. For this purpose, there is a wide range of methods available, depending on the nature of the sample (liquid or solid), the matrix complicity, and the elements of interest which is thoroughly addressed in the review of Schindl and Leopold (2015). For solid samples, especially geological samples, nickel sulfide fire assay method is well established for separation and pre-concentration of PGEs. However, a major drawback of this technique is that it requires several manually performed steps and involves high amounts of reagents and sample. Hence, very clean working conditions and accurate performance are required in order to minimize analyte loss and contamination. For samples with extremely low PGE content and/or low available sample amounts preferably wet digestion is performed in order to transfer the analytes into an aqueous phase, decompose natural organic matter and, in some cases, dissolve sparingly soluble matrix compounds, such as silica.

Separation of PGEs from liquid samples or digests can be performed by either selective pre-concentration of PGEs or elimination of interfering matrix components. Regarding the later, application of cation exchange resin is a popular tool to eliminate interfering elements from liquid samples. After pre-concentration and/or elimination of interferences, there are several instrumental detection techniques available for Pt determination in environmental samples. They include Inductively Coupled Plasma-Atomic Emission Spectrometry or-Mass Spectrometer (ICP-AES /ICP-MS), Flame or Electro Thermal Atomic Absorption Spectrometry (FAAS /ETAAS), or voltammetry.

Considering spectral interferences occurring in environmental samples analyzed by ICP-MS, depending on the matrix composition, either highly selective separation of the analytes or application of collision/reaction cell techniques must be applied. Application of isotope dilution in ICP-MS offers the opportunity of internal correction and can provide lower detection limits due to better signal/noise ratios. Alternatively tellurium (Te) co-precipitation for Pt detection was described in combination with ID-ICP-MS determination in airborne particulate matter. Moreover, laser ablation ICP-MS in well homogenized solid rock samples provide detection limits in the low ng.g^{-1} range (0.6 ng.g^{-1} Pt). Platinum detection by ICP-AES or by FAAS requires highly efficient pre-concentration and separation methods to achieve high sensitivity. For ETAAS, in comparison to ICP-MS detection limits are clearly higher, however, separation can be less selective, because possible spectral interferences are rare. Other rarer methods include chemiluminescence and neutron activation analysis. All those previously described methods are often only reserved to a certain type of samples displaying relatively high levels and therefore which do not require very sensitive method. Furthermore, the main issue of those techniques is to perform efficient separation and pre-concentration of Pt prior to analysis. In this regard, automated and simultaneous or at least sequential systems are preferable over manual techniques, in order to make the procedures more suitable for larger sample series, which is a main requirement in environmental monitoring and toxicological studies (Schindl and Leopold, 2015).

Currently, two methods for Pt determination in seawater are described in previous publications: Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Isotope Dilution Inductively Coupled Plasma-Mass Spectrometry (ID-ICP-MS) with respective detection limits (expressed as three times the standard deviation of blank measurements) for Pt_D in seawater of $3.9 \times 10^{-3} \text{ ng.L}^{-1}$ and $2.9 \times 10^{-3} \text{ ng.L}^{-1}$ (Cobelo-García et al., 2014b; Mashio et al., 2016). For the second technique, pre-concentration of Pt on an anion exchange resin is required to concentrate Pt and remove sea-salt and interfering metals present in seawater matrix (Obata et al., 2006). Sediment samples are often analyzed by ICP-MS following nickel-sulfur (NiS) fire-assay (e.g. Schäfer et al., 1998; Tuit et al., 2000) or by voltammetrical techniques (e.g. Cobelo-García et al., 2011). For biota samples, voltammetrical techniques are often applied (e.g. Neira et al., 2015; Ruchter and Sures, 2015) in order to avoid spectral interferences in the detection of low Pt concentrations by ICP-MS. One solution is also the application of high-resolution ICP-MS (e.g. Schäfer et al., 1998).

Since samples from the present study mainly originate from the natural environment, we applied the very sensitive AdCSV method as well as ICP-MS detection after performing signal correction. When possible some optimizations to adapt Pt determination in environmental and environmentally relevant set of samples were investigated. Accordingly, seawater Pt determination was performed by AdCSV method after UV-oxidation of the samples. Platinum concentrations in sediments cores and SPM were determined using AdCSV and Triple Quadrupole (TQ) ICP-MS respectively, after an ashing step for the first method. Biota samples were ashed and analyzed by both AdCSV and Single Quadrupole (SQ) ICP-MS.

Besides the development of more sensitive detection methods, a key issue regarding Pt analysis is validation (Schindl and Leopold, 2015). The only certified reference materials (CRMs) for Pt in environmental samples are the BCR®-723 road dust (IRMM) and Jsd-2 sedimentary rocks (indicative value from GSJ). Due to this lack of appropriate CRMs, verification of analytical data is frequently performed only by internal reference measurements like recovery experiments in spiked samples. In order to validate an analytical procedure, Schindl and Leopold (2015) suggest the application of another analytical method as a reference when appropriate CRMs are not available. This is what was performed in this study since AdCSV and ICP-MS methods were inter-compared for Pt determination in biological matrices.

All the laboratory material in contact with the samples was soaked in an acid bath (10 % v / v nitric acid: HNO₃, 65% Honeywell, or chloridric acid HCl, 65 % J.T. Baker) during 3 days, then thoroughly rinsed with ultrapure (MilliQ®) water, dried under a laminar flow hood and kept sealed in double polypropylene (PP) bags until use.

2.4.1. Analytical techniques

2.4.1.1. Voltammetry (*AdCSV*)

Voltammetrical determination of Pt is carried out using a μ Autolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm model 663 VA) equipped with three electrodes:

(i) a hanging mercury drop electrode (HMDE) which is the working electrode measuring the current passing through the solution. Mercury is used due to its wide negative potential range (due to the difficulty to reduce hydrogen ion or water at the mercury surface), and its surface is readily regenerated by producing a new drop (Kounaves, 1997).

(ii) a silver/silver chloride (Ag/AgCl) reference electrode which gives a known and stable potential and does not touch the sample solution. This electrode allows the application by the potentiostat of a pre-defined potential to the working electrode. Its potential is determined by the following reversible half- reaction: $\text{AgCl}_{(s)} + e^- = \text{Ag}_{(s)} + \text{Cl}^-$ (Kounaves, 1997).

(iii) an auxiliary (or counter) electrode which balances the charges generated by the current at the working electrode. Most often the counter electrode consists of a thin Pt wire (Kounaves, 1997). However since we measure this element, our counter electrode consists in a glassy carbon rod.

A polytetrafluoroethylene (PTFE) voltammetric cell serves in all experiments. The material of the cell was selected to minimize reaction with the sample (Kounaves, 1997). Furthermore, a PTFE rod, used at the highest rotation speed (3000 rpm), stirs the solution. The potentiostat is controlled using the NOVA 2.1 software (Metrohm®) which serves for routines/algorithms used for all data treatments. Sulphuric acid (H_2SO_4 , 93 – 98 % Trace metal grade, Fisher Chemical®), formaldehyde (37 – 41 % Analytical Reagent Grade, Fisher Chemical®), and hydrazine sulphate (Analytical Reagent Grade, Fisher Chemical®) are used. Solutions of 3.3 mM formaldehyde and 0.45 mM hydrazine were prepared using Milli-Q water and stored in 30 mL fluorinated ethylene propylene (PFE) bottles (Nalgene); these solutions are stable during several weeks. Nitrogen (N_2) (99.999 %; Alphagaz™ 1, Air Liquide®) is used to de-aerate the solution. Mercury used in the HMDE electrode is made for analysis and polarography (EMSURE, Merck®).

A 10 mL aliquot of sample is pipetted into the voltammetric cell. For Pt determination 30 μL of the two reagents formaldehyde and hydrazine sulphate are added directly to the solution as well as 300 μL of H_2SO_4 . In this matrix, formaldehyde and hydrazine sulphate form formazone.

Mostly, Pt exists in two forms of oxidation in nature: Pt(II) and Pt(IV) (Zhao and Freiser, 1986). However, only Pt(II) is detected by the analytical method. Hydrazine, which is introduced in excess, is a good reductant which will reduce any Pt(IV) into Pt(II) (Zhao and Freiser, 1986; van den Berg and Jacinto, 1988). The first step of the Pt chemical detection consists in the formation of the Pt(II)-formazone complex (Figure 20).

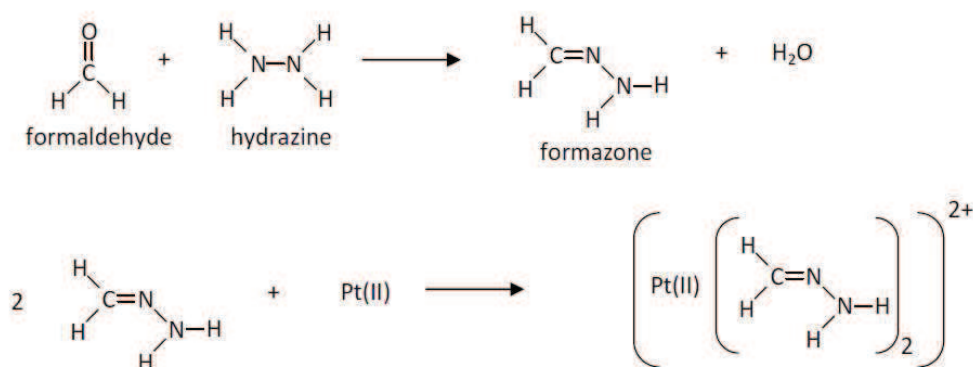


Figure 20: Reaction mechanisms of formation of the Pt(II)-formazone complex (Cobelo-García et al., 2014b).

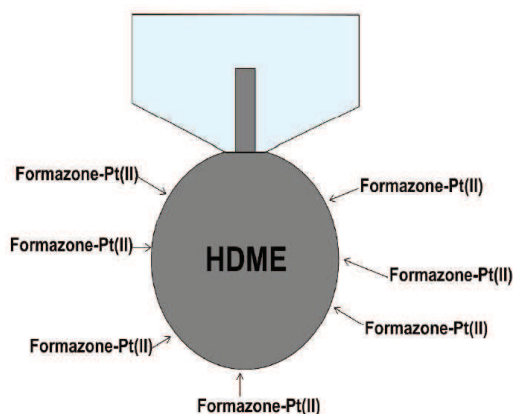


Figure 21: Adsorption of the Pt(II)-formazone complex on the surface of the HMDE during the accumulation time (Cobelo-García et al., 2014b).

Dissolved oxygen does interfere at the lowest Pt concentrations which can be determined with adsorptive stripping voltammetry. A deaeration period of 5 min is therefore recommended (van den Berg and Jacinto, 1988) using humidified N_2 . The largest drop size of Hg (0.52 mm^2) is selected and for each measure a new mercury drop is extruded. The accumulation is initiated with a potential set up at -0.3 V for 300 s, while the solution is stirred at 3000 rpm with the PTFE rod. This allows a constant flux of Pt complexes to reach the HMDE (Figure 21). At this potential, formazone-Pt(II) complexes is adsorbed at the surface of the HMDE.

After this time, stirring is stopped and an equilibration time of 10 s allows the formazone-Pt(II) complexes to stabilize. After the equilibration time, a cathodic stripping scan is carried out by changing the potential from - 0.5 V to - 1.1 V in the differential pulse (DP) mode. In this mode the potential is scanned with a series of pulses. Each potential pulse is fixed at the small amplitude of 4 mV and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse (Kounaves, 1997). The difference between current measurements at these points for each pulse is determined and plotted against the base potential (Kounaves, 1997). Other analytical parameters are a scan rate of 20 mV.s^{-1} with modulation time of 0.04 s, an interval time of 0.2 s, and modulation amplitude of 25 mV. This drop of potential causes the transformation of formazone-Pt(II) complexes into Pt(0) and formazone (Figure 22).

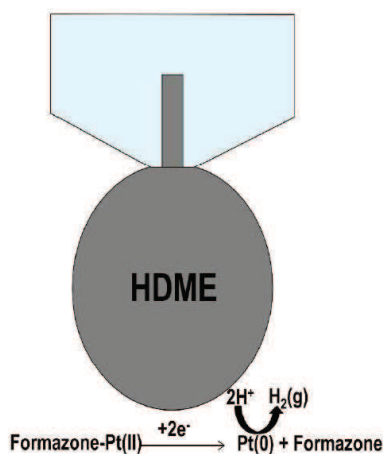


Figure 22: Hydrogen reduction catalyzed by metallic Pt during the cathodic stripping scan (Cobelo-García et al., 2014b).

The Pt(II)/Pt(0) reduction potential coincides with the catalytic hydrogen wave. Zhao and Freiser (1986) assumed that the catalytic hydrogen waves presumably arise by the decrease in the hydrogen overpotential on the active centres of metallic PGE reduced and deposited on the surface of the mercury electrode. Protons H^+ present in the acidic media of the solution are reduced to H_2 by Pt(0) (Figure 22). This mechanism explains the drop in H^+ production causing the peaked shape of the current-potential plot (van den Berg and Jacinto, 1988). The peak is consequently proportional to the Pt concentration of the sample.

A potential drawback of this voltammetric determination is that the catalytic analytical signal appears superimposed on a broad background wave due to formazone complex (Cobelo-García et al., 2014b). Longer accumulation times could solve this problem for instance by extending the adsorption time to 20 – 30 min for the determination of Pt in natural waters (van den Berg and Jacinto, 1988). In such conditions, the analysis of an aliquot by the method of standard addition may take several hours.

In order to overcome such inconvenience, the second derivative has been selected as it is more efficient in eliminating the background interferences than the first derivative, while keeping good peak-shaped transformed signals and better SNR than higher derivative orders (Cobelo-García et al., 2014b). Data were also smoothed using a smoothing factor of 2 which gives satisfactory results for the signal-to-noise ratio (SNR, Gordana et al., 2000). Peak height is then determined in the second derivative voltammogram using the peak–peak baseline method (Cobelo-García et al., 2014b; Gordana et al., 2000).

Determination of Pt concentrations is achieved by the method of standard addition i.e. by adding successive known amounts of the target substance adapted to the expected concentration (Franke et al., 1978). Dilution of standard stock solution of 100 ng.L^{-1} of Pt was used for additions (stock Pt standard solution $1000\text{ }\mu\text{g.mL}^{-1}$, PLASMACAL, SCP Science®, dilution with MilliQ water). Accordingly, the second measurement is preceded by a first spike of Pt stock solution, followed by two other Pt spikes (Figure 23).

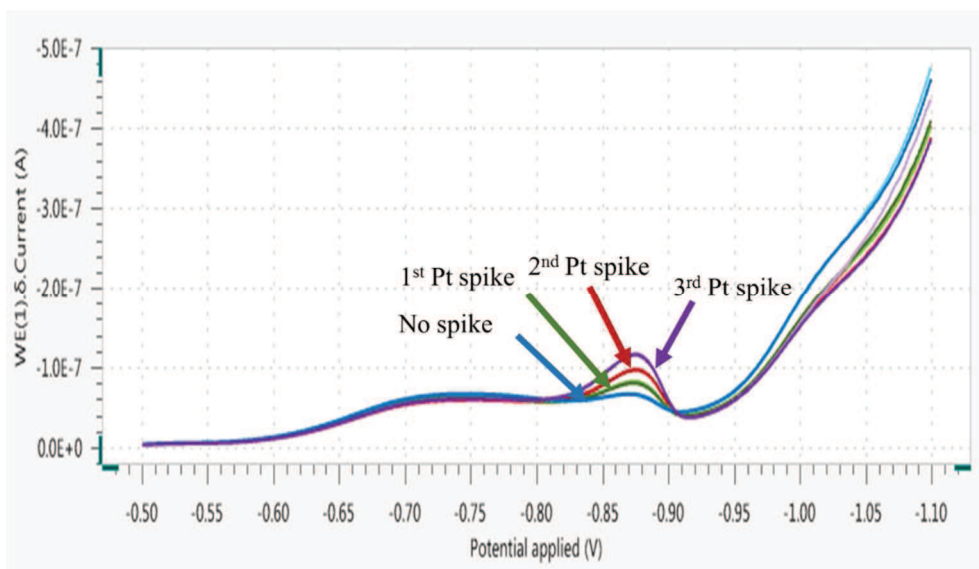


Figure 23: Original voltammograms of a seawater sample analysis. The two replicate measurements of the raw signal (blue lines), the first Pt spike (green lines), the second Pt spike (red lines), and the third Pt spike (purple lines) are represented.

Generally, two replicates of each measurement and three Pt spikes are performed, but this number is adjusted if the linearity of the curve is mediocre in order to increase the consistency and robustness of the results. Sample dilution, deposition time as well as Pt standard solution spike are adapted according to the type of sample analyzed and its expected Pt content.

2.4.1.2. ICP-MS

The ICP-MS technique principle is based on four consecutive steps:

1. *Nebulization*: the sample aliquot is sampled with an automatic sampler (ASX-520, CETAC®) and brought to the nebulizer (nebulization chamber) using a peristaltic pump. Sample is transformed into a fine aerosol through an argon (Ar) spray.

2. *Ionization*: the aerosol is injected into a quartz torch where it is ionized with the help of the Ar plasma (ionized Ar kept at a temperature between 6,000 and 8,000 °K).

3. *Separation according to the mass-to-charge ratio (m/Q)*: part of the formed ions (~ 10 %) pass through two consecutive vacuum chambers (10^{-3} Pa and 10^{-6} Pa respectively). In the second vacuum chamber, positively charged ions are extracted using electrostatic lenses. Those lenses guide the ion beam towards the quadrupole mass spectrometer which acts as a filter separating ions according to their mass-to-charge ratio. Only the ions with a ratio corresponding to the magnetic field applied to the quadrupole can pass through the filter.

4. *Detection*: the signal emitted by the ions reaching the detector is translated into a count number per second. A calibration of the apparatus, using the standard addition method allows the determination of the Pt concentration according to this value.

Two ICP-MS apparatus were used in this study: (i) a Single Quadrupole ICP-MS (X Series II, Thermo®) and (ii) a Triple Quadrupole ICP-MS (iCAP TQ, Thermo®, Figure 24).



Figure 24: Two analytical equipments used in this study. Left: SQ ICP-MS and right: TQ ICP-MS.

Main advantages of the TQ ICP-MS include its i) powerful interference removal, even in the toughest matrices, ii) low detection limits since it has a higher sensitivity, iii) very high accuracy and repeatability at sub ng.kg⁻¹ levels.

2.4.2. Determination of dissolved platinum in seawater

Sample preparation

For sample collection during the field campaigns, surface seawater was pumped using an electric pumping system connected to a Teflon tubing. Seawater samples were placed in acid-cleaned Teflon bottles previously rinsed with an aliquot of the filtrate obtained through filtration with 0.2 μm polycarbonate filters (Nucleopore®) and a filter-syringe (Sartorius®). In order to prevent losses by adsorption onto the container walls (van den Berg and Jacinto, 1988), samples were acidified to $\text{pH} = 1$ (36.5 – 38 % HCl Baker Instra®) and stored in the dark at 4 °C pending analysis.

Dissolved Pt in seawater was analyzed by means of voltammetrical techniques. However several types of interferences may occur when conducting voltammetrical analyses which requires a treatment of the sample. The presence of surface active compounds can interfere the signal by adsorbing competitively the labile Pt(II) ions that should be accumulated at the electrode surface. This is the case of organic complexing material present as traces in the sample (van den Berg and Jacinto, 1988). It is therefore necessary to treat seawater samples by Ultra Violet (UV) irradiation to get rid of surface active compounds before Pt determination. Aliquots of samples, placed in capped Teflon bottles, are irradiated overnight (i.e. ~ 12 h) in order to ensure the complete destruction of organic matter. Irradiation was carried out using two 64 W UV lamps (NIQ 60/35 XL, Heraeus) placed under a fume hood.

Analytical procedure

An aliquot of 10 mL of sample is placed in the measuring cell and a deposition time of 300 s is selected due to the very low Pt_D concentration. Accordingly, considering the purging, and the deposition times, of the two replicates of each of the three measurements (sample and sample + spike), Pt detection for one sample takes ~ 1 h.

Analytical quality control

No certified reference material for Pt_D in seawater (nor in any other aqueous matrix) exists. Accuracy of the voltammetric procedure was evaluated by means of the analysis of spiked CRM seawater CASS-6 as well as spiked seawater sampled in the Arcachon Bay. Recoveries greater than ~ 85 % were obtained with this approach. The detection limit for Pt_D (calculated as three times the blank standard deviation; $n = 20$) was estimated to 0.03 ng.L^{-1} .

2.4.3. Determination of particulate platinum in sediment and in suspended particulate matter

Sample preparation

Sediment cores

i) Lot River

The sediment cores were collected for the purpose of a previous study (Audry et al., 2004). Two continuous sediment cores of about 1.4 m-long were collected using a manual corer consisting of a 10 × 10 cm rectangular Plexiglas tube. The sediment cores were sliced in thin horizontal sections at 1 cm resolution from the surface to 25 cm. The lower part was sampled with a 5 cm resolution. Immediately after recovery, each sliced section was centrifuged in order to remove pore waters and then sealed in sampling-bags under nitrogen. Sediment samples were dried at 50 °C to constant weight and then powdered and homogenized with an agate mortar. Those samples were kept at room temperature, in the dark, pending analysis.

ii) Toulon Bay

Sediment cores from the Toulon Bay were also retrieved for the purpose of a previous study (Tessier et al., 2011). Cores of 10 cm diameter and 1 m long (Interface Corer, Plexiglas® tube) were transported to the laboratory PROTEE and sliced (2 cm length resolution) under inert atmosphere (N₂) to preserve redox conditions. The sliced sediment samples were pooled and homogenized (with a Teflon spoon), placed into pre-cleaned 1 L high-density polyethylene (HDPE) bottles and deep frozen (- 18 °C). Sediments were then sieved (2 mm) and kept in the dark at room temperature pending analysis.

Suspended Particulate Matter

Surface seawater was pumped using an electric pumping system connected to a Teflon tubing. Seawater samples were placed in acid-cleaned Teflon bottles, previously rinsed with an aliquot of the sample. Suspended Particulate Matter was obtained through seawater filtration on an acid-cleaned filtration unit with pre-weighed Teflon filters (47 mm, 0,45 µm FHLC filter, Merck Millipore®). Samples were dried in a drying oven at 50 °C, weighed again, and kept in the dark at room temperature prior analysis.

Selection of acid-digestion procedure

Sediment or SPM samples are processed through pseudo total digestion (*aqua regia* extraction, e.g. Tessier et al., 2011). Total trace metal concentrations in sediments are generally obtained by digestion of samples with strong acids (such as HNO_3 , HCl , perchloric: HClO_4 , and hydrofluoric acids: HF). The choice of the acid mixtures depends on the target element and the solid fractions to which it is associated. Acid digestion process can also affect the behavior of the target element, biasing the sediment extraction. Such is the case of Pt, for which volatile fluorine compound may form when heated up at 195 °C in the presence of HF acid (Patnaik, 2003; Weinstock et al., 1961).

Successful Pt determination using voltammetrical method with only *aqua regia* (i.e., HNO_3 and HCl mixture) for acid digestions of CRMs Jsd-2 (sedimentary rock, GSJ) and BCR-723 (road dust) are reported in the literature (Cobelo-García et al., 2014a) showing that the addition of HF to such samples is not essential (Sievers and Schuster, 2015). The results obtained for the BCR-723 were in good agreement with certified values after ICP-MS determination indicating that Pt is not bound to the silicate matrix (Leśniewska et al., 2005). Furthermore, some tests were performed in this study in order to compare Jsd-2 and BCR-723 CRM recoveries after acid digestions without (*aqua regia*) and with the addition of HF acid (tri-acid digestion: HNO_3 , HCl and HF mixture). Results show good recoveries for digestions without the addition of HF , obtaining ~ 101 % for Jsd-2 ($n = 2$) and ~ 98 % for BCR-723 ($n = 2$), whereas lower recoveries of only ~ 56 % and ~ 71 %, respectively, were obtained for the digestions with HF acid (AdCSV detection).

Selection of detection method

Generally, low expected levels of Pt_P in these environmental samples lead to the application of the most sensitive analytical method i.e. AdCSV. Sediment core samples from the Lot River and from the Toulon Bay were analyzed using this technique. However SPM samples collected on Teflon filters from the different sampling campaign were analyzed by TQ ICP-MS since i) those samples were necessary for the analysis of other trace metals (to the purpose of the SCHeMA Project) which is performed by ICP-MS method (including associated acid-digestion procedure), ii) the time required for voltammetrical analyses as well as the re-usable Teflon vials (requiring cleaning and possible contamination problems) were not realistic to apply to the large set of SPM samples (more than 130 samples).

2.4.3.1. Sediment cores – AdCSV

Sample preparation

Sediment samples from the cores are weighed using typically ~ 50 mg of material and processed following a protocol adapted from Cobelo-García et al. (2011). In order to remove all refractory organics that may cause interferences during voltammetric analyses, samples are ashed in a muffle furnace according to the heating scheme of $20\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ described by Nygren et al. (1990): 1 h at $200\text{ }^{\circ}\text{C}$, 30 min at $250\text{ }^{\circ}\text{C}$, 1 h at $350\text{ }^{\circ}\text{C}$, 30 min at $425\text{ }^{\circ}\text{C}$, and 3 h at $800\text{ }^{\circ}\text{C}$. Ashing is performed in acid-cleaned porcelain crucibles (Figure 25).

Those crucibles are retrieved and after cooling, 3 mL of concentrated nitric acid (HNO_3 , 65 % Suprapur, Merk®) is added together with 4 mL of concentrated hydrochloric acid (HCl , 30 % Suprapur, Merk®) for 1 h in order to completely dissolve particles that may stick on the crucibles (Figure 25). Thereafter, samples are transferred to perfluoroalkoxy (PFA) vials (Savillex®) and 1 mL HCl is added to rinse all the possible remaining material on the porcelain crucibles.

Vials are placed with their caps on a temperature controlled Teflon-coated heating block and samples are acid-digested at $195\text{ }^{\circ}\text{C}$ for 4 h. Vials are then removed and after cooling, caps are removed and the acids are evaporated at $195\text{ }^{\circ}\text{C}$ to near dryness. The residue is dissolved again with 1 mL of concentrated HCl and 1 mL of concentrated H_2SO_4 , evaporated at $195\text{ }^{\circ}\text{C}$ until no fumes are observed and a near constant volume, comprising mostly H_2SO_4 acid, is reached. This procedure removes remnant HNO_3 which interferes with the catalytic AdCSV Pt determination. Cooled contents are diluted using 0.1 M HCl , centrifuged at 4000 rpm for 10 min ($20\text{ }^{\circ}\text{C}$), and made up to 25 mL in acid-cleaned Teflon bottles.



Figure 25: Ashing of the samples in the muffle furnace (left) and acid addition to cooled crucibles (right).

Analytical procedure

For AdCSV method applied to sediment cores, aliquots of acid-digested sample are pipetted into the voltammetric cell (with or without dilution according to the Pt content). Platinum concentrations are determined applying the standard addition method as described previously, using an adapted deposition time (generally from 90 to 180 s).

Analytical quality control

For voltammetrical method, blank samples were processed including ashing and acid-digestion steps as performed for sediment samples. Typical blank concentration of $\sim 0.05 \text{ ng.L}^{-1}$ are obtained although most of the time Pt peak were not detectable for blank samples at the deposition time used for sediment Pt_p measurements. Detection limits is of $\sim 0.03 \text{ ng.g}^{-1}$ for a typical mass of 50 mg and sample volume of 25 mL.

The accuracy of the analytical procedure has been checked by analysing BCR-723 and Jsd-2 CRMs. Mean Pt concentration obtained were in good agreement with values obtained by other authors with recovery rates $> 90 \%$ for both CRMs.

2.4.3.3. Suspended Particulate Matter – ICP-MS

Sample preparation

The whole filter with typical SPM mass of $\sim 3.5 \text{ mg}$, is placed in PP tubes (DigiTUBEs®, SCP SCIENCE) together with 1 mL HNO₃ and 2 mL HCl (both concentrated, Suprapur, Merck®). Tubes with caps are placed in the Teflon-coated heating block, and digested at 110 °C for 3 h. Cooled contents were then diluted in 10 mL MilliQ water and centrifuged at 4000 rpm for 10 min (20 °C) prior to analyses.

Analytical procedure

Analyses were performed by TQ ICP-MS. After obtaining the raw signal, the first step is to correct for any possible spectral interference. For Pt analysis by ICP-MS, the most common mass interferences result from Hafnium-Oxygen: HfO⁺ species, like $^{178}\text{Hf}^{16}\text{O}$, $^{179}\text{Hf}^{16}\text{O}$, or $^{180}\text{Hf}^{16}\text{O}$ interfering with ^{194}Pt , ^{195}Pt , ^{196}Pt , respectively (Parent et al., 1997). The quantification of the interfering signal that overlaps the analyte signal and its subtraction by mathematical equation is limited by the intensity of the interference (Parent et al., 1997). For Pt analyses, this mathematical correction provides accurate results for Hf/Pt ratios of up to 50 (Parent et al.,

1997) which was applicable to some of the collected samples. Correction of HfO^+ interference is performed using $^{194}\text{Pt} / ^{195}\text{Pt}$ natural ratio and respective HfO^+ interference on both Pt isotopes, assuming that HfO^+ is the only spectral interference on those selected isotopes. We determined Pt concentrations following equations 1 and 2:

$$^{194}\text{Pt} = P \times A_{194\text{Pt}} + H \times A_{\text{HfO}/194}$$

1

$$^{195}\text{Pt} = P \times A_{195\text{Pt}} + H \times A_{\text{HfO}/195}$$

2

Where ^{194}Pt and ^{195}Pt correspond to measured (interfered) count numbers of both isotopes, P and H correspond to the total Pt and Hf concentrations respectively, $A_{194\text{Pt}}$ and $A_{195\text{Pt}}$ correspond to the natural abundances of isotope ^{194}Pt and ^{195}Pt respectively, $A_{\text{HfO}/194}$ and $A_{\text{HfO}/195}$ correspond to the abundance of HfO^+ interference on masses 194 and 195 respectively.

Using equations 1 and 2, calculations were performed on ^{195}Pt isotope which appears as the less interfered isotope. Interference corrected ^{195}Pt count number labelled $^{195}\text{Pt}_{\text{corr}}$ was determined as follows (equations 3 and 4):

$$P = (^{194}\text{Pt} \times A_{\text{HfO}/195} - ^{195}\text{Pt} \times A_{\text{HfO}/194}) / (A_{194\text{Pt}} \times A_{\text{HfO}/195} - A_{195\text{Pt}} \times A_{\text{HfO}/194})$$

3

$$^{195}\text{Pt}_{\text{corr}} = A_{195\text{Pt}} \times P$$

4

After signal correction, Pt concentration is determined from the slope of the standard addition calibration curve, which is performed each 5 samples, and considering the natural abundance of the ^{195}Pt isotope, the dilution, and the mass of the sample. About 150 SPM samples were collected, processed, and analyzed using this method. Unfortunately, for most SPM samples, sample mass and/or Pt concentrations were too low. This led to important HfO^+ interferences making the Pt determination impossible.

Analytical quality control

Each set of samples is processed with blank filters. These blanks are collected on the field using the same filtration unit and collection material as for other samples. Field blanks are also dried and acid-digested in order to correct for possible contamination (on-site, after drying, during storage, and/or during acid digestion). Mean Pt concentration obtained from the blanks is deduced from the value obtained for each sample. Typical blank concentration of $\sim 0.10 \text{ ng.L}^{-1}$ are obtained. Detection limits are of 0.34 ng.g^{-1} for typical mass of 3.5 mg and sample volume of 13 mL.

Similarly to AdCSV procedure, the accuracy of the analytical procedure has been checked by analyzing BCR-723 and Jsd-2 CRMs which were acid-digested together with a Teflon filter in the digestion tube. For minimal mass of 10 mg with Teflon filter, recovery results for CRM BCR-723 or of $\sim 100 \%$ and of 115% for Jsd-2. Despite lower environmental concentrations in our samples, these results, representing the analysis conditions applied to our samples, are encouraging regarding accuracy of Pt_T detection.

2.4.4. Determination of platinum in wild living biota

2.4.4.1. Sample preparation

Wild living and farmed organisms were sampled in various coastal sites. In both cases, sampling consisted in retrieving similar size, adult individuals. Preferentially biota were collected in winter in order to avoid “dilution” effect with reproductive tissues since tissue growth may mask or dilute trace metal levels (e.g., Andral et al., 2004). After sampling bivalves were depurated in natural seawater in order to clean the intestinal tract before chemical analysis, and to desorb loosely attached metals from tissue surfaces (Zimmermann and Sures, 2018). After depuration, oyster and mussel samples are opened and whole soft body or dissected organs are placed in acid-cleaned pre-weighed PP tubes (DigiTUBES®, SCP SCIENCE). Tubes are weighed in order to obtain the wet weigh of the organism. Shells of the individuals are also weighed and measured.

These values will help to determine the Condition Index which is calculated according to the following equation (5, after Strady et al., 2011):

$$\text{CI} = \text{Visceral Content (wet weight, g)} \div \text{Shell (wet weight, g)} \times 100$$

Samples are then deep-frozen (-80°C) using a freeze-dryer (Heto PowerDry LL 3000, Thermo®), grinded in an agate mortar and kept in the dark at room temperature pending analysis. For wild oyster samples from the RNO/ROCCH, a pool of fifteen individuals for each winter has been selected, lyophilized and grinded whereas for wild mussel samples the pool corresponded to ~ 240 individuals processed similarly.

Phytoplankton samples were collected on-board R/Vs in the different sampling sites by deploying a phytoplankton net of $20\text{ }\mu\text{m}$ mesh (a plankton net of $200\text{ }\mu\text{m}$ was deployed in the Gironde Estuary). Samples correspond to an integration of phytoplankton cells of horizontal or vertical sections in the water column according to the profile performed. Tests in the Arcachon Bay comparing vertical and horizontal profiles of phytoplankton did not show any difference in the results. Seawater containing concentrated phytoplankton cells is retrieved from the collector and centrifuged at 1,500 rpm. The supernatant was removed and the collected residue was rinsed with seawater, deep-frozen (-80°C), freeze-dried, and kept in the dark at room temperature pending analysis (Figure 26).



Figure 26: Phytoplankton sample collection and preparation with seawater collection (left) and sample centrifugation (right).

In order to evaluate the reliability of the method, including the centrifugation step, aliquots of supernatant from some phytoplankton samples ($n=5$) were retrieved. The risk with a centrifugation step is that the most fragile cells may break releasing their Pt content in the dissolved phase. Aliquots were analyzed by AdCSV after an UV-oxidation step. Results of this analysis show that only a minor fraction of P_{tp} might remain in the supernatant i.e. less than 2 % ($n=5$). Platinum concentrations in the supernatant are positively correlated with levels found in the different phytoplankton samples ($R^2 = 0.94$). This may reflect the effective loss of

part of the phytoplankton cells, the smallest and/or more fragile, yet of minor influence on the final determined concentrations.

Using the loss on ignition (8 hours at 800°C) as a proxy of the amount of organic material present in the samples, we have estimated Pt_P concentrations in the organic fraction as follows (6):

$$Pt_{PK} = \frac{[Pt_{tot} - Pt_{ss} \times (1 - \%OM)]}{\%OM}$$

6

With Pt_{PK} : the Pt concentration in the organic fraction (the planktonic fraction), Pt_{tot} : the Pt concentration in the total plankton net material sample, Pt_{ss} : the estimated Pt concentration in suspended sand, and $\%OM$: the percentage of organic matter determined using the loss on ignition.

2.4.4.2. Analytical procedure

Low Pt concentrations in natural environments result in generally low concentrations in biological tissues implying analytical challenges for their accurate determination. Therefore, several techniques allow the pre-concentration of trace metals in a given sample. For this purpose, ashing of powdered oyster tissues was implemented to pre-concentrate the Pt content (e.g. Schäfer et al., 1998), eliminating part of the sample matrix and allowing acid digestion of a more important mass of the sample. In comparison, classical biological sample acid digestion, without the ashing step, allows the preparation of 0.02 to 0.03 g with the same acid volume.

As aforementioned, the main problem using the ICP-MS method for environmental matrices are HfO^+ interferences which are formed in the plasma and can interfere with the detection of all Pt isotopes and prevent a correct analysis of Pt (Godlewska-Żyłkiewicz, 2004; Pyrzynska, 2015). This pre-concentration step allows for a significant increase of the signal and permits Pt concentration determination applying ICP- MS method. In order to validate Pt concentrations in the absence of biological CRM for Pt levels, we performed an inter-comparison of two completely independent methods: ICP-MS and AdCSV, including their respective mineralization procedures. This inter-comparison was performed using a pool of (non-contaminated) oysters (*Crassostrea gigas*) originating from the Gironde Estuary which was prepared by dissecting (~100 individuals), freeze-drying, sieving at 150 µm, and grinding/homogenizing in an agate mortar. Aliquots for each technique (n = 20 for ICP-MS; n = 13 for AdCSV), covering a wide range of sample mass i.e. between 0.05 g and 2 g, were

ashed in porcelain crucibles at 800°C according to the heating scheme described by Nygren et al. (1990). Samples were acid-digested according to the two respective method protocols as follows.

Voltammetry

For AdCSV analyses, after ashing, a mixture of 5 mL concentrated HCl and 3 mL concentrated HNO₃ (Suprapur, Merck®) is added to cooled crucibles which is then transferred to PFA vials as previously described for sediment core samples. Samples are acid-digested on a Teflon-coated heating block at 195 °C for 1 h with caps followed by an evaporation step (without caps). The residues are then dissolved adding 1 mL of concentrated H₂SO₄ (Fisher Chemical®), and evaporated again. Similarly to sediment samples, cooled contents are then diluted with 0.1 M HCl (Merck®) and centrifuged at 4000 rpm for 10 min (20 °C) prior to analyses.

Platinum voltammetric determinations is performed as previously explained for seawater and sediment samples, using standard addition method and a deposition time of 90 s.

ICP-MS

After ashing, samples are acid-digested according to an adapted protocol for trace element detection in biological matrices as described in Mikolaczyk et al. (2016). After crucibles had cooled down, 2 mL HCl and 1 mL HNO₃ (both concentrated) are added to the ashed residues. The mixture is then transferred in PP tubes with caps. Samples are then placed in the Teflon-coated heating block, and acid-digested at 110 °C for 3 h. Cooled contents are then diluted in 10 mL MilliQ water and centrifuged at 4000 rpm for 10 min (20 °C) prior to analyses.

Following ICP-MS measurements, the raw signal has to be corrected for HfO⁺ spectral interferences. In the pool oyster sample, Hf/Pt ratios were relatively low compared to those obtained for particle samples. Correction of HfO⁺ interference was performed using the ¹⁹³Ir signal that is highly interfered by HfO⁺ (Djingova et al., 2003) and given that Ir concentration in our samples was assumed negligible.

The following equation was applied 7:

$$^{195}\text{Pt}_{\text{corr}} = ^{195}\text{Pt}_{\text{meas}} - ^{195}\text{Pt}_{\text{inter}} \times A / B$$

Where $^{195}\text{Pt}_{\text{corr}}$ is the corrected signal of ^{195}Pt isotope, $^{195}\text{Pt}_{\text{meas}}$ the measured signal of ^{195}Pt isotope, $^{195}\text{Pt}_{\text{inter}}$ the signal of the interference (estimated from mass 193 signals representing HfO^+ interference), A is the % of formation of HfO^+ on the mass 195, and B the % of formation of HfO^+ on the mass 193 (modified from Djingova et al., 2003). Platinum concentrations are then determined using the standard addition method, considering natural ^{195}Pt abundance, the dilution factor, and the mass of the sample.

Inter-comparison

Results obtained from Pt analyses in the same oyster sample using two independent detection methods, AdCSV and ICP-MS, along with their respective digestion techniques were compared (Figure 27). Aliquots of the same dry, homogenized oyster soft tissue pool have been digested, ranging from 0.05 g to 2 g. Voltammetrical analyses gave similar Pt concentrations of $0.52 \pm 0.10 \text{ ng.g}^{-1}$ ($n = 13$; Figure 27), whatever the sample mass. When using the ICP-MS method, the results were similar to those obtained by voltammetry, only for sample masses $> 0.25 \text{ g}$ with average Pt concentrations of $0.51 \pm 0.04 \text{ ng.g}^{-1}$ ($n = 10$; Fig. 27). For sample masses below 0.25 g, the ICP-MS method produced results which were inconsistent, strongly overestimating the Pt concentration in the samples.

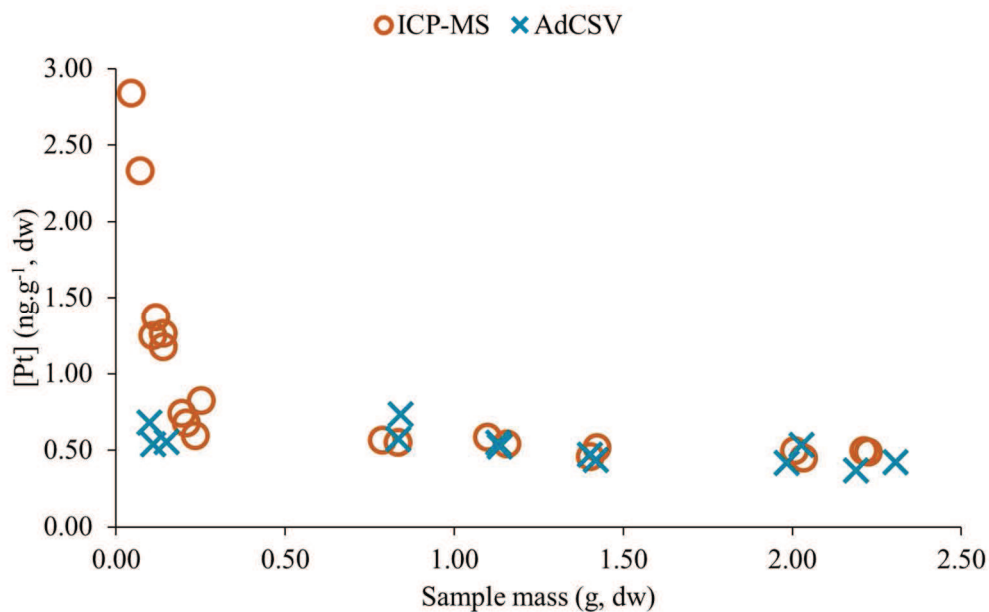


Figure 27: Comparison of Pt concentrations (ng.g^{-1} , dw: dry weight) measured in an oyster pool sample by ICP-MS (round symbols) and AdCSV (cross symbols) as a function of sample mass (g, dw).

Similar observations related to HfO^+ interference were already made in biological matrices (raptor feathers) following analysis through ICP-MS and application of mathematical corrections (Jensen et al., 2002). A limit ratio between interference / corrected concentration was determined above which the signal is mainly due to interferences rather than real Pt concentration in the sample.

Our results suggest limitations in controlling the influence of interferences and/or blanks, when using relatively low sample mass. Sample masses above 0.25 g (corresponding to at least 0.13 ng Pt) consistently allowed for reproducible and similar (< 3 % difference in average) results for both methods applied. Such convergence may indicate good accuracy, as classical accuracy measurements are impossible due to lacking suitable CRM (Pt in bivalves). Inter-method comparison was also performed with two plankton net samples providing similar results using both techniques (< 5 % differences in average).

These findings suggest that for the typical dry tissue mass range of biological samples analyzed within this study (i.e. between 0.5 and 2 g), both digestion- and analytical protocols produce similar results. It helps to select the best sample preparation and analytical procedure to apply to our biological samples. This selection is thus based on the mass of sample available. For fresh oyster samples, relatively important mass (up to 3 g) was available and ICP-MS method could be applied as well as for fresh wild mussel samples. When dried powder was available (oyster and mussel from the RNO/ROCCH), less sample was available and AdCSV method was applied.

In the absence of background knowledge on Pt concentrations in environmental phytoplankton samples, AdCSV method i.e. the most sensitive method was first applied to our samples (samples from the Genoa Harbor, SCHEMA-II-2016 field campaign). The relatively elevated Pt concentrations lead to the possible application of ICP-MS determination. Aliquots of plankton net material were analyzed using both techniques providing, as observed for bivalve samples, similar results between both methods ($\text{RSD} < 10 \%$, $n = 2$).

2.4.4.3. Analytical quality control

For voltammetrical method, blank samples were processed including ashing and acid-digestion steps as performed for biota samples. Typical blank concentration of $\sim 0.05 \text{ ng.L}^{-1}$ are obtained although, very often, Pt peak were not detectable for blank samples at the deposition time used for biota Pt measurements. Detection limits is of $\sim 0.03 \text{ ng.g}^{-1}$ and 0.01 ng.g^{-1} for a typical sample masses of 50 mg and 130 mg for mussel/oyster and phytoplankton samples respectively and sample volume of 25 mL. Since no CRM for biota Pt concentration exist, the accuracy of the analytical procedure has been checked by analyzing BCR-723 and Jsd-2 CRMs as aforementioned for Pt detection in sediment cores (recovery $> 90 \%$).

For ICP-MS method, blank samples were similarly processed as biota samples and typical blank concentrations of $\sim 0.4 \text{ ng.L}^{-1}$ are obtained. Detection limits range between ~ 3 and 6 pg.g^{-1} for typical masses comprised between 1 and 2 g and sample volume of 13 mL. Analysis of BCR-723 and Jsd-2 give satisfactory recovery results (recovery $\sim 100 \%$).

Furthermore inter-method comparison of ICP-MS and AdCSV methods was carried out on the same biological samples as aforementioned and suggest good accuracy and precision of Pt determination in this matrix.

2.4.5. Application of isotopically-labelled platinum in exposure experiments

2.4.5.1. Isotopically-labelled platinum spikes and sample preparation

In order to prepare our stock isotopically-labelled solution, solid metal shavings of ^{194}Pt (116.5 mg; Cortecnet®) were acid digested using a mixture of 4 mL concentrated HCl and 2 mL of concentrated HNO_3 (both Suprapur®, Merck). Acid digestion was performed on a hot plate at 110°C for 4 h. The isotopically-labelled solution was diluted using ultrapure water (MilliQ®). The concentration and isotopic composition were controlled before starting the experiment by ICP-MS analysis. Stock isotopically-labelled ^{194}Pt solution had a concentration of $532 \pm 1 \text{ mg.L}^{-1}$ ($n = 5$), corresponding to a nominal total Pt concentration of 650 mg.L^{-1} . The isotopic abundance of isotopically-labelled ^{194}Pt is of 81.9 % while natural abundance of ^{194}Pt is of 32.9 %.

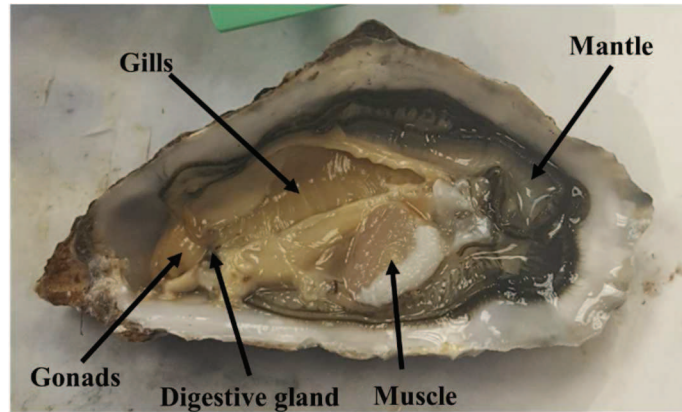


Figure 28 : Oyster organism with the different organ dissected for organotropism study.

At time of sampling, 5 individuals per tank (pseudo-replicates) were sampled for chemical analysis of Pt concentrations in total soft tissue for each exposure condition and in both tanks serving as respective replicates. Ten other individuals were sampled for chemical analysis of Pt concentrations in the different organs and for histopathological analyses. Oysters were sampled, opened, the water inside the shell was discarded and the soft body rinsed with uncontaminated seawater. Shells were weighed and measured in order to determine the CI (Figure 29).

For total soft tissue chemical analyses, entire soft bodies were placed in acid-cleaned PP tubes. For organotropism analyses, organs (gills, digestive gland, gonads, muscle, and mantle; Figure 28) were dissected and placed separately in acid-cleaned PP tubes (Figure 29). Gonads could not be systematically isolated from digestive glands for all samples. Pieces of pure digestive gland and the remaining digestive gland and gonads were separated in two sample types.



Figure 29: Oyster dissection at the PiE Marine Station. From left to right: shell and soft tissue weighing, whole organism dissection, organotropism dissection.

Soft tissues were deep-frozen (-80°C), freeze-dried, crushed in an agate mortar in order to obtain a homogenous powder, and stored in the dark at room temperature, pending analysis. Sampling for histopathological and histochemical analyses was also performed (PiE laboratory).

In addition to dissections, faeces were collected four times ($T = 7, 21, 28$, and 35) during the experiment in each feeding tank (uncontaminated seawater in order to avoid Pt adsorption on faeces). Sampling was done using a Pasteur syringe (Figure 30). All visible faeces at time of collection were sampled but this sampling cannot be considered as fully quantitative since we might miss some material excreted later on. Samples were collected in a Teflon bottle, filtered on pre-weighed Teflon filters (47 mm, $0.45\ \mu\text{m}$ FHLC filter, Merck Millipore®, Figure 30), deep-frozen (-80°C), and dried in a drying oven at 50°C prior analysis.



Figure 30: Faeces collection. On the left faeces collection with the Pasteur syringe, on the right Teflon filters with faeces from the different feeding tanks.

Seawater (homogenized by continuous airflow) was sampled manually from the tanks into 250 mL Teflon bottles. Water samples were filtered immediately through $0.2\ \mu\text{m}$ polycarbonate filters (Nucleopore®) with a filter-syringe (Sartorius®). Filtrates were collected in acid-cleaned 60 mL Teflon bottles previously rinsed with an aliquot of the filtrate, acidified to $\text{pH} = 1$ (36.5 – 38 % HCl Baker® Instra) and stored in the dark at 4°C pending analysis.

2.4.5.2. Analytical procedure

The exposure experiment was performed with a spike of Pt stable isotope in order to measure Pt accumulation kinetics by ICP-MS. After validation of the inter-comparison AdCSV / ICP-MS showing similar results for both independent methods (see *Section 2.4.4.2*), the ICP-MS protocol (digestion and analysis) was applied to all biological samples of the exposure experiment. Samples were ashed and acid-digested as described previously (*Section 2.4.4.2*). Same procedure was performed on faeces collected on Teflon filters but without the ashing step. Analyses were performed by quadrupole ICP-MS (Thermo, X Series II) applying the standard addition method (using mono-elementary Pt standard solution 1000 µg.mL⁻¹ PLASMACAL, SCP Science) to each sample. After obtaining the raw signal, the first step is to correct for any possible spectral interference as aforementioned. Considering Hf / Pt ratios < 50, mathematical correction provided accurate results and HfO⁺ correction were performed using the ¹⁹³Ir signal, given that Ir concentration in our samples was assumed negligible.

The following equation was applied (8):

$$S_{\text{corr}} = S_{\text{meas}} - (S_{\text{inter}} \times A / B)$$

8

Where S_{corr} is the corrected signal of the analyte (¹⁹⁴Pt or ¹⁹⁵Pt), S_{meas} the measured signal of the analyte (¹⁹⁴Pt or ¹⁹⁵Pt), S_{inter} the signal of the interference (estimated from mass 193 signals representing HfO⁺ interference), A is the % of formation of HfO⁺ on the masses 194 and 195, and B the % of formation of HfO⁺ on mass 193 (modified from Djingova et al., 2003). After signal correction, the respective contributions of natural and isotopically-labelled Pt in the oyster tissues were assessed as follows (equations 9 and 10):

$$^{194}\text{Pt}_{\text{corr}} = L \times A^{194}\text{Pt}_L + N \times A^{194}\text{Pt}_N$$

9

$$^{195}\text{Pt}_{\text{corr}} = L \times A^{195}\text{Pt}_L + N \times A^{195}\text{Pt}_N$$

10

Where $^{194}\text{Pt}_{\text{corr}}$ and $^{195}\text{Pt}_{\text{corr}}$ are the interference-corrected count numbers of ¹⁹⁴Pt and ¹⁹⁵Pt corresponding to the sum of natural and isotopically-labelled Pt signals, E corresponds to the count number for isotopically-labelled Pt, N corresponds to the count number for natural Pt, $A^{194}\text{Pt}_L$ corresponds to the abundance of isotope ¹⁹⁴Pt in the isotopically-labelled solution,

$A^{195}\text{Pt}_L$ corresponds to the abundance of isotope ^{195}Pt in the isotopically-labelled solution, $A^{194}\text{Pt}_N$ and $A^{195}\text{Pt}_N$ correspond to the respective natural abundances of the isotopes ^{194}Pt and ^{195}Pt .

Then L and N were determined as follows (equations 11 and 12):

$$L = \frac{{}^{194}\text{Pt}_{\text{corr}} \times A^{195}\text{Pt}_N - {}^{195}\text{Pt}_{\text{corr}} \times A^{194}\text{Pt}_N}{A^{194}\text{Pt}_L \times A^{195}\text{Pt}_N - A^{195}\text{Pt}_L \times A^{194}\text{Pt}_N} \quad 11$$

$$N = \frac{{}^{194}\text{Pt}_{\text{corr}} \times A^{195}\text{Pt}_L - {}^{195}\text{Pt}_{\text{corr}} \times A^{194}\text{Pt}_L}{A^{195}\text{Pt}_L \times A^{194}\text{Pt}_N - A^{194}\text{Pt}_L \times A^{195}\text{Pt}_N} \quad 12$$

The concentrations of both, Labelled Pt (CL) and Natural Pt (CN) were determined from the corrected signal L and N using the standard addition method. Finally, the total Pt concentrations in oyster samples were obtained from the sum CL + CN.

The main advantage of applying isotope spike techniques is the simultaneous determination of metal concentrations accumulated from isotopically-labelled spikes and the amount of metal naturally present in the same organism, i.e. excluding biases due to variability between individuals. Such information is not possible to obtain with other analytical methods measuring total element concentrations (Mikolaczyk et al., 2016). Accurate determination of CN was only possible for the B and C exposure conditions i.e. 50 ng.L⁻¹ and 100 ng.L⁻¹ spiked concentrations. In the D tanks Pt was spiked at 10,000 ng.L⁻¹ and the oysters accumulated high amounts of Pt very rapidly making the determination of CN impossible because $^{194}\text{Pt}/^{195}\text{Pt}$ ratios in the oysters were similar to the $^{194}\text{Pt}/^{195}\text{Pt}$ ratio in the isotopically-labelled solution throughout the whole experiment. Platinum concentrations in oysters from tanks D and in faeces were determined using the CL value only.

Considering the very low Pt content of isolated organs from non-exposed individuals (T = 0), Pt was determined by AdCSV method applying already described protocol for biota matrices (deposition time of 90 s). Seawater samples from control tanks were also analyzed by voltammetry after elimination of organic matter by UV-oxidation as previously described using a deposition time of 300 s. Seawater samples from exposure tanks were diluted 20, 40 and 4000-fold (for Pt spike concentrations of 50, 100, and 10,000 ng.L⁻¹ respectively) and analyzed by ICP-MS using standard addition method.

2.5. Conclusion

Various samples from contrasting sampling sites were collected in the frame of this study. They differ in terms of matrix and of Pt content. As mentioned in literature, reliable determination of Pt trace concentrations in environmental samples remains a challenging task (Schindl and Leopold, 2015). Several analytical procedures were developed and tested within the last decade, but only few of them proved realistic approaches for investigation of large sample set and determination of environmental Pt levels. In this study, we apply mainly two different analytical approaches being the voltammetry and the ICP- MS techniques. According to sample matrix and to Pt content, adapted analytical procedures for sample preparation and Pt detection are applied. Method selection, according to sample type, is summarized in the diagram Figure 31 together with associated detection limits and analytical quality control results.

Taking into consideration recent advances in the data treatment of voltammetrical signals we applied this technique especially to seawater samples, allowing to reach lower detection limits (Cobelo-García et al., 2014b). Performing different optimization steps (material cleaning, UV-oxidation and/or deposition time) adapted to our samples, we managed to determine Pt_D levels in seawater from various coastal sites. Sediment cores were also analyzed by AdCSV methods. Considering the large number of SPM samples and the need to perform other analyses on these samples, we applied TQ ICP-MS method. A large part of our sample set consisted in biota samples including environmental and experimentally-exposed organisms. Environmental samples were primarily analyzed by AdCSV providing accurate and precise data on Pt levels in wild living and farmed marine bivalves. Despite the very efficient analytical procedure that represents the AdCSV, regarding the numerous samples deriving from the exposure experiment (in total more than 500 samples), we worked at optimizing Pt determination to make the procedure more suitable for larger sample series. This constitutes the main requirement in environmental monitoring and toxicological studies according to (Schindl and Leopold, 2015).

By implementing a pre-concentration step (ashing) the ICP-MS response signal was increased, over-passing interference difficulties (after mathematical corrections). Our approach was validated through successful inter-comparison of AdCSV and ICP-MS results in biota samples (sufficient sample mass and relative Pt content are required). Furthermore, application of stable isotopes in the exposure experiment allowed, to apply principles commonly used in isotope dilution methods permitting to differentiate naturally present and accumulated Pt.

In summary, the collection of original material combined with the application and validation of analytical techniques allowed the acquisition of valuable environmental data that will be introduced and discussed in the following chapters.

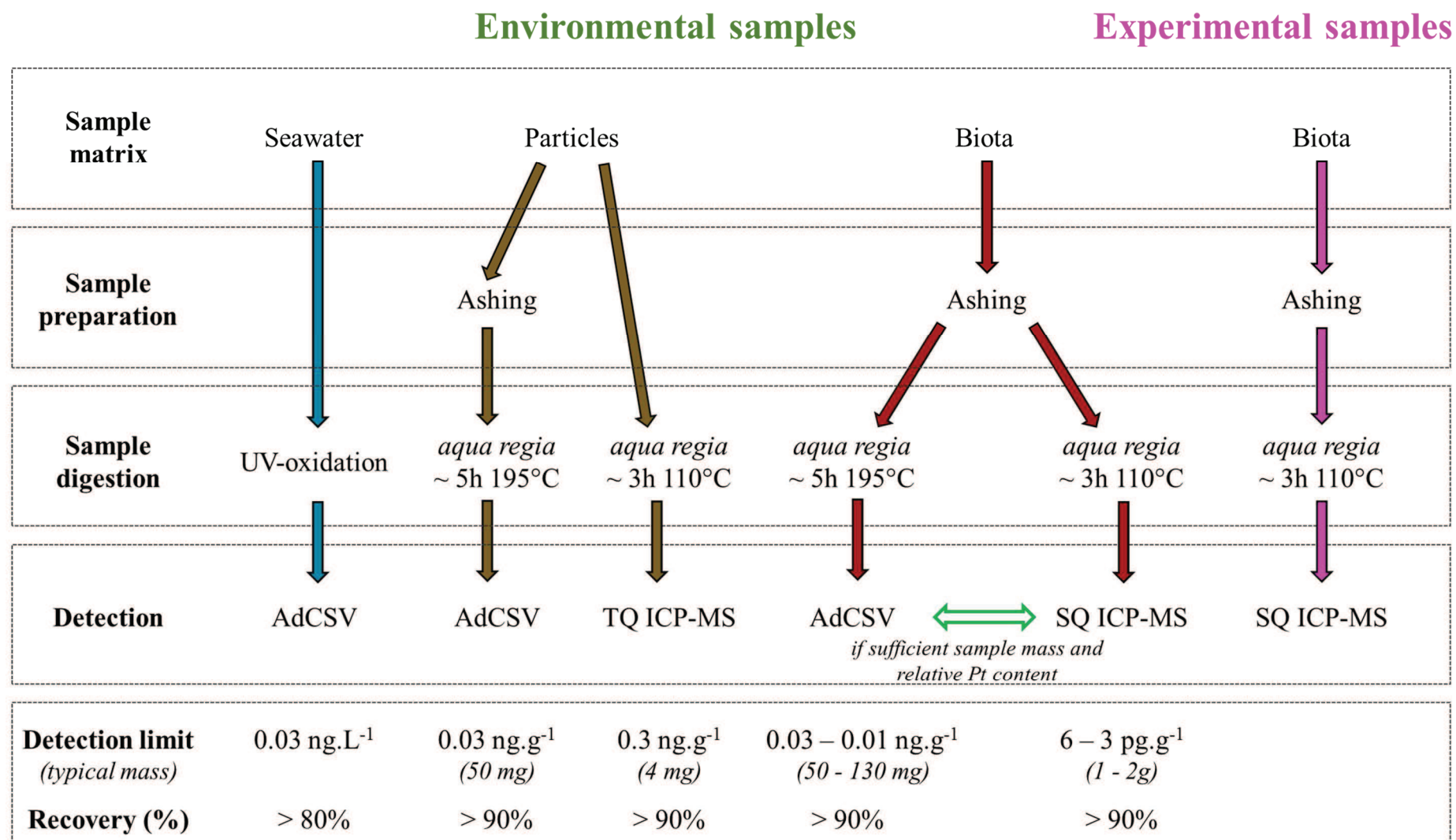


Figure 31: Summary of the analytical procedures applied to the different matrices analyzed in the present study. Voltammetrical and ICP-MS methods were inter-compared (green arrow) for biota sample allowing to use both technique if sufficient sample mass and relative Pt content are available.

Chapter 3

Platinum accumulation and physiological response in oyster *Crassostrea gigas*

Chapter 3: Platinum accumulation and physiological response in oyster *Crassostrea gigas*

3.1. Introduction

Representative living organisms have been used for biomonitoring since several decades. In particular, bivalves are used for the monitoring of anthropogenic pressure of organic and inorganic substances for which they represent a quantitative indicator (Cossa, 1989). The Mussel Watch concept has existed since the late 1970s, using mussels (*Mytilus*) and oysters (*Ostrea* or *Crassostrea*) as surveillance organisms (Goldberg et al., 1978). Numerous studies worldwide have successfully addressed oysters as biomonitors for “classical” trace metals, particularly species of the genus *Crassostrea* such as *C. gigas* (Baudrimont et al., 2005; Burioli et al., 2017). Accordingly, the Pacific oyster, *C. gigas* with its wide geographical distribution (being one of the world’s most widespread bivalves) provides an excellent “sentinel” species (Burioli et al., 2017). It is available all year round, and has a sufficiently long life-time to allow sampling of more than one year-class (Cossa, 1989). In addition, this species is abundant in numerous marine areas, sessile (and therefore is an authentic representative of the study area), can be easily collected, and is extremely resistant to environmental stress (Rainbow and Phillips, 1993). Oysters are also large enough to provide sufficient tissue for chemical analysis (Cossa, 1989). As it is also an edible mollusk, concentrations of harmful elements in its tissues must be monitored (Burioli et al., 2017).

Biomonitoring already is an established tool for “classical” trace metal surveillance, but **what about the potential application of regular biomonitoring studies for Pt in coastal systems?**

In order to be measured in living organisms, a contaminant must be bioavailable in the ambient environment. The bioavailability of an element (or contaminant) is defined as the fraction of a chemical present in the environment that is available for accumulation in organisms (Amiard-Triquet and Rainbow, 2009). The environment includes water, sediments, suspended particulate matter, and food.

Bioavailability might be determined through three complementary approaches according to Amiard-Triquet and Rainbow (2009):

- Regular chemical monitoring of the distribution of the element in different environmental compartments (e.g. water, sediments...) from which its fate would be forecast.
- Measurements of bioaccumulated contaminants in biota exposed in the field or in the laboratory that reflect the bioavailable concentrations in the environment (applied procedure in biomonitoring programs such as Mussel Watch).
- Measurements of physiological responses (at different levels of biological organization) associated with accumulated doses in biota exposed to contaminants under laboratory conditions or in the field.

In the field, measurements of Pt levels in wild mussels *Mytilus galloprovincialis* confirmed the potential bioavailability of Pt to mussels that could be extrapolated to marine bivalves in general (Neira et al., 2015).

Oyster *C. gigas* could therefore be regarded as a possible sentinel species for Pt contamination. For this, other factors must be considered as reported by Cossa (1989) and Ruchter (2012):

- The organism should accumulate the element without showing increasing mortality.
- A high bioconcentration factor for the investigated element should be exhibited by the organism to facilitate quantification.
- A simple correlation should exist between the metal content of the organism and the average metal concentration in the ambient environment (e.g. water, sediments). This correlation should be similar for all individuals in all study areas under all biotic or abiotic conditions.

When considering metal uptake in marine bivalves, apart from their potential application for biomonitoring processes, one should also investigate the potential adverse physiological effects that may occur in living organisms. In this sense, there is also a lack of knowledge concerning **the response of marine organisms, at different levels of biological organization, following exposure to Pt.**

Concerning uptake mechanisms, exposure to metal may occur through the dissolved phase which is known as the direct pathway or through the particulate material via food which is the trophic pathway. For trace metal intracellular uptake, elements must first bind with (i.e., form coordination complexes with) ligands present on the cell surface (Sunda, 1989). Following

binding of a metal ion to the transport ligand, the metal may either dissociate back into the medium or be transported across the membrane. Crossing plasma membrane of the cell most likely occurs through facilitated diffusion or through energy-dependent processes (Marigómez et al., 2002). Once within the cell, metal binds to a variety of existing ligands. The rates of intracellular accumulation of metals are determined by the number and binding characteristics of the available ligands and their accessibility (Marigómez et al., 2002).

When exposed to a pollutant, living organisms may develop adaptive strategies (defense and/or tolerance) according to the degree of exposure. Those strategies consist in the control of metal accumulation, a decrease of their uptake, an increase of their excretion (e.g. Mikolaczyk, 2016). Such biological effects of pollutants on organisms are called “biomarkers” consisting of biochemical and/or physiological changes in organisms, and thus represent initial responses to environmental perturbations and contamination (de Lafontaine et al., 2000). The selected biomarkers should indicate that the organism has been exposed to pollutants (exposure biomarkers) and/or the magnitude of the organism’s response to the pollutant (effect biomarkers or biomarkers of stress, Cajaraville et al., 2000).

Regarding the scarcity of data on Pt bioaccumulation in marine organisms and in order to investigate the aforementioned characteristics, the following laboratory exposure experiment was performed:

- Test organism: Oyster *Crassostrea gigas* collected from a non-polluted estuary.
- Route of exposure: Direct pathway.
- Test system and exposure medium: Daily renewed seawater with pre-equilibrated tank walls.
- Platinum source: Isotopically-labelled ^{194}Pt solution, dissolved Pt(IV). Adapted spikes performed daily in renewed seawater.
- Exposure concentration: 50 and 100 ng.L^{-1} nominal Pt concentrations, considered as environmentally relevant levels; and 10,000 ng.L^{-1} Pt considered as relatively high concentration.
- Exposure period: 35 days
- Feeding: in separate tanks with daily renewed unspiked seawater

Outcomes from this exposure experiment have been divided into two parts addressing different aspects of Pt bioaccumulation in marine bivalves: (i) platinum accumulation kinetics and (ii) organotropism and biomarker response.

3.2. Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species in coastal marine environments

The ecotoxicological endpoints introduced in the present section are the uptake and accumulation kinetics throughout the exposure period. For this, oysters were sampled on a weekly-basis, and whole organisms were analyzed in order to measure Pt concentrations in soft tissues. The use of isotopically-labelled spikes is related to the application of ICP-MS technique. Platinum measurements in biota matrices through quadrupole ICP-MS is often hindered by spectral interferences generally related to Hafnium Oxides (HfO^+ , (Godlewska-Żyłkiewicz, 2004; Pyrzynska, 2015)). Therefore AdCSV method is rather applied for Pt levels detection in such matrices. Yet, a particular interest exists in applying the ICP-MS method for experimentally-derived samples since this method is less time consuming in terms of i) sample preparation and ii) sample analysis. Above all, the major strength of stable isotope Pt spikes is the potential for the determination of both natural/initial Pt content and the Pt accumulated from spikes **in the same individual**, measured simultaneously by ICP-MS.

This analytical technique must be validated. In the absence of suitable Certified Reference Material, an inter-method comparison using two independent digestion and measurement techniques (ICP-MS and AdCSV methods) has been performed.

Overall, the objective is to **assess whether oyster *C. gigas* is a relevant species for Pt exposure monitoring in coastal environments?**

Accordingly, the study focuses on:

- The determination of the rate and kinetics of dissolved Pt accumulation in soft tissues of oysters.
- The observation of bioconcentration factors obtained after direct exposure to environmentally relevant Pt concentrations.
- The assessment of Pt accumulation at relatively high Pt concentrations that could be considered in case of a heavy pollution event.

Results from this study have been published in the peer-reviewed journal *Science of the Total Environment*.

Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species in coastal marine environments

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Abstract

Platinum Group Elements (PGEs) are extremely scarce in the Earth's Crust and of strong interest for high-end technologies due to their specific properties. They belong to the Technology Critical Elements (TCEs) for which use is forecast to increase, implying growing emissions into the environment in the following years. In particular, with the intensive use of platinum (Pt) in car catalytic converters, the anthropogenic geochemical cycle of this element has surpassed the natural cycle. Yet, environmental Pt levels are still in the sub picomolar range, making its analytical detection a challenge. Few studies cover the behavior of Pt in marine waters in terms of speciation, reactivity and possible transfer to the biota. In this study, oysters (*Crassostrea gigas*) from an unpolluted estuary were exposed to the stable isotope ¹⁹⁴Pt in seawater at a range of concentrations during 35 days. Seawater was renewed daily and spiked to three nominal Pt concentrations (50, 100, and 10,000 ng.L⁻¹) for two replicate series. In addition, control conditions were monitored. Five oysters from each tank were dissected after 3, 7, 14, 21, 28, 35 days of Pt exposure, and analyzed by ICP-MS. Accuracy of this analytical method applied to biological matrix was checked by an inter-method comparison with a voltammetrical technique. A concentration-dependent accumulation of Pt in oysters increasing with exposure time occurred. After 28 days, oyster Pt accumulation from low and intermediate exposure conditions reached a plateau. This was not the case of the highest exposure condition for which oyster tissues showed increasing concentrations until the last day of the experiment. A linear correlation exists between seawater concentrations and Pt content in oysters for low and intermediate exposure concentrations i.e. closer to environmental concentrations. By showing high Pt accumulation potential, oysters may serve as sentinels, ensuring biomonitoring of Pt concentrations in marine coastal waters.

Keywords: PGE; Exposure study; Bivalve; Seawater; ICP-MS

1. Introduction

With the ongoing changes in resource use and technological progression, many elements undergo major disturbance of their geochemical cycles. This is the case of a group of elements named Technology Critical Elements (TCEs). These trace elements have the particularity of being scarce at the Earth surface but have a great interest in terms of economy since they offer peculiar characteristics applied to modern technologies. In this group, the Platinum Group Elements (PGEs) draw attention since their Earth's surface anthropogenic fluxes exceed their natural geochemical fluxes (Sen and Peucker-Ehrenbrink, 2012). In particular, platinum (Pt) is used for jewelry and anti-cancer drugs. However, the major demand for Pt is for automobile catalytic converters. Catalytic properties of Pt are used to reduce vehicle emissions representing > 50% of the end use market for PGEs (Bossi and Gediga, 2017).

Regularly introduced in cars from the early 1990's, different environmental compartments have recorded this ongoing change in Pt use. Accordingly, in highly urbanized areas, very high concentrations of Pt are found in road-dusts and roadside soils (Schäfer and Puchelt, 1998). Yet environmental records of Pt increase include also airborne particulate matter such as in Mexico or Germany where elevated Pt concentrations were attributed to automobile catalysts (Rauch et al., 2006; Zereini et al., 2001). Increasing Pt concentrations are also observed in sedimentary cores from (i) an urban lake showing a major increase in Pt accumulation rates from the 1990's to the 2000's (Rauch and Hemond, 2003), (ii) urban estuaries that record strong anthropogenic Pt sources (Mashio et al., 2016) and (iii) even very remote areas such as Antarctica (Soyol-Erdene et al., 2011) since airborne particles are not only present in areas close to emissions but can be transported over longer distances (Zereini et al., 2001). Beyond those abiotic environmental archives, organisms have also been studied to assess environmental changes of trace metal concentrations. This is particularly the case of bivalves that are sedentary sentinel organisms for many trace elements, especially in coastal environments (e.g. Goldberg et al., 1978). Bivalves have already been used to detect Pt contamination in aquatic ecosystems (Abdou et al., 2016; Neira et al., 2015; Ruchter and Sures, 2015). Those studies have shown that wild bivalves (respectively oysters *Crassostrea gigas* and mussels *Mytilus edulis*, and freshwater clams *Corbicula* sp.) seem to be suitable biomonitors for Pt contamination reflecting emission variations over time. Such organisms may bioconcentrate Pt up to a factor of $5 \cdot 10^3$ (Neira et al., 2015). This is a very valuable feature, considering the analytical challenge that represents Pt analysis in samples (e.g. water, particles, and organisms) from natural aquatic environments. Concentrations are very low in such natural samples (i.e. in the $\text{ng} \cdot \text{L}^{-1}$ range) and often close to detection limits whatever the analytical technique. The strong and complex matrix of coastal waters implies additional analytical limitations, which may explain why only few studies report Pt levels in seawater and coastal environments. Currently, two methods for Pt determination in seawater are described in previous publications: Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID-ICP-MS) with respective detection limits (expressed as three times the standard deviation of blank measurements) for seawater of $3.9 \cdot 10^{-3} \text{ ng} \cdot \text{L}^{-1}$ and $2.9 \cdot 10^{-3} \text{ ng} \cdot \text{L}^{-1}$ Pt (Cobelo-García et al., 2014a; Mashio et al., 2016). For the second technique, pre-concentration of Pt on an anion exchange resin is required to concentrate Pt and remove sea-salt and interfering metals present in seawater matrix (Obata et al., 2006). Platinum

concentrations in biological materials collected in the field are most commonly analyzed by voltammetry (Ruchter and Sures, 2015; Neira et al., 2015; Abdou et al., 2016). Matrix effects (spectral interferences with Hafnium Oxygen: HfO^+ particularly) and generally low content of Pt analyte may lead to analytical difficulties when using quadrupole ICP-MS (Godlewska-Zylkiewicz, 2004; Pyrzynska, 2015). However, this technique presents several advantages compared to voltammetrical techniques that is in particular interfered by the presence of organic matter and other interfering trace metals (Cobelo-García et al., 2014b). Advantages of ICP-MS method include the fact that this analytical technique is less time consuming in terms of i) sample preparation (organic matter elimination not compulsory, no evaporation required, single-use vessel) and ii) sample analysis (automated sample injection, rapid measurement etc.). In this study, determination of Pt concentrations in biota was done by ICP-MS analyses after an ashing step of the samples. Accuracy of Pt determination was cross-checked by an inter-method comparison between ICP-MS and voltammetry, as no appropriate Certified Reference Material (CRM) is available.

There is still a lack of knowledge concerning Pt speciation in coastal environments. Literature reports that in seawater, the inorganic equilibrium speciation of Pt(II) and Pt(IV) is dominated by PtCl_6^{2-} and $\text{PtCl}_5(\text{OH})^{2-}$, respectively (Gammons, 1996) with Pt(IV) being the most important oxidation state (Cobelo-García et al., 2013). Yet, metal source or metal speciation can considerably influence biological availability (Zimmermann et al., 2015) and several exposure studies have proved the potentiality of Pt to accumulate in aquatic organisms. Literature reports that soluble Pt is more bioavailable to zebra mussels than particle-bound Pt (Sures and Zimmermann, 2007). Platinum uptake by the freshwater isopod *Asellus aquaticus* was found to be higher for Pt(IV) than for Pt(II) (Rauch and Morrison, 1999) while the reverse was observed for other freshwater organisms (e.g. Zimmermann et al., 2002). Other aquatic organisms exposed to dissolved Pt include fish species such as eel *Anguilla anguilla* (Zimmermann et al., 2004), or chub *Squalius cephalus* (Ruchter, 2012) reporting the accumulation capacity of these freshwater animals. Mulholland and Turner (2011) addressed for the first time Pt accumulation in natural seawater in an aquatic organism, the gastropod *Littorina littorea*. Accumulation of Pt from dissolved form uptake and diet was studied and suggested that Pt is mainly accumulated from the aqueous phase. This paper is to the best of our knowledge the first addressing Pt accumulation kinetics in a marine bivalve by exposing wild oysters (*Crassostrea gigas*) to isotopically-labelled Pt (^{194}Pt) in seawater for 35 days. Platinum uptake kinetics were investigated in oysters using a wide range of Pt concentrations including environmentally relevant concentrations. Previous studies have already proved the ability of wild oysters to accumulate trace metals integrating and amplifying the environmental signal (e.g. Baudrimont et al., 2005; Lanceleur et al., 2011). More particularly *C. gigas* species already served as monitors to study historical records of recent Pt contamination in an estuary (Abdou et al., 2016). This species represents therefore a promising Pt accumulating sentinel for bio-monitoring studies. This work aims at determining the rate and kinetics of dissolved Pt accumulation in soft tissues of oysters. In addition, results will help to determine the potentiality of using wild oysters as sentinel organisms for Pt in marine coastal environments.

2. Material and Methods

All the laboratory material in contact with the samples was soaked in an acid bath (HCl 65% J.T. Baker 10% v/v or HNO_3 65% Honeywell 10% v/v

v) during 3 days, then thoroughly rinsed with ultrapure (MilliQ®) water, dried under a laminar flow hood and kept sealed in double polypropylene bags until use.

2.1. Preparation of isotopic solutions

Solid metal shavings of ^{194}Pt (116.5 mg; Cortecnet®) were acid digested using a mixture of 4 mL concentrated HCl and 2 mL of concentrated HNO_3 (both Suprapur®, Merck). Acid digestion was performed on a hot plate at 110 °C for 4 h. The isotopically-labelled solution was diluted using ultrapure water (MilliQ®). The concentration and isotopic composition were controlled before starting the experiment by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis at the UMR EPOC 5805, University of Bordeaux, France. Stock isotopically-labelled ^{194}Pt solution had a concentration of $532 \pm 1 \text{ mg} \cdot \text{L}^{-1}$ ($n = 5$), corresponding to a nominal total Pt concentration of $650 \text{ mg} \cdot \text{L}^{-1}$ Pt (isotopic abundance of ^{194}Pt : 81.9%). Natural abundance of ^{194}Pt is 32.9%.

2.2. Experimental setup

Oysters used for the experiment were purchased from the “OSTRANOR” oyster farm in a relatively pristine area San Vicente de la Barquera, Cantabria, Spain. A total of 545 oysters with similar characteristics ($\sim 90 \pm 5 \text{ mm}$ shell length, adult individuals) were acquired. The exposure experiments took place at the Plentzia Marine Station (PiE, UPV/EHU, Basque Country, Spain). Seawater is naturally filtered by sand in the uptake wells aided with a pump that sends the water to the Marine Station. Seawater gas balance is controlled in the Station and then passes through a decantation/inertial tank. Seawater in the experimental tanks contains around 1000 particles/ml that are smaller than $3 \mu\text{m}$ (as measured in a Beckman Coulter Counter Z2). All the conductions in contact with seawater are metal-free PVC tubing. For the remainder of this paper, the term ‘seawater’ refers to this water. Oysters were acclimatized and depurated in seawater with a continuous water and air flow during 12 days and photoperiod was established at 12 h:12 h (light:dark cycle). In parallel, the different exposure tanks were filled with seawater, spiked to the respective nominal total Pt concentrations for 12 days with daily renewal in order to equilibrate tank walls with the experimental seawater prior to the exposure experiment limiting adsorption processes. Three exposure conditions were set by spiking ^{194}Pt to seawater: $50 \text{ ng} \cdot \text{L}^{-1}$ Pt (B condition); $100 \text{ ng} \cdot \text{L}^{-1}$ Pt (C condition); $10,000 \text{ ng} \cdot \text{L}^{-1}$ Pt (D condition). As to the best of our knowledge, the present study is the first experimental work on direct Pt uptake in marine bivalves, no previous information on suitable exposure conditions was available. Therefore, we arbitrarily set the lowest and the intermediate exposure conditions (tanks B and C) at $50 \text{ ng} \cdot \text{L}^{-1}$ Pt and $100 \text{ ng} \cdot \text{L}^{-1}$ Pt respectively. These concentrations represent about 500 and 1000 times the Pt concentrations encountered in clean coastal environments (mean Pt estuarine concentrations $\sim 0.1 \text{ ng} \cdot \text{L}^{-1}$; Cobelo-García et al., 2014a). Yet they represent only 10 and 20 times Pt concentrations found in urban polluted coastal areas (e.g. the Tokyo Bay $\sim 7 \text{ ng} \cdot \text{L}^{-1}$ Pt, Obata et al., 2006). They can therefore be considered as environmentally relevant levels. Accordingly, the exposure concentration in tanks D, $10,000 \text{ ng} \cdot \text{L}^{-1}$ Pt, represented from 2,000 to 100,000 times environmental concentrations. Such concentrations were selected to (i) cover a wide range of concentrations, from environmentally relevant to relatively high values, (ii) ensure an observable uptake and potential effect on the biota (ongoing work) and (iii) compare accumulation kinetics patterns for one oyster population in comparable living conditions at very different exposure levels. Each condition was carried out in replicate (i.e. in separate tanks), as reflected by the tank numbers: B1 and B2, C1 and C2, D1 and D2. The tanks A1 and A2 corresponded to control conditions without Pt addition.

The experimental design accounted for three types of analyses: i) chemical analyses of Pt concentration in total soft tissue, ii) chemical analyses of Pt distribution between oyster organs (organotropism), and iii) histological impact of Pt exposure. The present paper focuses on the Pt concentrations in total soft tissue (i), while organotropism and histological responses (ii and iii) will be studied in a subsequent paper. Twenty-five oysters were isolated and dissected, representing the T0, i.e. the initial condition for the three types of analyses. Then, 520 individuals were evenly (65 individuals per tank) distributed in 8 polypropylene experimental 45 L tanks filled with 40 L seawater, aerated by continuous air flow, in a temperature-controlled room (17 °C) with an artificial photoperiod (12 h:12 h, light:dark cycle).

Each day, control and experimental tanks were emptied (12 pm). The oysters were rinsed with non-spiked seawater and transferred into separate clean “feeding tanks” (one for each exposure condition), filled with non-spiked seawater and fed for 4 h using commercial food (SERA MARIN, “Coraliquid” Sera GmbH Heinsberg, Germany). During this time, control and exposure tanks were rinsed and filled again with non-spiked seawater. After 4 h of equilibration, physical and chemical parameters (temperature, salinity, pH, and dissolved O_2 level) were measured and seawater was then spiked with ^{194}Pt to nominal total Pt exposure concentrations. Spikes were performed using the isotopically-labelled stock solution of ^{194}Pt ($650 \text{ mg} \cdot \text{L}^{-1}$ total Pt) for the D tanks and a diluted solution ($6.5 \text{ mg} \cdot \text{L}^{-1}$ total Pt; dilution with MilliQ water) for the B and C tanks. Along the experiment seawater volume in the tanks, the related amounts of ^{194}Pt spiked and food provided were adapted to the number of individuals remaining after the dissections keeping exposure and feeding conditions of individual oysters at the same level throughout the experiment. Accordingly, the following proportions were kept constant throughout the experiment: $0.5 \pm 0.07 \text{ L}$ seawater $27 \pm 4 \text{ ng}$ total Pt in tanks B, $53 \pm 7 \text{ ng}$ total Pt in tanks C, $5,310 \pm 737 \text{ ng}$ total Pt in tanks D, and $7 \cdot 10^7$ algae cells per individual oyster and per day. Feeding tanks were then emptied, the oyster batches were rinsed and placed again in their respective control and experimental tanks containing freshly-spiked seawater. In this way, exposure to ^{194}Pt was maintained as constant as possible from day to day, minimizing experimental biases (e.g. due to adsorption to container walls) which would have been much greater throughout the whole experiment without regular (daily) renewal of the experimental conditions. Despite these precautions, water analyses show that a significant decrease occurs 20 h after the spike which was not only related to Pt uptake by oysters and is discussed further in details in Section 4.3. Direct exposure of oysters to Pt was performed 20 h per day, simulating a realistic scenario of exposure in a natural intertidal environment.

In order to observe early stage contamination processes, individuals were sampled in each tank and dissected after 3 days of exposure ($T = 3$). Weekly dissections were performed $T = 7$, $T = 14$, $T = 21$, $T = 28$ and $T = 35$ days after exposure. At time of sampling, 5 individuals per tank (pseudo-replicates) were sampled for chemical analysis of Pt concentrations in total soft tissue for each exposure condition and in both tanks serving as respective replicates. Ten other individuals were sampled for chemical analysis of Pt concentrations in the different organs and for histopathological analyses. Oyster mortality was checked daily; individuals were considered dead when they were opened and their valves failed to close after physical stimulation. Seawater from the different tanks was sampled after 20/21 and 29/30 days of exposure, before and after daily renewal and three other times after daily renewal (33, 34, and 35 days after exposure). Seawater (homogenized by continuous airflow) was sampled manually from the tanks into 250 mL Teflon bottles. Water samples were filtered immediately through $0.2 \mu\text{m}$ polycarbonate filters (Nucleopore®) with a filter-syringe (Sartorius®). Filtrates were collected in acid-cleaned 60 mL Teflon tubes previously rinsed with an aliquot of the filtrate, acidified to $\text{pH} = 1$ (36.5–38% HCl Baker Instra) and stored in the dark at 4 °C pending analysis.

2.3. Sample preparation

Oysters were sampled, opened, the water inside the shell was discarded and the soft body rinsed with uncontaminated seawater. For total soft tissue chemical analyses, entire soft bodies were placed in acid-cleaned (10% HNO₃; 65% p.a. Honeywell), polypropylene (PP) tubes (DigiTUBES®, SCP SCIENCE), and wet weight was determined. Each valve was also weighed and measured (length, width, and thickness). This allows for the determination of a Condition Index (CI) of the oysters. The CI was calculated for each individual according to the equation (Strady et al., 2011a):

$$CI = \text{Visceral Content (wet weight; g)} / \text{Shell (wet weight; g)} * 100. \quad (1)$$

Soft tissues were deep-frozen (−80 °C), freeze-dried, crushed in an agate mortar to obtain a homogenous powder, and stored in the dark at room temperature, pending analysis.

2.4. Analytical procedure

The exposure experiment was performed with a stable isotope of Pt spike in order to measure Pt accumulation kinetics by ICP-MS. Low Pt concentrations in natural environments result in generally low concentrations in biological tissues implying analytical challenges for their accurate determination. Therefore, several techniques allow the pre-concentration of trace metals in a given sample. For this purpose, ashing of powdered oyster tissues was implemented to pre-concentrate the Pt content (e.g. Schäfer et al., 1998), eliminating part of the sample matrix and therefore allowing acid digestion of a more important mass of the sample (up to 3 g in our case). In comparison, classical biological sample acid digestion, without the ashing step, allows the preparation of 0.02 to 0.03 g with the same acid volume. Samples were ashed in porcelain crucibles at 800 °C during 3 h according to the heating scheme described by Nygren et al. (1990). Then samples were acid-digested according to an adapted protocol for trace element detection in biological matrices as described in Mikolaczyk et al. (2016). After crucibles had cooled down, 2 mL HCl and 1 mL HNO₃ (30% HCl and 65% HNO₃ Suprapur®, Merck) were added to the ashed residues. The mixture was then transferred in polypropylene (PP) tubes (DigiTUBES®, SCP SCIENCE) with caps, placed in the Teflon-coated heating block, and digested at 110 °C for 3 h. Cooled contents were then diluted in 10 mL MilliQ water and centrifuged at 4000 rpm for 10 min (20 °C) prior to analyses. Analyses were performed by quadrupole ICP-MS (Thermo, X Series II) applying the standard addition method (using mono-elementary Pt standard solution 1000 µg·mL^{−1} PLASMACAL, SCP Science) to each sample. Using the principles commonly applied in isotope dilution methods we can differentiate naturally present Pt and accumulated Pt as described below.

After obtaining the raw signal, the first step is to correct for any possible spectral interference. For Pt analysis by ICP-MS, the most common mass interferences result from Hafnium-Oxygen: HfO⁺ species, like ¹⁷⁸Hf¹⁶O, ¹⁷⁹Hf¹⁶O, ¹⁸⁰Hf¹⁶O or ¹⁸¹Hf¹⁶O interfering with ¹⁹⁴Pt, ¹⁹⁵Pt, ¹⁹⁶Pt, respectively (Parent et al., 1997). The quantification of the interfering signal that overlaps the analyte signal and its subtraction by mathematical equation is limited by the intensity of the interference (Parent et al., 1997). For Pt analyses, this mathematical correction provides accurate results for Hf/Pt ratios of up to 50 (Parent et al., 1997), which was applicable to the samples of the exposure experiment (¹⁷⁸Hf/¹⁹⁴Pt and ¹⁷⁹Hf/¹⁹⁵Pt < 50). Correction of Hf oxide (HfO⁺) interference was performed using the ¹⁹³Ir signal that is highly interfered by HfO⁺ (Djingova et al., 2003) and given that Ir concentration in our samples was assumed negligible.

The following equations were applied:

$$S_{\text{corr}} = S_{\text{meas}} - (S_{\text{inter}} * A/B) \quad (2)$$

where S_{corr} is the corrected signal of the analyte (¹⁹⁴Pt or ¹⁹⁵Pt), S_{meas} the measured signal of the analyte (¹⁹⁴Pt or ¹⁹⁵Pt), S_{inter} the signal of the interference (estimated from mass 193 signals representing HfO⁺ interference), A is the % of formation of HfO⁺ on the masses 194 and 195, and B the % of formation of HfO⁺ on mass 193 (modified from Djingova et al., 2003).

After signal correction, the respective contributions of natural and isotopically-labelled Pt in the oyster tissues were assessed as follows:

$$^{194}\text{Pt}_{\text{corr}} = L * A^{194}\text{Pt}_L + N * A^{194}\text{Pt}_N \quad (3a)$$

$$^{195}\text{Pt}_{\text{corr}} = L * A^{195}\text{Pt}_L + N * A^{195}\text{Pt}_N \quad (3b)$$

where $^{194}\text{Pt}_{\text{corr}}$ and $^{195}\text{Pt}_{\text{corr}}$ are the interference-corrected count numbers of ¹⁹⁴Pt and ¹⁹⁵Pt corresponding to the sum of natural and isotopically-labelled Pt signals, E corresponds to the count number for isotopically-labelled Pt, N corresponds to the count number for natural Pt, $A^{194}\text{Pt}_L$ corresponds to the abundance of isotope ¹⁹⁴Pt in the isotopically-labelled solution, $A^{195}\text{Pt}_L$ corresponds to the abundance of isotope ¹⁹⁵Pt in the isotopically-labelled solution, $A^{194}\text{Pt}_N$ and $A^{195}\text{Pt}_N$ correspond to the respective natural abundances of the isotopes ¹⁹⁴Pt and ¹⁹⁵Pt.

Then L and N were determined as follows:

$$L = \frac{(^{194}\text{Pt}_{\text{corr}} * A^{195}\text{Pt}_N - ^{195}\text{Pt}_{\text{corr}} * A^{194}\text{Pt}_N)}{(A^{194}\text{Pt}_L * A^{195}\text{Pt}_N - A^{195}\text{Pt}_L * A^{194}\text{Pt}_N)} \quad (4a)$$

$$N = \frac{(^{194}\text{Pt}_{\text{corr}} * A^{195}\text{Pt}_L - ^{195}\text{Pt}_{\text{corr}} * A^{194}\text{Pt}_L)}{(A^{195}\text{Pt}_L * A^{194}\text{Pt}_N - A^{194}\text{Pt}_L * A^{195}\text{Pt}_N)} \quad (4b)$$

The concentrations of both, Labelled Pt (CL) and Natural Pt (CN) were determined from L and N using the standard addition method (addition of mono-elementary Pt stock solution with natural isotopic composition).

Finally, the total Pt concentrations in oyster samples were obtained from the sum CL + CN. This enables an estimate of the total Pt uptake over exposure time at the individual scale, as described elsewhere (Mikolaczyk et al., 2016). However, these calculations were only possible for the B and C exposure conditions i.e. 50 ng·L^{−1} and 100 ng·L^{−1} spiked concentrations. In the D tanks Pt was spiked at 10,000 ng·L^{−1} and the oysters accumulated high amounts of Pt very rapidly making these calculations impossible because ¹⁹⁴Pt/¹⁹⁵Pt ratios in the oysters were similar to the ¹⁹⁴Pt/¹⁹⁵Pt ratio in the isotopically-labelled solution throughout the whole experiment. The use of this technique, successfully applied to other trace metals in oysters (e.g. Ag and Cu, Mikolaczyk et al., 2016), would have needed lower Pt spikes, producing intermediate ¹⁹⁴Pt/¹⁹⁵Pt ratios in the oysters (i.e. values between natural and spike ¹⁹⁴Pt/¹⁹⁵Pt ratios). However, as aforementioned, no major background knowledge exists concerning dissolved Pt accumulation kinetics in seawater organisms. For oysters not exposed to isotopically-labelled Pt (T0 and oysters from Tanks A), calculations were based only on the interference-corrected ¹⁹⁵Pt signal (¹⁹⁵Pt_{corr}) using standard addition method (addition of mono-elementary Pt stock solution).

Literature reports that the detection of low Pt concentrations in natural biological samples by ICP-MS may be difficult due to spectral interferences (Godlewska-Żyłkiewicz, 2004; Pyrzynska, 2015). Voltammetric analyses are often preferred to ICP-MS for this type of matrix (e.g. Ruchter and Sures, 2015). We therefore performed an inter-method comparison of two completely independent methods: ICP-MS and AdCSV, including their respective mineralization procedures. In the absence of a Certified Reference Material for Pt in mollusks or other marine organisms, analytical quality was checked by analyzing a pool of (non-contaminated) oysters (*C. gigas*) originating from the

Gironde Estuary that was prepared by dissecting (~100 individuals), freeze-drying, sieving at 150 µm, and grinding/homogenizing in an agate mortar. Aliquots for each technique ($n = 20$ for ICP-MS; $n = 13$ for AdCSV), covering a wide range of sample mass i.e. between 0.05 g and 2 g, were ashed and acid-digested according to the two respective method protocols.

For voltammetry, after ashing, a mixture of 5 mL concentrated HCl and 3 mL concentrated HNO₃ (30% HCl and 65% HNO₃ Suprapur®, Merck) was added to cooled crucibles. After 1 h, the mixture was transferred to PFA vials (Savillex®) and placed in a Teflon-coated heating block at 195 °C for 1 h with caps followed by an evaporation step (without caps). The residues were then dissolved adding 1 mL of concentrated H₂SO₄ (93–98% Trace metal™ grade, Fisher Chemical), and evaporated again until no more fumes were observed (i.e. only H₂SO₄ was present). Cooled contents were then diluted with 0.1 M HCl (Suprapur®, Merck) and centrifuged at 4000 rpm for 10 min (20 °C) prior to analyses. Platinum voltammetric determinations were carried out using a µAutolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm® 663 V.A.) equipped with three electrodes: i) a hanging mercury drop electrode (HMDE; the working electrode), ii) a Ag/AgCl reference electrode, and iii) a glassy carbon auxiliary electrode. A polytetrafluoroethylene (PTFE) voltammetric cell served in all experiments and the potentiostat was controlled using the NOVA 2.1 software. Aliquots of acid-digested sample were pipetted into the voltammetric cell, together with two reagents, 3.3 mM formaldehyde (37–41% Analytical Reagent Grade, Fisher Chemical), and 0.45 mM hydrazine sulfate (Analytical Reagent Grade, Fisher Chemical). Analytical procedure described by Cobelo-García et al. (2014b) was applied to our samples using a deposition time of 90 s. Platinum concentrations were determined by standard addition method (using mono-elementary Pt standard solution 1000 µg·mL⁻¹ PLASMALAC, SCP Science). Standard additions were adapted to each sample. After validation showing similar results for both independent methods (see Section 3.3), the ICP-MS protocol (digestion and analysis) was applied to all biological samples of the exposure experiment.

Seawater samples from control tanks were analyzed by voltammetry after elimination of organic matter by UV oxidation (Obata et al., 2006). The samples were placed in capped Teflon tubes after adding 50 µL of H₂O₂ for 25 mL of solution, and irradiated overnight using two 64 W UV lamps (NIQ 60/35 XL, Heraeus) placed under a fume hood. Aliquots (10 mL) of UV-digested sample were pipetted into the voltammetric cell and Pt concentrations were determined as described previously, using a deposition time of 300 s. Seawater samples from exposure tanks were diluted 20, 40 and 4000-fold (for Pt spike concentrations of 50, 100, and 10,000 ng·L⁻¹ respectively) and analyzed by ICP-MS using standard addition method.

2.5. Platinum uptake and bioconcentration factor calculations

The overall Pt uptake (PU) by oysters expressed as the fraction (%) of the amount of spiked Pt at the tank scale has been estimated by mass balance calculations following the Eq. (5):

$$PU_t(\%) = (\text{Oyster Pt mass})_t / (\text{Spiked Pt mass})_t \quad (5)$$

with t : the sampling time; $(\text{Oyster Pt mass})_t = (\text{Mean oyster [Pt]}_t - \text{Mean oyster [Pt]}_{-1}) * \text{number of individuals at } t * \text{total individual mass}$; and $\text{Spiked Pt mass} = \text{nominal [Pt]}_{\text{spiked}} \text{ at } t * \text{seawater volume} * \text{number of exposure days between the sampling times}$.

Furthermore Bioconcentration Factor (BCF; Arnot and Gobas, 2006) were determined according to the Eq. (6):

$$BCF = [Pt]_{\text{oyster}} / [Pt]_{\text{seawater}} * 10^3 \quad (6)$$

with $[Pt]_{\text{oyster}}$ the total Pt concentration in the oyster soft tissue (ng·g⁻¹) and $[Pt]_{\text{seawater}}$ the Pt concentration in seawater (ng·L⁻¹).

2.6. Quality control and statistics

Since no CRM for Pt in biological matrices are available, efficiency of acid digestion of the ICP-MS protocol was checked by analyzing the CRM DORM-2 (dogfish muscle, NRCC). Recoveries > 90% were observed for Cd, Cu, Co, and Zn, and >80% for Cr and Ni ($n = 5$). The only available CRMs for Pt concentrations are the BCR®-723 road dust (IRMM) and Jsd-2 sedimentary rocks (indicative value from GSJ). Analyses of these CRMs by ICP-MS gave satisfactory recovery values of 87% and 101% respectively ($n = 3$). Platinum concentrations in these CRMs were also analyzed by AdCSV and gave satisfactory recovery of 89% for BCR®-723 and 98% for Jsd-2 ($n = 3$). Furthermore inter-method comparison of ICP-MS and AdCSV methods were realized on the same biological samples in order to assess accuracy and precision of Pt determination in this matrix. Limit of detection of Pt in biological matrix for AdCSV and ICP-MS methods (calculated as $3 * \text{blank standard deviation}$) were of respectively 0.8 pg·g⁻¹ ($n = 5$), and 6.4 pg·g⁻¹ ($n = 50$), for typical dry tissue weight of 1.9 g.

In the absence of CRM for dissolved Pt in seawater, precision of the voltammetric procedure was evaluated by means of the analysis of spiked seawater laboratory-internal standard giving recoveries >95% and precision expressed as Relative Standard Deviation (RSD) below 10% ($n = 3$). The detection limit for dissolved Pt (calculated as $3 * \text{blank standard deviation}$; $n = 20$) was estimated to 0.04 ng·L⁻¹.

In order to assess for significant changes between physical-chemical parameters and between Pt concentrations between the different sampling times, one-way ANOVA tests were run for parametric data and Kruskal-Wallis for non-parametric data. Homoscedasticity was checked for all the data using Bartlett test. Holm-Bonferroni or Mann-Whitney pairwise post-hoc tests were performed. In all cases, significant differences correspond to p -values < 0.05.

3. Results

3.1. Physical and chemical parameters of the exposure medium

Major physical and chemical parameters were monitored throughout the experiment. Temperature, pH, salinity, and dissolved O₂ concentration were measured daily, 4 h after water renewal and before moving the oysters from the feeding tanks to the exposure tanks. We observed temperature values of 15.2 ± 0.42 °C, pH of 7.93 ± 0.02 , salinity of 32.6 ± 0.68 , and dissolved O₂ concentrations of 8.19 ± 0.1 mg·L⁻¹. Holm-Bonferroni tests ran on the data showed that temperature, pH, salinity and dissolved oxygen concentrations were similar in all tanks and throughout the whole experiment.

3.2. Mortality and Condition Index of oysters

Mortality was very low, as only one organism died during the experiment period (tank D1, after 15 days of exposure). At each sampling time, oyster size and tissue mass were controlled to determine if any difference in bioaccumulation would depend on the size and mass of the organism and to assess the individual health status. To this purpose, Condition Index (CI) was determined for the different tanks at each sampling time according to the Eq. (1). Despite some minor fluctuations over time, CI showed overall similar values ($CI = 20 \pm 4$; $n = 480$) in all the tanks and throughout the experiment.

3.3. Inter-method comparison of ICP-MS/voltammetry protocols for Pt quantification in bivalves

Results obtained from Pt analyses in the same oyster sample using two independent detection methods, ICP-MS and voltammetry

(AdCSV), along with their respective digestion techniques were compared.

Aliquots of the same dry, homogenized oyster soft tissue pool have been digested, ranging from 0.05 g to 2 g. Voltammetrical analyses gave similar Pt concentrations of $0.52 \pm 0.10 \text{ ng} \cdot \text{g}^{-1}$ ($n = 13$; Fig. 1), whatever the sample mass. When using the ICP-MS method, the results were similar to those obtained by voltammetry, yet only for sample masses $>0.25 \text{ g}$ with average Pt concentrations of $0.51 \pm 0.04 \text{ ng} \cdot \text{g}^{-1}$ ($n = 10$; Fig. 1). For sample masses below 0.25 g, the ICP-MS method produced results which were inconsistent, strongly overestimating the Pt concentration in the samples. These findings suggest that for the typical dry tissue mass range of samples analyzed within the exposure experiment (i.e. between 0.8 and 2 g), both digestion- and analytical protocols have produced similar results.

3.4. Platinum concentrations in the exposure media

Seawater was sampled several times 1 h and 20 h after spiking the water in order to estimate Pt concentrations before and after the daily exposure period (Table 1).

Seawater Pt concentrations determined in tanks A control are relatively low with mean concentrations of $0.25 \pm 0.06 \text{ ng} \cdot \text{L}^{-1}$ ($n = 4$) 1 h after the spike performed in the exposure tanks and remained low 20 h after the spike performed in the exposure tanks ($0.22 \pm 0.07 \text{ ng} \cdot \text{L}^{-1}$; $n = 4$). Analyses of seawater Pt concentrations confirm that the spikes in the different tanks were close to the nominal exposure concentrations for the tanks B, C and D with respective mean values of 52 ± 2.6 , 102 ± 3 , and $9900 \pm 270 \text{ ng} \cdot \text{L}^{-1}$, 1 h after the spikes were performed ($n = 4$ for each exposure condition; Table 1). A decrease of $\sim 30\%$ in dissolved Pt concentration occurred 20 h after spiking seawater in each exposure tank ($n = 10$ for each exposure conditions; Table 1). A mass balance calculation to estimate the extent of the biological uptake is discussed in Section 4.3.

3.5. Isotopic ratio $^{194}\text{Pt}/^{195}\text{Pt}$ in oyster tissues

The use of stable isotope spiking allows for tracing metal uptake and provide information on the “natural” Pt content part and the contribution of isotopically-labelled Pt accumulated from the spikes in the same individual oyster. The “natural” part of Pt concentrations of oysters from tanks B and C showed similar values compared to average concentrations obtained for T0 samples, as well as those obtained for oysters from tanks A (controls, Table 2). The high isotopically-labelled Pt uptake by oysters from tanks D impedes the determination of “naturally” present Pt levels for oysters from this exposure condition.

Natural $^{194}\text{Pt}/^{195}\text{Pt}$ ratio is 0.973. The isotope ratio in the isotopically-labelled solution was $^{194}\text{Pt}/^{195}\text{Pt} = 5.51$. Isotopic ratios in oyster tissues

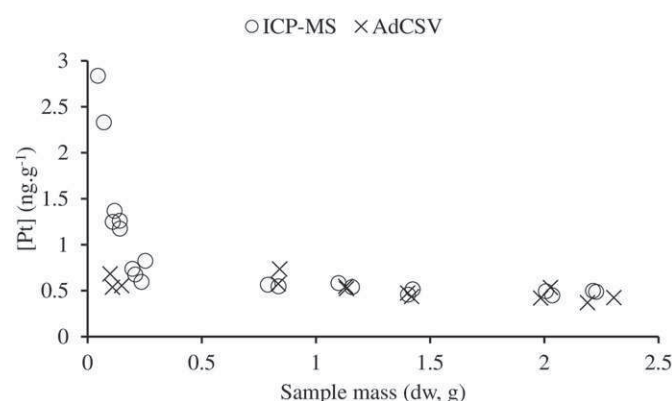


Fig. 1. Comparison of Pt concentrations ($\text{ng} \cdot \text{g}^{-1}$, dry weight) measured in an oyster pool sample by ICP-MS (round symbols) and AdCSV (cross symbols) as a function of sample mass (dw: dry weight).

Table 1

Dissolved Pt concentrations in the control tanks and in the exposure media. Means and standard deviations (SD) were calculated from the data of two replicate tanks per condition at two sampling times ($n = 2 \times 2$) 1 h after the spike, and at five sampling times ($n = 2 \times 5$; $n = 2 \times 2$ for control tanks) 20 h after the spike.

		Tanks A	Tanks B	Tanks C	Tanks D
1 h after the spike	Mean Pt concentrations ($\text{ng} \cdot \text{L}^{-1}$)	0.25	52	102	9910
	SD ($\text{ng} \cdot \text{L}^{-1}$)	0.06	2.6	3.0	270
	n	4	4	4	4
20 h after the spike	Mean Pt concentrations ($\text{ng} \cdot \text{L}^{-1}$)	0.22	36	70	7170
	SD ($\text{ng} \cdot \text{L}^{-1}$)	0.07	3.4	5.1	610
	n	4	10	10	10

exposed to labelled ^{194}Pt spikes were clearly modified from the first sampling time, i.e. three days after the beginning of the exposure experiment in all exposure conditions. Even in tanks B, with the lowest Pt exposure, the $^{194}\text{Pt}/^{195}\text{Pt}$ ratio reached values up to 3.3 at $T = 3$. The ratio continuously increased until reaching the value of the pure isotopically-labelled solution at $T = 35$. Similar trends occurred in tank C with $^{194}\text{Pt}/^{195}\text{Pt}$ ratio = 4.9 at $T = 3$ and $^{194}\text{Pt}/^{195}\text{Pt}$ 5.5 at $T = 35$. In contrast, oysters from tanks D displayed $^{194}\text{Pt}/^{195}\text{Pt}$ ratios of 5.5 from $T = 3$ in all individuals.

3.6. Platinum concentrations in oyster tissues

Evolution of total Pt concentrations in oyster soft tissues was monitored through time for the 35 days of exposure experiment. Oysters from the control tanks (A tanks) showed average values between 0.2 and $0.4 \text{ ng} \cdot \text{g}^{-1}$, i.e. remained similar to initial values throughout the experiment (about $0.25 \text{ ng} \cdot \text{g}^{-1}$; Fig. 2). Slightly different values were independent from the replicate series and most likely due to inter-individual variability and/or generally very low concentrations compared to method-inherent detection limits (LOD). Results from statistical analysis (one-way ANOVA) showed that no significant differences exist between Pt concentrations in oysters from tanks A at the different sampling times.

Analyses of total Pt concentrations showed rapid and efficient Pt accumulation in oyster tissues over time depending on their exposure conditions in both replicate series compared to control tanks (Fig. 2). At the lowest exposure conditions (i.e. $50 \text{ ng} \cdot \text{L}^{-1}$ Pt; B tanks), Pt concentrations in total oyster soft tissues increased with time and were 10 times higher than those in control individuals after only 3 days of exposure ($\sim 4 \text{ ng} \cdot \text{g}^{-1}$ Pt, significant difference). Concentrations increased continuously thereafter to values of $\sim 25 \text{ ng} \cdot \text{g}^{-1}$ after 35 days, i.e. ~ 100 times greater than those in non-exposed individuals. No significant differences are observed between Pt concentrations of oysters from tanks B sampled at one week interval i.e. between $T = 3$ and $T = 7$, $T = 7$ and $T = 14$, $T = 14$ and $T = 21$, $T = 21$ and $T = 28$, $T = 28$ and $T = 35$. Yet, significant differences exist for all sampling time intervals of two weeks and more. Oysters exposed to intermediate Pt concentrations of $100 \text{ ng} \cdot \text{L}^{-1}$ (C tanks) showed a similar pattern of accumulation kinetics. After 3 days, Pt concentrations in the C tanks were similar to those observed for the B tanks i.e. ten times higher than in non-exposed organisms (significant difference). Subsequently, the Pt

Table 2

Comparison of natural Pt concentrations ($\text{ng} \cdot \text{g}^{-1}$, dry weight) in oysters from tanks B and C with Pt concentrations in T0 individuals and control oysters (tanks A).

	T0	Tanks A	Tanks B	Tanks C
Mean natural Pt concentrations ($\text{ng} \cdot \text{g}^{-1}$)	0.236	0.263	0.223	0.227
SD ($\text{ng} \cdot \text{g}^{-1}$)	0.071	0.095	0.125	0.115
n	10	60	60	60

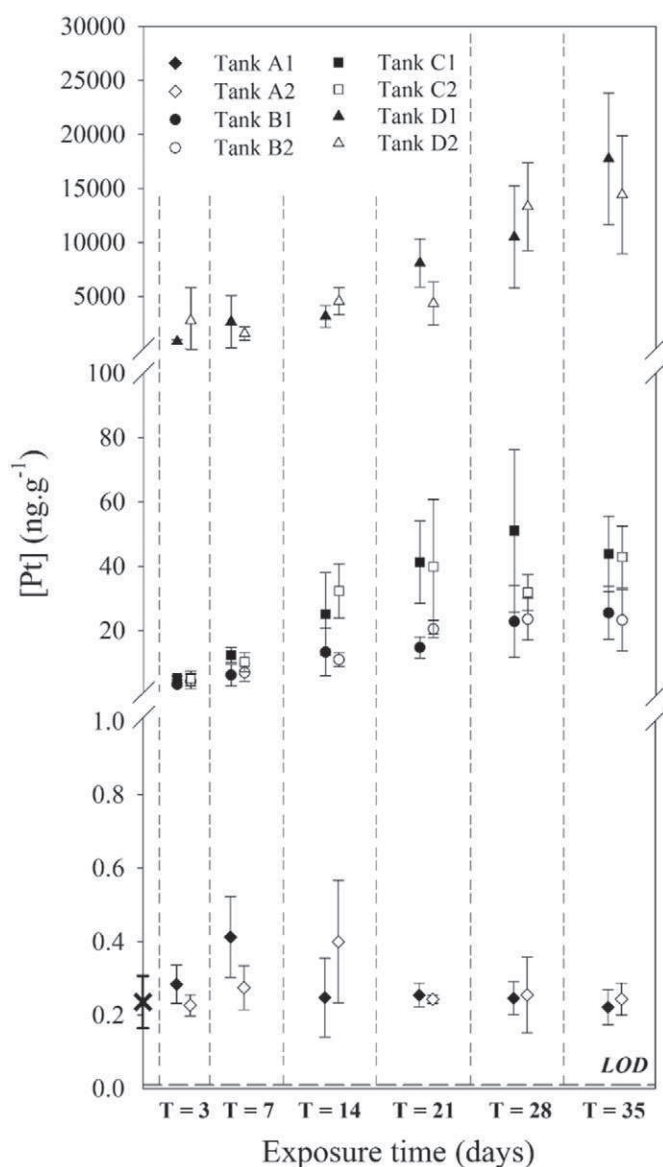


Fig. 2. Accumulation kinetics of Pt in oyster soft tissues. Oyster mean Pt concentrations ($n = 5$, dry weight) for each replicate (replicate 1: full symbols, replicate 2: empty symbols) of tanks B (round symbols), C (square symbols), and D (triangular symbols) as well as in control tanks (A, diamond symbols). Cross symbols represent initial Pt concentrations in oysters at $T = 0$ ($n = 10$). The dashed line represents the analytical limit of detection ($LOD = 0.8 \text{ ng} \cdot \text{g}^{-1}$). Note the discontinued scale of the concentration axis. Error bars represent standard deviation (SD).

concentration in oyster tissues increased more steeply than for the B tanks during the following weeks (significant differences from one week to the other i.e. between $T = 3$ and $T = 7$, and $T = 7$ and $T = 14$). Platinum concentrations in oysters from tanks C reached values of about $50 \text{ ng} \cdot \text{g}^{-1}$, i.e. ~ 200 times the control concentrations after only 14 days of exposure. After $T = 21$, Pt concentrations in oyster tissues from tanks B and C seemed to show an accumulation plateau (no significant differences between $T = 21$, $T = 28$ and $T = 35$ for both tanks; Fig. 2).

The series of oysters exposed to $10,000 \text{ ng} \cdot \text{L}^{-1}$ Pt (D tanks) accumulated rapidly high amounts of Pt in their soft tissues. After only 3 days of exposure, oysters in D tanks had Pt concentrations $\sim 4,000$ times higher than non-exposed individuals. However, no significant differences exist between Pt concentrations at $T = 3$ and at $T = 7$. Oysters continuously accumulated Pt (significant differences between sampling times) reaching values of about $15,000 \text{ ng} \cdot \text{g}^{-1}$ at the end of the experiment

(Fig. 2). No significant differences exist between Pt concentrations at $T = 28$ and at $T = 35$, which might be related to one relatively lower value measured at $T = 35$ in tank D1 and two lower values in tank D2.

3.7. Platinum uptake kinetics

The overall Pt uptake kinetics was determined with the Eq. (5) in oysters from each exposure conditions. The relative Pt uptake by oysters in the tanks B and C compared to the total amount of Pt spiked was $\sim 5\text{--}10\%$ until $T = 28$ days when the plateau was reached. At $T = 35$, Pt uptake for oysters from tanks B is near 0% and reaches only 1% for oysters from tanks C. In oysters from tanks D, % Pt uptake was more variable and clearly higher in most cases (Fig. 3).

4. Discussion

4.1. Platinum analysis in biological matrices – ICP-MS vs AdCSV methods

Results from analyses of non-contaminated environmental samples by ICP-MS were cross-checked by applying AdCSV as a different, independent analytical method. The results suggest that for the range of sample masses tested (from 0.05 to 2 g), Pt concentrations in oyster tissues obtained by the AdCSV method are independent from sample mass and reproducible ($0.52 \text{ ng} \cdot \text{g}^{-1} \pm 0.10$; $n = 13$; Fig. 1). In contrast, the reliability of the ICP-MS analysis depends on the sample mass for sample masses below 0.25 g. Here, the Pt concentrations appeared as increasingly overestimated with decreasing sample mass. This observation has been attributed to limitations in controlling the influence of interferences and/or blanks, when using relatively low sample mass. However, in the present work sample masses above 0.25 g (corresponding to at least 0.13 ng Pt) consistently allowed for reproducible and similar ($<3\%$ difference in average) results for both methods applied. Such convergence may indicate good accuracy, as classical accuracy measurements are impossible due to lacking suitable CRM (Pt in bivalves). Precision, estimated from repeated measurements of the same oyster pool was of 19% RSD ($n = 13$) for the voltammetry method and 8% RSD ($n = 10$) for the ICP-MS method.

Validation of Pt analysis in biological samples without CRM (using different methods) has been performed in previous studies (e.g. Haus et al., 2009; Ruchter, 2012). Zimmermann et al. (2001) compared High Pressure Ashing (HPA)/AdCSV with Sector Field (SF)-ICP-MS. Comparison between both analytical methods gave satisfactory results (2–10% difference) only for Pt concentrations higher than $1 \text{ ng} \cdot \text{g}^{-1}$ which is higher than the natural concentrations observed in this study. Leśniewska et al. (2004) validated Pt values measured in grass samples exposed to road Pt emissions with both HR (High

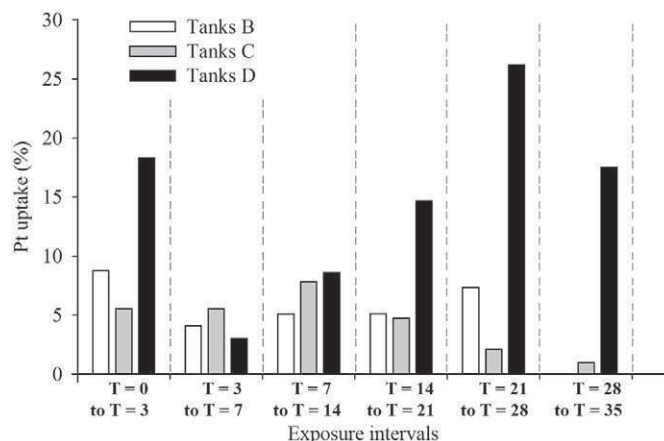


Fig. 3. Percentage of calculated Pt uptake by oysters in tanks B, C and D for the different exposure intervals (i.e. between sampling times). Mean Pt uptake of the two replicates of each exposure condition ($n = 10$ for each bar).

Resolution)-ICP-MS and quadrupole ICP-MS. However, those samples presented much higher Pt concentrations ($\sim 10 \text{ ng} \cdot \text{g}^{-1}$). The present findings are valuable for several reasons. Acid digestion of such high sample masses greatly benefits from the ashing step which reduces sample volume and matrix (pre-concentration) for both methods and is mandatory for voltammetry measurements (organic matrix suppression). Furthermore, the existence of two reliable analytical methods implies the choice of the more adequate method, depending on sample characteristics and experimental features. The AdCSV method provides higher sensitivity than the ICP-MS method for a given sample mass (typically below 0.25 g). Yet, the ICP-MS method presents several advantages including digestion of ashed samples in single-use PP tubes at 110°C . Voltammetry method requires the use of PFA vials to ensure complete acid-digestion of the sample at 195°C (acid evaporation step). This implies that the cleaning procedure of PP tubes can be run in parallel to the analyses avoiding stock problems and potential memory effects that may occur when using a limited stock of PFA vials. In addition to the time saved by the ICP-MS digestion protocol (no acid evaporation step needed), the instrumental procedure is also less time-consuming than AdCSV analyses, which sometimes need relatively long accumulation times to concentrate Pt at the electrode.

4.2. Interest of stable isotope spikes for Pt detection in biological matrices

The ICP-MS method allows for the detection of isotopes implying the possibility of using isotope-specific spikes and detection which has great advantages over classical methods using mono-elementary spikes and total Pt detection in biological accumulation studies. The main advantages as described in previous works (e.g. Mikolaczyk et al., 2016) are: i) the measurement of changed isotope ratios allows for the measurement of metal bio-uptake when the respective change in total element concentrations is not detectable due to analytical uncertainties, and ii) ability to simultaneously determine metal concentrations accumulated from isotopically-labelled spikes and the amount of metal naturally present in the same organism, i.e. excluding biases due to variability between individuals. Such information is not possible to obtain with other analytical methods measuring total element concentrations (Mikolaczyk et al., 2016). Naturally present Pt concentrations were determined for oysters from exposure tanks B and C. These Pt levels are similar to average concentrations determined in oysters from T0 and from tanks A control (Table 2). These 'natural' Pt concentrations were compared to the few data in field studies reporting Pt concentrations in wild-living marine bivalves. The values obtained in the present work were close to, but somewhat lower than average Pt concentrations ($0.332 \text{ ng} \cdot \text{g}^{-1}$) in oysters (*Crassostrea gigas*) from the Gironde Estuary collected in 2013 (Abdou et al., 2016) and in mussels (*Mytilus galloprovincialis*) from a remote location away from anthropogenic pressure in Galicia, Spain ($0.31 \text{ ng} \cdot \text{g}^{-1}$; Neira et al., 2015). These findings suggest that oysters used in the present experiment were largely pristine, implying that seawater at both sites (the oyster farm and the Plentzia Bay) had relatively low Pt concentrations ($0.12\text{--}0.25 \text{ ng} \cdot \text{L}^{-1}$). However, due to the very strong accumulation of the isotopically-labelled Pt by the oysters from tanks D (4,000 times greater than natural Pt after only 3 days exposure), the ^{195}Pt in the isotopically-labelled spike solutions totally masked the signal of the initially present natural ^{195}Pt . This suggests that isotopically-labelled spike experiments have an outstanding potential to trace precisely and sensitively metal accumulation, therefore covering a very wide range of exposure conditions. However, they are limited for higher exposure conditions by the purity of the isotopically-labelled solutions available.

4.3. Platinum accumulation kinetics

In order to assess Pt accumulation in oysters, several parameters were monitored along the experiment, because variations of physical-chemical parameters may influence Pt uptake and accumulation by

the test organisms. For instance, both toxicity and accumulation of Pt have been shown to be temperature-dependent (Veltz et al., 1996). In the present work, daily measured physical-chemical parameters displayed no major differences between tanks, suggesting that general conditions were similar for different exposure conditions and replicate series. The constant level of Condition Indices (CI), determined at each sampling time, supports the good physiological state of the oysters along the experiment (Geffard et al., 2007). This result also supports the adult age of the oysters that are not in growing phase during which those filter-feeders might accumulate more metals due to higher filtration activity (Baudrimont et al., 2016).

Platinum concentrations in oysters from control tanks (tanks A) remained very low throughout the experiment (Fig. 2) i.e. close to environmental values for marine bivalves (see Section 4.2). Few individuals had Pt concentrations below the limit of detection, whereas few others had somewhat higher Pt concentrations (about $0.5 \text{ ng} \cdot \text{g}^{-1}$) in both replicates. Seawater concentrations were monitored in control tanks and show relatively low values of about $0.23 \text{ ng} \cdot \text{L}^{-1}$ (Table 1). Previous work has shown that inter-individual differences in tissue metal concentrations occur even within a population of similar-sized individuals exposed to trace metals in controlled, uniform conditions, which may explain the observed variations (Langston and Bebianno, 1998). Overall, the results obtained from the control tanks clearly suggest that Pt contamination from tank material, food, water supply, pumping system etc. was negligible during the experiment.

However, only three days after the start of exposure to ^{194}Pt , all oysters had accumulated measurable amounts of Pt in all exposure conditions (Fig. 2), showing that oysters (i.e. marine bivalves) readily accumulate dissolved Pt from seawater, similarly to previous works on freshwater bivalves (e.g. Ruchter, 2012; Sures and Zimmermann, 2007). All exposure experimental groups showed inter-individual variations as observed in oysters populations used for similar exposure studies on Ag and Cu accumulation (Mikolaczyk et al., 2016). However, those differences in Pt concentrations between individuals of the same exposure conditions were in general clearly lower than obtained from differing exposure conditions. Furthermore, both replicates of each condition generally showed similar accumulation patterns.

In the tanks B and C, Pt concentrations in oysters steadily increased with time, following a linear pattern until $T = 21$. However, oysters from the same tanks sampled at the following exposure times (i.e. $T = 28$ and $T = 35$) showed similar Pt concentrations, suggesting that Pt concentrations in oysters might have reached a plateau of accumulation, at which metal uptake is compensated by excretion (Singer et al., 2005). Since no significant differences exist between Pt concentrations measured at $T = 21$, 28, and 35 both within tanks B and within tanks C, a plateau of accumulation must have started since 21 days after exposure. The plateau trend in metal bioaccumulation suggests the existence of efficient regulation processes to eliminate trace metals (e.g. Cd and Zn in freshwater bivalves; Marie et al., 2006). In contrast, oysters exposed to the highest concentration of Pt ($10,000 \text{ ng} \cdot \text{L}^{-1}$ total Pt; tanks D) showed different accumulation kinetics. Here, average Pt concentrations in oysters increased with time throughout the whole experiment (i.e. until $T = 35$ days after the start of exposure) and no plateau occurred for this exposure concentration. On the contrary, an "exponential" increase seems to best describe Pt accumulation in oysters from these tanks (Fig. 2). Platinum accumulation has been addressed in several exposure experiments held on freshwater organisms (e.g. Rauch and Morrison, 1999; Zimmermann et al., 2002; Zimmermann et al., 2004...). Ruchter (2012) exposed the freshwater clam *Corbicula* sp. to the same environmentally relevant Pt concentrations i.e. 50 and $100 \text{ ng} \cdot \text{L}^{-1}$ for a longer exposure time period (70 days). These treatment groups showed low accumulation rates up to day 40 and following increasing Pt concentrations until the last sampling day. This suggests that steady state was therefore not reached even after 70 days of exposure (Ruchter, 2012). On the contrary, Pt uptake in zebra mussels

Dreissena polymorpha exposed to tap water containing $100 \mu\text{g}\cdot\text{L}^{-1}$ Pt reached a plateau of accumulation after only two weeks of exposure (Sures and Zimmermann, 2007). Discrepancies in Pt uptake kinetics in freshwater organisms compared to seawater organisms can be related to different factors. These factors include water replacement as performed in this study opposed to static exposure experiments (e.g. Sures and Zimmermann, 2007) that leads to decreasing available Pt levels to biota. Furthermore, another important factor of metal bioavailability is its speciation. It is supposed that Pt(II) is the most important oxidation state in freshwater while Pt(IV) dominates in seawater (Cobelo-García et al., 2013). The isotopically-labelled Pt solution used in this study probably contained Pt(IV), given the dissolution protocol (Gammons, 1996). This and the probable dominance of Pt(IV) in marine waters suggest that the oysters in the present experiment were exposed to Pt(IV). Literature reports that zebra mussels demonstrate a significantly higher uptake for Pt(II) than for Pt(IV) in freshwater (Zimmermann et al., 2015). In contrast, the freshwater isopod *Asellus aquaticus* has been reported to accumulate more Pt(IV) than Pt(II) (Rauch and Morrison, 1999). To the best of our knowledge, no similar information comparing availability of different Pt species exists for marine organisms. Overall, there is an evident lack of data on Pt speciation in aquatic systems.

Percentages of Pt uptake were determined according to the Eq. (5) for each exposure condition through time (Fig. 3). Since seawater in the tanks was renewed and spiked to nominal Pt concentrations daily and seawater volume was adapted to the decreasing number of oysters left, exposure in terms of both nominal Pt concentrations and absolute Pt amount per oyster was supposedly constant over time for a given experimental group. The similar and constant Pt uptake percentage until $T = 21$ for oysters from tanks B and C (i.e. 50 and $100 \text{ ng}\cdot\text{L}^{-1}$) may reflect this constant exposure (Fig. 3). The above findings in oysters are consistent with results obtained from mass balance calculations applied to freshwater clams *Corbicula* sp. exposed to Pt in previous work (Ruchter, 2012).

Seawater concentrations in the exposure tanks B, C, and D were measured several times 1 h and 20 h after Pt spike was added to tanks. The data suggest systematic removal of 30% of the initially spiked Pt concentrations 20 h after the spike was performed (Table 1). Although part of this Pt removal from seawater is due to uptake in oyster soft bodies, the mass balances suggest that an important part of the Pt losses might be related to other processes than biota uptake. Previous work has shown that Pt concentrations in spiked freshwater decreased from $100 \mu\text{g}\cdot\text{L}^{-1}$ to $\sim 70 \mu\text{g}\cdot\text{L}^{-1}$ in one day, when only shells of *D. polymorpha* were present, suggesting that non-biological processes such as precipitation, adsorption of the metals onto surfaces of the aquaria and mussel shells also may remove Pt from the water (Sures and Zimmermann, 2007). Although the concentration ranges in Sures and Zimmermann (2007) were one to three orders of magnitude higher than those in the present work, this 30% removal of dissolved Pt in their freshwater experiment is similar in magnitude to results obtained for seawater. In the present work, the experimental tanks were filled up with seawater and spiked to respective nominal concentrations 12 days (with daily renewal) before the beginning of the experiment for equilibration purposes. In addition, water renewal was performed daily during the exposure experiment in an attempt to minimize Pt adsorption on tank material. Platinum compounds have a tendency towards sorption to the wall of storage material especially for polyethylene-polypropylene containers (Godlewska-Żyłkiewicz, 2004). Accordingly, we cannot exclude Pt adsorption from seawater onto tank material, or shells. Other trace metals such as Cd have been reported to accumulate on shells of the marine clam *Macoma balthica* (Langston and Zhou, 1987). However, Cd adsorption by the shells showed lower saturation kinetics than soft tissues and release from the shells was relatively rapid when shells were placed into clean seawater (Langston and Zhou, 1987). The exposed oysters were fed for 4 h in separate feeding tanks with clean seawater. Assuming Pt

adsorption onto shells during exposure, the feeding step might allow for partial Pt removal from the shells and, to a lesser extent, a possible Pt excretion of oyster soft tissues. Sures and Zimmermann (2007) have shown that the storage of previously exposed mussels in clean water for 2 days does not lead to a significant metal elimination from soft tissues, but results in an initial elimination of superficial contamination.

Preliminary results obtained from the analysis of oyster faeces and pseudo-faeces of oysters from tanks D collected in feeding tanks (filled with clean seawater and algae) suggest that such excretion material can represent up to 5% of the amount of Pt initially spiked (unpublished data). Accordingly, in addition to adsorption on tank material and shells, possible detoxification mechanisms and excretion may contribute to the mass balance in exposure experiments. The accumulation plateau observed in oysters from tanks B and C after 21 days (Fig. 2) is consistent with clearly lower Pt accumulation (Fig. 3), suggesting the activation of excretion or elimination mechanisms. Such mechanisms can include increased production of metallothioneins, metal-chelating agents typically activated in *C. gigas* when exposed to an excess of trace metals (Mouneyrac et al., 1998). Intense metallothionein production occurred within 7 days in oysters *C. gigas* exposed to Ag and/or Cu at environmentally relevant exposure levels, suggesting a rapid onset of excretion or elimination mechanisms that were maintained through time (up to 28 days; Rementeria et al., 2016).

In contrast, oysters from tanks D showed different uptake kinetics. Platinum concentrations of oyster soft tissues sampled three days and seven days after exposure do not show significant differences. However, a significant increasing Pt accumulation kinetics can be observed until the last sampling day $T = 35$, without reaching an accumulation plateau, although detoxification mechanisms through faeces seem to exist (unpublished data). Accordingly, we hypothesize that at relatively low Pt exposure (50 and $100 \text{ ng}\cdot\text{L}^{-1}$), Pt accumulation is controlled by oyster elimination mechanisms, whereas at exposure up to $10,000 \text{ ng}\cdot\text{L}^{-1}$ Pt, oysters are not able to efficiently eliminate Pt from their tissues resulting in continuous accumulation. The only oyster mortality occurred in tank D1 after 15 days of exposure, but we cannot exclude that by continuing this experiment for a longer period, increasing Pt accumulation and mortality would have occurred in oysters from the tanks D.

4.4. Environmental relevance

Few publications on environmental Pt concentrations in estuarine areas and the open ocean suggest higher Pt concentrations in coastal water bodies than in the open ocean (Mashio et al., 2016). Reported Pt concentrations range from ~ 0.004 to $0.1 \text{ ng}\cdot\text{L}^{-1}$ in the Lérez Estuary, the Gironde Estuary, and in the North Pacific Ocean (Cobelo-García et al., 2014a, 2013; Mashio et al., 2016; Suzuki et al., 2014). Yet, anthropogenic inputs may locally lead to higher dissolved Pt concentrations reaching $7 \text{ ng}\cdot\text{L}^{-1}$ in the Tokyo Bay (Obata et al., 2006). Considering such generally low levels, highly sensitive and sophisticated detection methods are necessary to determine accurate Pt concentrations in estuarine and marine waters. Bivalves such as mussels (*Mytilus* sp.) and oysters (*Ostrea* sp. or *Crassostrea* sp.) have been used for long time as surveillance or sentinel organisms of water quality due to their ability to integrate and concentrate numerous pollutants to a valuable degree over seawater levels (Goldberg et al., 1978). The present work has shown that oysters *C. gigas* rapidly accumulate dissolved Pt from seawater at different concentrations including environmentally relevant exposure levels. The use of *C. gigas* as a sentinel species for Pt implies that a simple correlation exists between the Pt content in the organism and the average Pt concentration in seawater (as for freshwater bivalves; Ruchter, 2012). Results from this experiment suggest that oysters from tanks B and C, i.e. exposed to 50 and $100 \text{ ng}\cdot\text{L}^{-1}$ respectively, display a plateau of accumulation. This suggests that the

ratio between Pt concentration in the organism and Pt concentration in seawater reached a steady state (Arnot and Gobas, 2006).

Assuming this steady state, a linear relation between seawater Pt concentrations in exposure tanks B and C and Pt concentrations in oysters at $T = 35$ were determined (Fig. 4). The linear pattern suggests a positive correlation ($R^2 > 0.99$) and the ability of oysters to serve as sentinel of Pt seawater concentrations at environmentally relevant levels. Furthermore, considering that oyster Pt accumulation from tanks B and C reached a steady state allows the estimation of a Bioconcentration Factor (BCF; Arnot and Gobas, 2006) according to the Eq. (6). Following this calculation, both oyster groups exposed to environmentally relevant Pt concentrations of 50 and 100 $\text{ng} \cdot \text{L}^{-1}$ had an average BCF of ~ 500 . No BCF was estimated for oysters in the tanks D, since Pt accumulation did not reach steady state (no accumulation plateau). Data obtained for tanks D at $T = 35$ ($[\text{Pt}]_{\text{seawater}} = 10,000 \text{ ng} \cdot \text{L}^{-1}$; $[\text{Pt}]_{\text{oyster}} = 16,060 \text{ ng} \cdot \text{g}^{-1}$, $n = 10$) clearly suggest that oysters exposed to such high Pt concentrations do not reproduce the linear pattern observed for environmentally relevant exposure levels. This also suggests that exposure to relatively high Pt levels may result in other processes and kinetics governing Pt accumulation in oysters.

The BCFs obtained from direct exposure experiments in this study (tanks B and C) is lower than BCFs observed in wild oysters *C. gigas* from the Gironde Estuary (BCF $\sim 3 \cdot 10^3$; Abdou et al., 2016) and in control oysters of the present experiment (BCF $\sim 1.3 \cdot 10^3$). Accumulation kinetics of bivalves is best described by an asymptotic curve reaching pseudo-equilibrium (Casas et al., 2008). This pseudo-equilibrium state might differ from the environmental equilibrium because of the instability and the complexity of real environmental conditions (in terms of hydrodynamics, geochemistry, or ecology) that are neglected in exposure studies (Casas et al., 2008). Furthermore, the trophic exposure pathway could also represent a route of contamination for wild oysters even though direct uptake has often been described as the dominant trace metal contamination pathway, at least for exposure experiments lasting no longer than one month (e.g. Cd; Ettajani et al., 2001; Strady et al., 2011b). Trophic transfer of Pt has been studied in the gastropod *Littorina littorea* fed with Pt contaminated marine macroalgae *Ulva lactuca* (Mulholland and Turner, 2011). Limited Pt accumulation from contaminated food was observed suggesting that this trace metal is bound in an inaccessible or indigestible form in *U. lactuca*. However these findings do not necessarily imply that the diet is an unimportant source of Pt to biota (Mulholland and Turner, 2011). Therefore, longer exposure experiments including the trophic pathway would therefore be needed to potentially approach the actual environmental BCF. Furthermore, in estuarine environments (seasonal), physical and chemical gradients affect biogeochemistry and organism physiology including reproduction cycles impacting organism exposure and toxicity of contaminants (de Souza Machado et al., 2016).

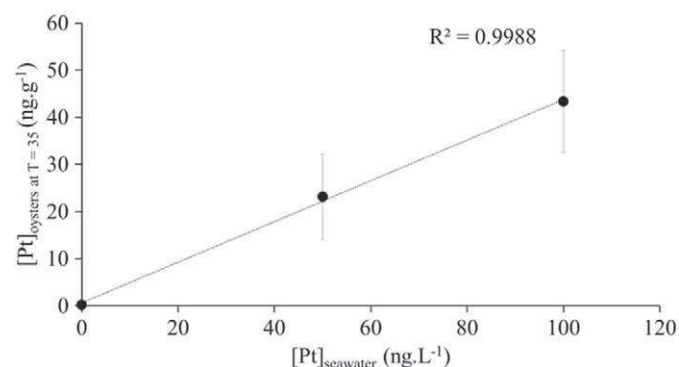


Fig. 4. Correlation between Pt concentration in seawater ($\text{ng} \cdot \text{L}^{-1}$) and mean Pt concentration ($n = 10$) in oyster tissues from tanks A (control), and from exposure tanks B and C after 35 days of experiment ($\text{ng} \cdot \text{g}^{-1}$, dry weight). Error bars represent standard deviation.

Given the increasing amounts and diversity of Pt forms released into the environment, the need for environmental assessment of Pt contamination is growing. Platinum and PGEs uptake by biota can be influenced by the type of human activity and subsequent chemicals release on the watershed. This takes into account, for instance the potential of anion-emitting activities to enhance the environmental bioaccessibility of PGEs, particularly in urbanized areas (Zereini et al., 2017). The determination of Pt concentrations in adult wild oysters (with sufficiently long exposure periods to integrate ambient conditions) seems to provide a promising tool for the assessment of seawater Pt levels. Monitoring of anthropogenic Pt impacted environments could include the sampling of native wild oysters, as well as, the use of caged organisms which is a particularly useful approach to determine site-point effects (e.g. close to a high traffic road, or hospital effluents...), and time-point pollution (e.g. road runoff...). This method gives a highly sensitive and rapid response (Marigómez et al., 2013) providing relevant information about chemical contaminants levels, especially trace metals, in seawater (e.g. Capolupo et al., 2017; Marigómez et al., 2013).

5. Conclusions and perspectives

Oysters (*C. gigas*) originating from an uncontaminated environment were exposed to dissolved ^{194}Pt spiked in seawater during a period of 35 days. Isotopically-labelled Pt spikes allowed for the determination of the dynamic Pt bioaccumulation between oysters at varying exposures, as well as, the determination of natural/initial Pt content and the Pt accumulated from isotopically-labelled spikes in the same individual. Results from an inter-method comparison using two completely independent digestion and measurement techniques show that Pt concentrations in natural oysters (not exposed to Pt spike addition) can be reliably measured by ICP-MS when sufficient sample mass (i.e. $>0.25 \text{ g}$ dry weight, 0.13 ng Pt) is available. This method can therefore be an alternative to AdCSV for detection of Pt in biological samples. Platinum accumulation kinetics at three Pt exposure concentrations (50, 100, and $10,000 \text{ ng} \cdot \text{L}^{-1}$) revealed that at environmentally relevant levels (50 and $100 \text{ ng} \cdot \text{L}^{-1}$) Pt accumulation may be controlled by excretion mechanisms, whereas at $10,000 \text{ ng} \cdot \text{L}^{-1}$ uncontrolled Pt accumulation and mortality is to be expected. Bioconcentration factors of ~ 500 determined experimentally for this exposure experiment, were somewhat lower than natural BCFs in wild oysters ($\sim 10^3$) consistent with the assumption that accumulation follows an asymptotic curve with an actual steady state being reached after longer exposure time. These findings suggest that according to the definition by Ruchter (2012), *C. gigas* is a good sentinel species for Pt in seawater at environmentally relevant levels since, this organism i) accumulates Pt without suffering mortality, ii) shows a high Pt BCF, and iii) a linear correlation exists between Pt content in the organism and Pt in ambient seawater. Therefore, the analysis of wild oyster Pt concentrations integrating and increasing the environmental signal has great potential to assess the local dissolved Pt levels in seawater. Future works should address toxicological effects in marine bivalves caused by Pt uptake at environmentally relevant exposure levels. Furthermore, given the strong and linear Pt accumulation observed in this study, future experiments should explore even lower exposure levels in order to get closer to environmental seawater Pt concentrations.

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The experimental study of Pt accumulation kinetics in Oysters (*C. gigas*) from an uncontaminated environment, exposed to dissolved ^{194}Pt spiked in seawater during a period of 35 days has produced the following outcomes:

From an **analytical perspective**, this study represents a successful application of Pt stable isotope spikes under laboratory conditions since:

- ✓ A clear distinction in the isotopic ratio ($^{194}\text{Pt} / ^{195}\text{Pt}$) was observed between non-exposed organisms and organisms exposed to the different concentrations (including the lowest), after only three days of exposure.
- ✓ Results from analyses of non-contaminated environmental samples by ICP-MS were cross-checked by applying AdCSV as a different, independent analytical method. The results suggest that sample masses above 250 mg (corresponding to at least 0.13 ng Pt in our samples) consistently allowed for reproducible and similar (< 3 % difference in average) results for both methods applied. Such convergence may indicate good accuracy, as classical accuracy measurements are impossible due to lacking suitable CRM.
- ✓ After signal interference corrections, the use of stable isotope spikes applying natural and enriched isotopic ratios, allowed the determination of natural/initial Pt content and the Pt accumulated in the same individual. The “natural” part of Pt concentrations of oysters exposed to environmentally-relevant Pt concentrations showed similar values compared to average concentrations obtained for non-exposed/control organisms. Such information is not possible to obtain with other analytical methods measuring total element concentrations, as often applied in exposure experiments (Mikolaczyk et al., 2016).

From an **ecotoxicological point of view**, the experiment provides reliable environmentally-relevant information on Pt bioconcentration in marine organisms.

- ✓ After only three days of exposure, all oysters had accumulated measurable amounts of Pt, including the series at lowest exposure levels. Accordingly, this result suggests a clear and rapid uptake of Pt since the first days of exposure which confirms the bioavailability of Pt from the dissolved phase, as already observed in freshwater bivalves (Ruchter, 2012; Sures and Zimmermann, 2007).

- ✓ At environmentally-relevant Pt concentrations, oyster tissue Pt levels increased following a linear pattern with time until 21 days of exposure. A plateau of accumulation (steady state) is reached after this time suggesting that metal uptake is compensated by excretion and efficient regulation processes (Singer et al., 2005).
- ✓ A linear relation exists between seawater Pt concentrations and oyster tissue Pt concentrations with a positive correlation ($R^2 > 0.99$) for oysters exposed to environmentally-relevant levels. This result supports the ability of oysters to serve as sentinel of Pt seawater concentrations in nature.
- ✓ Bioconcentration factors of ~ 500 were determined in those exposed oysters, which is lower compared to control oysters and wild bivalves (Abdou et al., 2016; Neira et al., 2015). This observation might be explained by a pseudo-equilibrium state that might differ from the environmental equilibrium including instable and complex natural parameters (in terms of hydrodynamics, geochemistry...) that are neglected in exposure studies (Casas et al., 2008).
- ✓ At higher exposure levels, no steady state was reached. Different uptake processes and kinetics may govern Pt accumulation in oysters. Oysters were not able to efficiently eliminate Pt from their tissues resulting in continuous accumulation. One oyster died in these exposure tanks and we cannot exclude that higher mortality would have occurred when continuing exposure under these experimental conditions.

3.3. Organotropism and biomarker response in oyster *Crassostrea gigas* exposed to platinum in seawater

Under the same exposure experiment, Pt distribution between the different organs was investigated. For this, oysters were dissected in six sub-samples being: muscle, gills, mantle, pure gonads, pure digestive gland, and a mix digestive gland + gonads. Monitoring of Pt organotropism may provide knowledge on Pt physiological response following Pt exposure in oysters. In addition Pt concentrations were also measured in excretion materials being the faeces. This assessment may help to understand if marine bivalves are actually activating detoxification mechanisms to face Pt exposure.

Except from the chemical determination of Pt levels in soft tissues and in excretion materials, living organisms might exhibit modification of biomarkers.

- At cellular levels: lipofuscin and neutral lipid contents were measured.

Lipofuscins are mainly produced due to lipid peroxidation; originating from poly-saturated fatty acids oxidation by Reactive Oxygen Species (ROS); and are accumulated in lysosomes as insoluble lipoprotein granules (Viarengo and Nott, 1993). Moreover, in marine bivalves, neutral lipids contribute to energy resources and help in the gamete development during maturation. Depletion of such material may occur following trace metal exposure and suggest that energy resources are required to face pollutant exposure (Séguin et al., 2016).

- At tissue level: histopathological alterations were monitored including digestive gland integrity.

Histopathological alterations include the presence of Black Silver Deposits (BSD). Black Silver Deposits reveal softly bound metals localized within cellular compartments (Danscher, 1991; Soto et al., 2002). Lysosomes in the digestive cells, which represent target sites for many contaminants (Cajaraville et al., 1995; Izagirre and Marigómez, 2009), were also monitored.

- At the organism level: the IBR indices were determined.

The Index of Biological Response (IBR) has been successfully applied to exposed sentinel bivalve species in order to assess their health status (Brooks et al., 2011; Cravo et al., 2012; Garmendia et al., 2011; Rementería et al., 2016).

Overall, the objective of this second study is **to evaluate Pt accumulation effects including the role of the different organs and the physiological effects in marine organisms such as oysters**. Results from this study are ready for submission in the peer-reviewed journal *Environmental Science and Pollution Research*.

Organotropism and biomarker response in oyster *Crassostrea gigas* exposed to platinum in seawater

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Abstract

Platinum (Pt) is a Technology Critical Element (TCE) for which biogeochemical cycles are still poorly understood. This lack of knowledge includes Pt effects on marine organisms, which proved to be able to bioconcentrate this trace element. Oysters *Crassostrea gigas* were exposed to stable Pt isotope spiked daily in seawater for 35 days. Organotropism study revealed that gills, and to a lesser extent mantle, are the key organs regarding Pt accumulation, although a time- and concentration-dependent linear increase in Pt levels occurred in all the organs investigated (i.e. digestive gland, gonads, gills, mantle, and muscle). In oysters exposed to Pt concentrations of 10,000 ng.L⁻¹, significant biomarker impairments occurred, especially at cellular levels. They reflect altered lipofuscin and neutral lipid contents, as well as intralysosomal metal accumulation. These observations were attributed to activation of excretion / detoxification mechanisms, including Pt elimination through faeces and clearly support the importance of the digestive gland in the response to direct Pt exposure.

Keywords: Platinum; Isotopes; Seawater; *Crassostrea gigas*; Organotropism; Biomarker; Ecotoxicology

1. Introduction

The Platinum Group Elements, including platinum (Pt) and five other rare metals, belong to the Technology Critical Elements (TCEs), considered as elements of great relevance in the development of emerging key technologies (Cobelo-García et al., 2015). The extensive use of TCEs has led to a global disturbance of their natural geochemical cycles with anthropogenic fluxes being dominant over natural fluxes (Sen and Peucker-Ehrenbrink, 2012). Increasing Pt concentrations in the environment have been attributed to various anthropogenic sources such as industries, anti-cancer drugs and, more importantly, car catalytic converters (Moldovan et al., 2002). Catalytic car exhaust transformation is an efficient green key technology, but goes along with the emission of Pt along the roadsides, resulting in elevated Pt concentrations in many environmental compartments. Maximum Pt contamination occurs in highly populated and urbanized areas, but anthropogenic Pt anomalies are also recorded in very remote sites (e.g. polar ice, Soyol-Erdene et al., 2011). Only little information on Pt concentrations in natural water bodies is available, but field observations and laboratory exposure studies have shown Pt uptake and bioaccumulation by different aquatic organisms such as annelids, crustaceans, bivalves, snails and various fish species (e.g. Zimmermann and Sures, 2018). The small number of field studies include data on Pt concentrations in bivalves, such as freshwater clams, as well as seawater mussels and oysters (Ruchter and Sures, 2015; Neira et al., 2015; Abdou et al., 2016, respectively). Those widely distributed organisms are sedentary, easy to sample and continuously available throughout the year (Zimmermann and Sures, 2018). Considering their high potential for metal bioconcentration, bivalves are preferentially used in biomonitoring programs reflecting trace metal contamination over decades (e.g. Mussel Watch Program; Goldberg et al., 1978). Ideal sentinel organism accumulate contaminant without suffering mortality and showing high accumulation capacity (Ruchter, 2012). Literature reports that a couple of adverse effects including deleterious effects at molecular, organelle, and tissue/organ levels occurring in aquatic organisms following Pt exposure under laboratory conditions. Physiological adverse effects include the induction of heat shock proteins (hsp70, stress conditions; Singer et al. 2005) or tissue alteration (e.g. cytoplasm vacuolization; Osterauer et al., 2010). Although such exposure studies provide valuable knowledge, test organisms are generally exposed to relatively high Pt concentrations exceeding by far environmental levels (Sures et al., 2015). Previous work has reported on short-term Pt bioaccumulation kinetics in oysters (*Crassostrea gigas*) exposed to isotopically-labelled Pt (^{194}Pt) in seawater covering a wide concentrations range, as an approach to environmentally relevant levels (Abdou et al.,

2018). The present work introduces results from the same experiment, focusing on Pt organotropism and toxicological effects in oysters using a battery of cell and tissue level biomarkers and their integration into the Integrative Biological Response Index (IBR Index) for environmental health assessment (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006).

The objectives are to assess Pt effects at i) cellular level, with the measurement of lipofuscin and lipid contents; ii) tissue level, including quantification of histopathological alterations and digestive gland integrity; and iii) organism level, in light of Pt distribution in the different organs and possible excretion through faeces. Accordingly, this work aims at characterizing Pt accumulation/distribution and the related physiological effects in marine organisms such as oysters.

2. Material and Methods

Experimental setup as well as sampling protocols and analyses for Pt quantification in soft tissues were already described in a previous paper (Abdou et al., 2018). They are briefly summarized in the following sections, together with the description of protocols for histological and biomarker studies.

2.1. Experimental setup

Stock isotopic solution was prepared using solid metal shavings of ^{194}Pt (116.5 mg; Cortecnet®) which were acid digested using 4 mL of concentrated HCl and 2 mL of concentrated HNO_3 (both Suprapur®, Merck) heated up at 110 °C for 4 h. The stock solution was diluted using ultrapure water (MilliQ®) and ^{194}Pt final concentration ($532 \pm 1 \text{ mg.L}^{-1}$) was quantified through Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo® X Series II; n = 5).

Clean oysters (*Crassostrea gigas*, n= 545) obtained from a commercial seller (“OSTRANOR S.L.”, San Vicente de la Barquera, Cantabria, Spain), with similar shell length ($\sim 90 \pm 5 \text{ mm}$) were transported to the Plentzia Marine Station of the University of the Basque Country (PiE-UPV/EHU) to perform exposure experiments under controlled conditions. Prior the beginning of exposure, oysters were maintained in naturally filtered seawater in an open flow system (12 days). Exposure tanks were equilibrated spiking the respective nominal total Pt concentrations in seawater (12 days, daily renewal). The different tank conditions namely A, B, C, and D consisted in: control tanks, where no Pt was spiked; and exposure tanks with Pt spikes of 50 ng.L^{-1} Pt; 100 ng.L^{-1} Pt; and $10,000 \text{ ng.L}^{-1}$ Pt respectively. Literature reports that

environmental concentrations range from $\sim 0.1 \text{ ng.L}^{-1}$ in relatively clean coastal environments (Cobelo-García et al., 2014a) to $\sim 7 \text{ ng.L}^{-1}$ Pt in contaminated areas (Obata et al., 2006). Therefore, as explained by Abdou et al. (2018) in a previous paper, the different concentrations were chosen in order to cover a range of concentrations, from environmentally relevant (B and C conditions) to relatively high exposure levels (D conditions). Replicate of each condition (in separate tank) were performed and mentioned 1 and 2 (e.g. A1 and A2).

Before starting Pt exposure, we isolated and dissected twenty-five oysters which constitute the $T = 0$ (initial conditions) for the targeted parameters. Thereafter, we performed an even distribution of the 520 individuals in eight 45 L polypropylene (PP) tanks (65 individuals per tank) which were at first filled with 40 L aerated seawater. The experimental room was temperature-controlled (17°C) and equipped with an artificial photoperiod set up on a 12 h : 12 h light : dark cycle. Chemical and physical parameters including temperature, salinity and pH were measured daily during the experiment using a TetraCon 96® and a Sentix ® 41 probes (PROFILINE, WTW®). On a daily basis and after mortality checking, oysters were rinsed, fed and transfer in clean separate tanks while exposure tanks were rinsed, filled up with clean seawater and spiked to nominal Pt concentrations using ^{194}Pt stock solution. After 4 h feeding and spike equilibration, oysters were transferred again in the control and exposure tanks. Seawater, spike, and food volumes were adapted according to the number of oyster organisms left after each dissection in order to maintain exposure and feeding conditions constant by individual.

2.2. Sampling protocols

Organotropism analyses were performed on oysters from tanks D, sampled at $T = 3$, $T = 7$, $T = 28$, and $T = 35$. Histological and cellular studies were performed on oysters from each exposure condition at $T = 3$, $T = 7$, and $T = 28$. At time of sampling, five individuals per tank (pseudo-replicates) were sampled for both types of analysis. Oysters were opened, water remaining inside the shell was removed, and soft body rinsing was performed using uncontaminated seawater.

For organotropism analyses, organs (gills, digestive gland, gonads, muscle, and mantle) were dissected and placed separately in acid-cleaned (10 % HNO_3 ; 65 % Honeywell), PP tubes (DigiTUBEs®, SCP SCIENCE). Gonads could not be systematically isolated from digestive glands. Therefore, pieces of pure digestive gland and the remaining digestive gland and gonads were mineralized and analyzed separately. Wet weight of each organ was determined. In addition, each valve was weighed and measured (thickness, width, and length), allowing the

determination of the Condition Index (CI) calculated for each oyster according to the equation (1) from Strady et al., (2011):

$$CI = \text{Flesh weight (wet weight; g)} / \text{Shell weight (wet weight; g)} \times 100. \quad (1)$$

As CI has been already discussed in Abdou et al. (2018), no results are presented herein but the CI data have been used in the integrative biological response index (IBR, see section 2.4.). After dissection, soft tissues were deep-frozen (- 80 °C). Tissues were thereafter freeze-dried, crushed in an agate mortar, and stored at room temperature, in the dark, until Pt quantification.

For histological analyses, a ~ 5 mm thick cross-section of the soft body was performed using a sharp blade. This section included main organs and tissues (digestive gland, gills, and gonad) of the oysters. Sections were immersed in seawater buffered with formalin and kept at 4 °C for 24 h. Samples were rinsed with ethanol (70 %), processed in the tissue processor (Leica ASP300®), finally embedded in paraffin (Leica Surgipath® Paraplast) and sectioned in a microtome (Leica® RM2125 RTS). Sections of 5 µm were placed in a microscope slide and were stained for histopathology and autometallography analyses. Histochemical analyses were obtained from the same tissue samples: digestive glands were excised, put in cryovials, and frozen in liquid nitrogen rapidly. We kept the frozen samples at – 80 °C pending sectioning and staining as described in section 2.3.3.

In addition to dissections, faeces were collected four times (T = 7, 21, 28, and 35) during the experiment in each feeding tank (uncontaminated seawater in order to avoid Pt adsorption on faeces). Sampling was performed using a Pasteur syringe. All visible faeces at time of collection were sampled but this sampling cannot be considered as fully quantitative since we might miss some material excreted later on. Samples were collected in a Teflon bottle, filtered on pre-weighed Teflon filters (47 mm, 0,45 µm FHLC filter, Merck Millipore), deep-frozen (- 80 °C), then oven-dried at 50 °C, and stored in sealed containers pending analysis.

2.3. Analytical procedure

2.3.1. Determination of Pt concentrations

Soft tissues of organ were prepared for ICP-MS Pt quantification as described in Abdou et al., 2018. The steps consisted in ashing (800 °C) followed by acid digestion using *aqua regia* (3 h at 110°C). Same procedure (without the ashing step) was applied to faeces collected on Teflon filters. We applied the standard addition method (mono-elementary Pt standard solution 1,000 µg.mL⁻¹ PLASMACAL, SCP Science). Spectral interference (hafnium-oxygen species) corrections were applied on the ¹⁹⁴Pt and ¹⁹⁵Pt isotopes as detailed in Abdou et al. (2018). According to an inter-method comparison between ICP-MS and Adsorptive Cathodic Stripping Voltammetry (AdCSV) methods, sufficient sample mass (i.e. > 0.25 g dry weight, 0.13 ng Pt) is necessary to reliably measure Pt concentrations in natural oysters (not exposed to Pt spikes) by quadrupole ICP-MS (Abdou et al., 2018). As for organotropism measurements, organ masses were generally too low (e.g. ~ 0.1 g for digestive gland) to allow for ICP-MS analyses in non-exposed oysters, these samples were analyzed by AdCSV as described in Abdou et al., (2018). Briefly, after ashing, samples were acid-digested using *aqua regia* (1 h at 195 °C), followed by an evaporation step and dissolution with sulfuric acid (H₂SO₄). Samples are thereafter evaporated again and diluted with hydrochloric acid (HCl). Platinum voltammetric quantification was performed using a µAutolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm® 663 V.A.) and controlled using the NOVA 2.1 software. Aliquots of acid-digested sample were added to the voltammetric cell, together with formaldehyde and hydrazine sulfate as well as H₂SO₄ and concentrations of Pt were determined applying the standard addition method and considering the second derivative of the voltammogram (Cobelo-García et al., 2014b).

2.3.2. Histopathological alterations

In order to assess histopathological alterations, paraffin embedded tissue sections were stained with haematoxylin-eosin (Gamble and Wilson, 2002) using a stainer integrated workstation (Leica® ST5010 Autostainer XL; Leica® CV5030). Microscopic slide observations were performed under a light microscope (Olympus BX-61).

Connective-to-diverticula (CTD) ratio was quantified in order to assess the integrity of the digestive gland tissue. Five randomly selected fields of digestive gland were acquired per individual using a digital camera coupled to the microscope. Images were processed through Image J program (Image Processing and Analysis in Java, Maryland, USA) as described in

Rementeria et al. (2016). The CTD ratio is the area (extent) occupied by the interstitial connective tissue relative to the area occupied by the digestive diverticula. This ratio was obtained using the following equation (2) from Brooks et al. (2011):

$$\text{CTD ratio} = c / (b + d + l) \quad (2)$$

With: (c) interstitial connective tissue, (b) basophilic cells, (d) digestive cells and (l) diverticular lumen.

Atrophy of the digestive tubules was calculated using a semi-quantitative scale according to Kim et al. (2006), with values in a range from 0 (no atrophy) to 4 (severe atrophy in most tubules).

2.3.3. Histochemical analyses

In order to determine the lipofuscin content in the digestive gland, sections of 8 µm thickness corresponding to the digestive gland were obtained using a cryostat with a – 26 °C chamber temperature. Frozen samples were stained according to the Schmorl method (Pearse, 1985). Five randomly selected pictures of each sample were acquired using a digital camera coupled to a microscope (Leitz Laborlux S, Leica Microsystems, Wetzlar, Germany). Using the BMS (Biological Measurement System) program, lipofuscins were optically segmented. Using the SPSS/PC program, the relative proportion between lipofuscin surface and the remaining extent of the digestive tissue was determined (Rementeria et al., 2016; Zorita et al., 2006).

For neutral lipid content determination, frozen 8 µm sections of digestive gland were similarly obtained using the cryostat at – 26 °C. Samples were thereafter processed following the ORO (Oil Red O) staining method (Culling, 1974). Image analysis was performed following the same method as aforementioned for lipofuscin content but applied to neutral lipid (Marigómez et al., 2013, Rementeria et al., 2016).

For autometallographical staining, a 5 µm thick sections of paraffin embedded samples were dewaxed in xylene, re-hydrated and dried at 37 °C for 24 h. Samples were thereafter covered using the commercial silver enhancement kit (Silver Enhancement Kit, BBI Solutions) at room temperature (in the dark) and reaction was stopped after 20 min. Slides were washed using distilled water (Elix®) and mounted in Kaiser's gelatin-glycerin (Merck®). Metals, including Pt, appeared as Black Silver Deposits (BSD). Those deposits were quantified using the same method as for previous histochemical parameters, and measured as volume density of BSD (V_{VBSD} ; Soto et al., 2002).

2.4. Integrative Biological Response (IBR/n) index

Integrative Biological Response (IBR) index was calculated according to Devin et al. (2014), based on the method by Beliaeff and Burgeot (2002). Five biomarkers, at different biological organization levels, were used for the index calculation (lipofuscin content, neutral lipid accumulation, atrophy, CTD ratio and Condition Index). Since the IBR value depends on the number of applied biomarkers, the IBR/n value presented in the results section was obtained dividing the IBR by 5 (Brooks et al., 2011; Marigómez et al., 2013).

2.5. Quality control and statistics

We analyzed the only available Certified Reference Materials (CRMs) for Pt: the Jsd-2 sedimentary rocks (indicative value from Geological Survey of Japan) and the BCR®-723 road dust (Institute for Reference Materials and Measurements). The ICP-MS analysis of these CRMs provided satisfactory recoveries of 101 % and 87 %, respectively ($n = 3$). They were also quantified through AdCSV providing satisfactory recovery of 98 % for Jsd-2 and 89 % for BCR®-723 ($n = 3$). The detection limit for Pt measured in biological samples by ICP-MS ($3 \times$ blank standard deviation, $n = 10$) ranged between 0.06 and 0.006 ng.g^{-1} for typical sample mass comprised between 0.1 and 1 g, while it is of 0.01 ng.g^{-1} for sample mass of 0.1 g analyzed through AdCSV.

To evaluate significant differences (p -values < 0.05) in (i) physical-chemical parameters and (ii) Pt concentrations between the different sampling times and the different organs, we performed one-way ANOVA tests for parametric data and we applied Kruskal-Wallis tests for non-parametric data. In addition, we checked for the homoscedasticity of the data applying Bartlett test. Pairwise post-hoc tests were carried out (Mann-Whitney or Holm-Bonferroni).

For histological measurements, statistical analyses were performed using the SPSS v 23.0 (IBM®). Data normality and homogeneity (Kolmogorov Smirnov and Levene's tests) were checked and analyses of variance and Duncan's post hoc test were carried out in order to determine significant differences between conditions ($p < 0.05$). Kruskal-Wallis tests and Dunns post hoc test were applied to non-parametric data.

3. Results

3.1. Platinum accumulation kinetics in the different organs

Temporal aspects

Platinum concentrations determined separately in each organ from non-exposed organisms ($T = 0$) and from exposed oysters ($T = 3$ to $T = 35$), covered several orders of magnitude (Fig. 1; Table 1). For oysters exposed to isotopically-labelled Pt, both replicate series gave similar Pt concentration evolution through time for each organ studied, showing a steep increase in concentrations throughout the experiment (Fig. 1). Determination coefficients (R^2) revealed a significant linear accumulation of Pt over time with values > 0.9 in each organ (Table 1). All Pt concentrations in oysters exposed to Pt spikes throughout the experiment (from $T = 3$) were statistically different ($p < 0.002$) from those in non-exposed oysters. In fact, soft tissues showed Pt concentrations at least 100 times higher than initial conditions after only three days of exposure ($T = 3$; Table 1). At the end of the experiment ($T = 35$), Pt concentrations in muscle were $\sim 2,000$ -fold higher than at $T = 0$ (Table 1). For digestive gland, gonads and mantle final Pt values were $\sim 20,000$ -fold and in digestive gland + gonads $\sim 50,000$ -fold greater than at $T = 0$. The greatest increase occurred in gills where Pt concentrations were $\sim 65,000$ -fold higher at $T = 35$ (Table 1). For most exposure times and organs, results were significantly different ($p < 0.05$) from the previous sampling time (values labelled * in Fig. 1). Note that, for all the organs studied, no significant differences were observed between $T = 28$ and $T = 35$ for both replicate series.

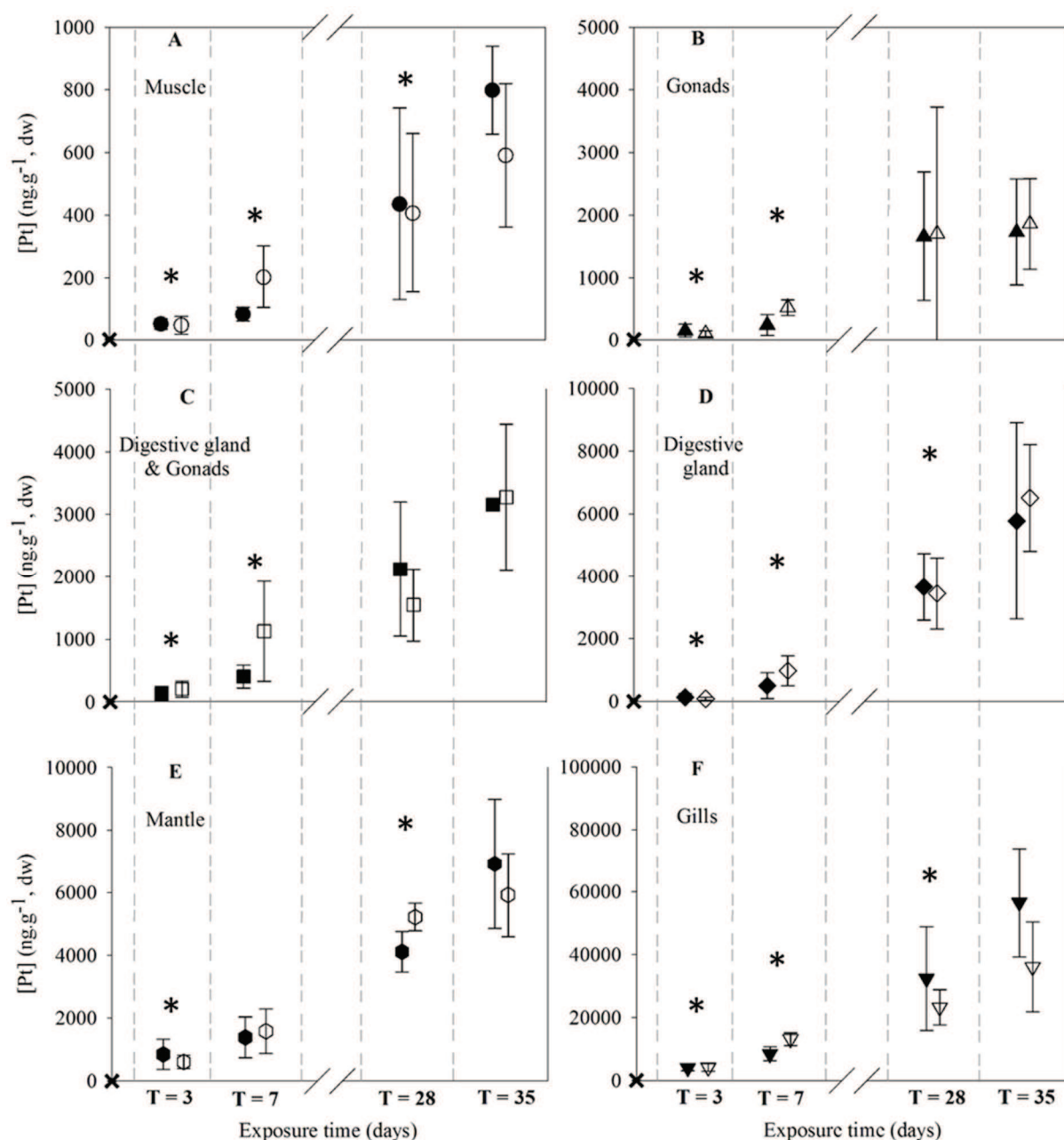


Fig. 1 Platinum concentrations in the different organs throughout the exposure period. Organ average Pt concentrations ($n = 5$, dry weight) for each replicate series of the tanks D (replicate 1: full symbols, replicate 2: empty symbols). Cross symbols represent initial Pt concentrations in organs from oysters at $T = 0$ (i.e. non-exposed individuals; $n = 5$) for each organ. Note the discontinued scale of the time axis. Error bars represent standard deviation (SD) and asterisks (*) significant difference from the previous sampling time.

Organ comparison

At initial conditions, differences of Pt concentrations between muscle and digestive gland and between muscle and mantle were non-significant although digestive gland + gonads and gonads alone tended to show the lowest Pt concentrations (Table 1). Whole organism Pt concentrations have been estimated from Pt values in the different organs and considering the respective organ masses. Platinum distribution between organs expressed as average percentage ($n = 10$) with respect to total organism Pt content (Fig. 2) showed that at initial conditions gills contained $\sim 40\%$ of the total Pt content, whereas muscle and mantle had similar percentages of $\sim 20\%$. The other organs, digestive gland + gonads, and gonads and digestive gland alone showed similar values of $\sim 7\%$.

After exposure, gills generally showed the highest Pt concentrations, while similar Pt concentrations occurred in muscle, mantle, and digestive gland. At all exposure times, significant differences occurred between mantle and the other organs as well as between gills and the other organs. Platinum concentrations in exposed oysters followed the general trend: gills > mantle > digestive gland \sim digestive gland + gonad > gonads > muscle (Table 1). Compared to estimated whole organism concentrations, gills reached the highest percentage of Pt of up to 70 % at all times (Fig. 2). Platinum percentages in muscle dropped from $\sim 20\%$ to $\sim 3\%$ and remained stable. The percentages of all other organs showed only minor variations compared to initial conditions and throughout the exposure period. The lowest Pt contribution occurred in digestive gland at $T = 3$ with only $\sim 0.5\%$. The comparison of “reconstituted” whole organism Pt concentrations with those measured in whole individuals from the same experiment, analyzed without organ dissection (Abdou et al., 2018) showed overall good fit of both datasets (less than 20 % difference), confirming the previously observed linear trend in Pt accumulation with high coefficients of determination for whole oysters from tanks D1 ($R^2 = 0.94$) and D2 ($R^2 = 0.97$).

Table 1 Platinum concentrations in the different oyster organs and in faeces. Mean Pt concentrations (ng.g⁻¹, dry weight) in organs of oysters from both replicate series of tanks D (n = 10); platinum concentrations determined in oyster faeces from each replicate. *n.d.*: not determined; R²: determination coefficients determined from linear regressions of time-dependent Pt concentration increase in each organ.

	T = 0	T = 3	T = 7	T = 21	T = 28	T = 35	R²
Muscle	0.289 ± 0.127	48.1 ± 23.2	141 ± 93.9	<i>n.d.</i>	421 ± 282	683 ± 220	0.967
Gonads	0.090 ± 0.030	117 ± 82	372 ± 204	<i>n.d.</i>	1,680 ± 1,610	1,800 ± 781	0.988
Digestive gland + gonads	0.083 ± 0.027	184 ± 128	766 ± 681	<i>n.d.</i>	1,870 ± 933	4,090 ± 2,280	0.948
Digestive gland	0.341 ± 0.053	113 ± 100	712 ± 503	<i>n.d.</i>	3,540 ± 1,110	6,460 ± 2,500	0.960
Mantle	0.313 ± 0.027	731 ± 388	1,490 ± 681	<i>n.d.</i>	4,660 ± 781	6,350 ± 1,760	0.993
Gills	0.677 ± 0.006	4,090 ± 1,120	10,800 ± 3,140	<i>n.d.</i>	28,300 ± 13,600	45,200 ± 18,700	0.961
Faeces A1- A2	<i>n.d.</i>	<i>n.d.</i>	< 0.285 - 3.25	0.498 - 3.59	<i>n.d.</i>	1.63 - <i>n.d.</i>	
Faeces B1- B2	<i>n.d.</i>	<i>n.d.</i>	27.4 – 51.4	33.2 - 55.7	23.4 – 34.5	14.3 - 44.0	
Faeces C1- C2	<i>n.d.</i>	<i>n.d.</i>	77.1 - 58.6	82.0 - 140	49.3 – 91.3	61.7 - 90.9	
Faeces D1- D2	<i>n.d.</i>	<i>n.d.</i>	7,080 – 7,850	44,000 - 38,400	<i>n.d.</i>	10,000 – 20,400	

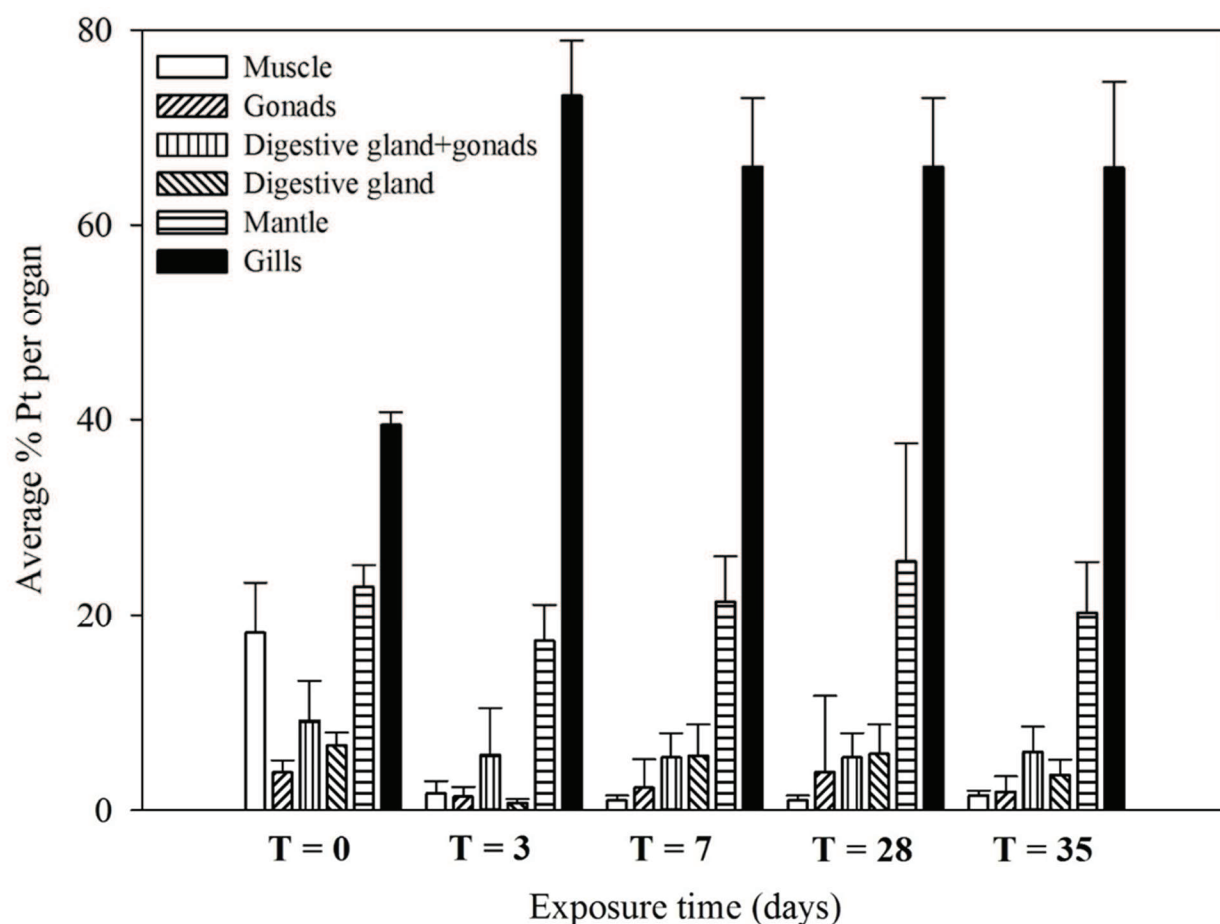


Fig. 2 Evolution of Pt distribution between the different organs. Average Pt percentage per organ (from both replicates; $n = 10$) with respect to total organism Pt content. Error bars represent standard deviation (SD).

3.2. Platinum concentrations in faeces

Platinum concentrations in faeces were monitored through the exposure period. Control tanks showed very low, similar Pt concentrations along the experiment ($\sim 1.1 \text{ ng.g}^{-1}$ for A1 and $\sim 3.4 \text{ ng.g}^{-1}$ for A2; Table 1). Faeces from oysters exposed to Pt showed clearly higher Pt concentrations, reflecting the order of exposure levels in the different tanks, but not the proportions of exposure concentrations. Values between replicate series were similar. Faeces did not display a clear trend over time. In fact, at $T = 7$ oysters faeces showed mean Pt concentrations of $\sim 40 \text{ ng.g}^{-1}$ in tanks B, $\sim 70 \text{ ng.g}^{-1}$ in tanks C, and $\sim 7,000 \text{ ng.g}^{-1}$ in tanks D. After 21 days of exposure, similar Pt concentrations are determined for tanks B while slightly higher Pt concentrations occurred in oyster faeces from tank C. Yet, at this sampling time, faeces mean Pt concentrations in tanks D reached $\sim 40,000 \text{ ng.g}^{-1}$. At the following sampling times, there was a slight decrease in Pt concentrations in faeces from tanks B, no major change for tanks C, and a clear decrease for those from tanks D. Accordingly, at the final sampling time

(T = 35), faeces showed Pt concentrations of ~ 30 and 80 ng.g^{-1} in tanks B and C respectively, and $\sim 15,000$ in tanks D (Table 1).

3.3. Histopathological alterations

Histological observation of the digestive gland indicated the presence of some minor histopathological alterations such as the presence of ciliated organisms, *Mytilicola intestinalis* or the presence of necrotic tubules and haemocytic infiltrations. However, prevalence and intensities were in general low and did not present a clear pattern regarding differences between control and exposed groups. These results are in agreement with tissue level biomarkers (CTD ratio and atrophy levels) for which no significant differences between experimental groups occurred, but rather modest trends were observed. The atrophy level at T = 0 was 1.93 ± 0.78 , while the highest level was recorded in group D after 28 days of exposure with a value of 2.68 ± 0.51 . Similarly, for CTD ratio no significant differences occurred between groups and time but dose-response trends occurred after 7 days of exposure with values ranging from 0.158 ± 0.05 in oysters from tanks A, to 0.181 ± 0.04 in the oysters from tanks D. At T = 21, CTD ratio varied from 0.175 ± 0.05 in control individuals while it was of 0.199 ± 0.04 in tanks D (Fig. 3a, b).

Concerning histochemical measurements, lipofuscins were generally detected in the digestive epithelium as dark spots (Fig. 3c, d). In general a significant increase in the lipofuscin content was observed in oysters from tanks C and D from T = 7 onwards (Fig. 4a) with values higher than control (tanks A) and tanks B oysters. It should also be noted that even in control oysters, a modest increase of lipofuscin content was measured after 28 days of exposure (Fig. 4a).

Neutral lipids in the digestive gland were observed as red deposits mainly located in the interstitial connective tissue of the digestive gland of oysters (Fig. 3e, f). The quantification of the neutral lipid accumulation indicated a significant decrease of lipids through time in oysters from tanks C and D (Fig. 4b), with more marked differences at longest exposure periods.

Metals were detected as Black Silver Deposits (BSD) and were mainly located in the gills, stomach, intestine, haemocytes and, to a lesser extent, in the digestive epithelium (Fig. 3g, h). The quantification of the volume density of BSD could only be performed in oysters from tanks C and D but not in tanks A and T = 0, nor in tanks B due to very low accumulation (Fig. 4c). A significant increase in the BSD was observed in oysters from tanks D compared to those from tanks C and these differences increased through time. Differences mainly occurred in the basal lamina at the beginning of the experiment and increased in digestive tubules with exposure time.

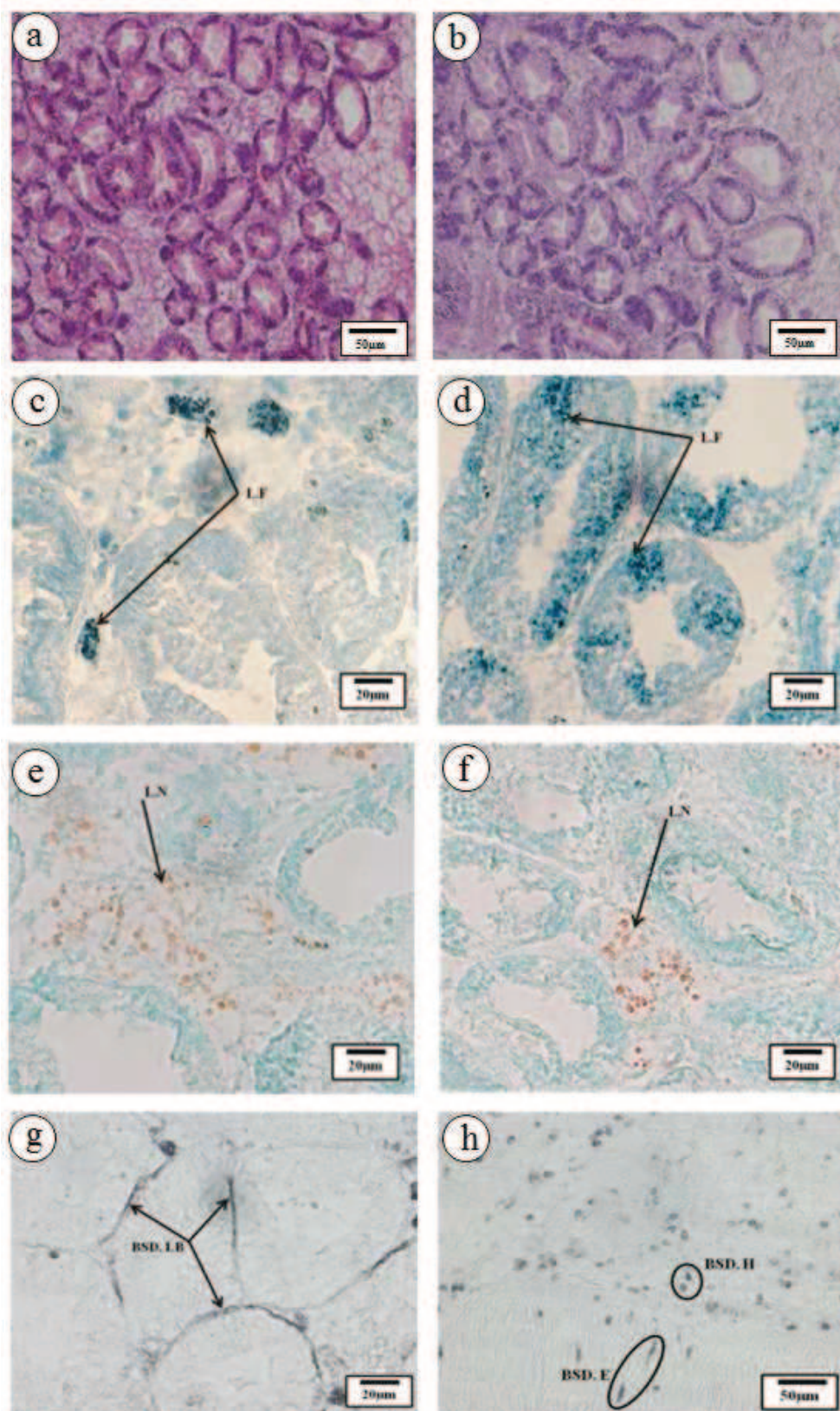


Fig. 3 Microscopic observations of the digestive gland of oysters from initial condition $T = 0$ (A), and control tanks A (G), exposed organisms at $T = 28$ for tanks B (C and E) and tanks D (B, D, F and H). Hematoxylin-Eosin staining (A and B), Lipofuscin Schmorl staining (C and D), ORO staining (E and F) and autometallographical staining (G and H), (LP) Lipofuscin; (LN) Neutral Lipids, (BSD) Black Silver Deposits in the Basal Layer (LB), epithelium of the digestive tract(E) and hemocytes (H).

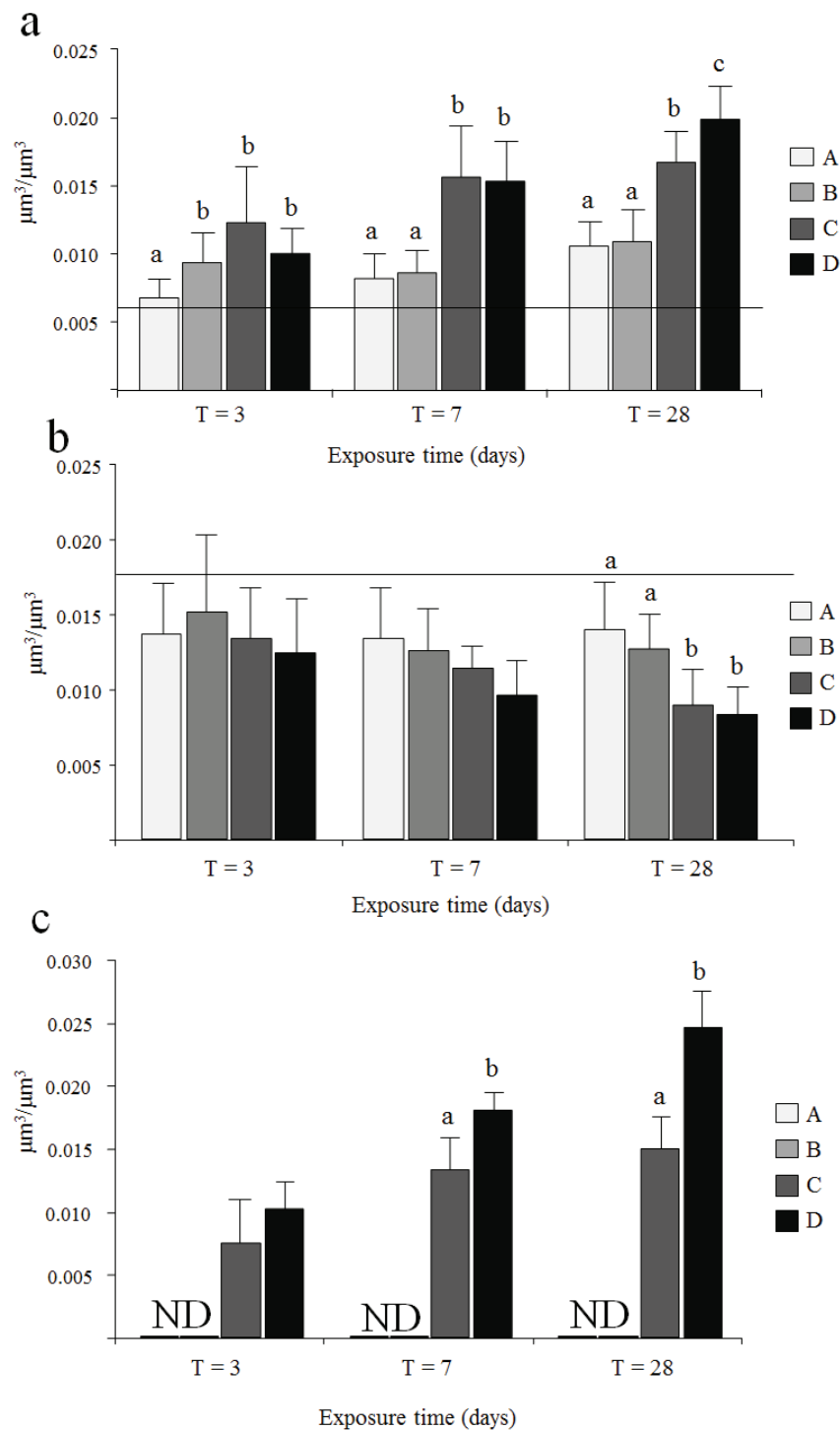


Fig. 4 Lipofuscin contents in digestive cells (A), Neutral lipid accumulation (B) and Intralysosomal metal accumulation (V_{VBSD}) (C) in oysters from the different tanks after 3, 7 and 28 days of exposure. The horizontal line indicates values at T = 0. Letters on top of the bars indicate significant differences between groups at the same exposure-time ($p < 0.05$), ND: not detectable.

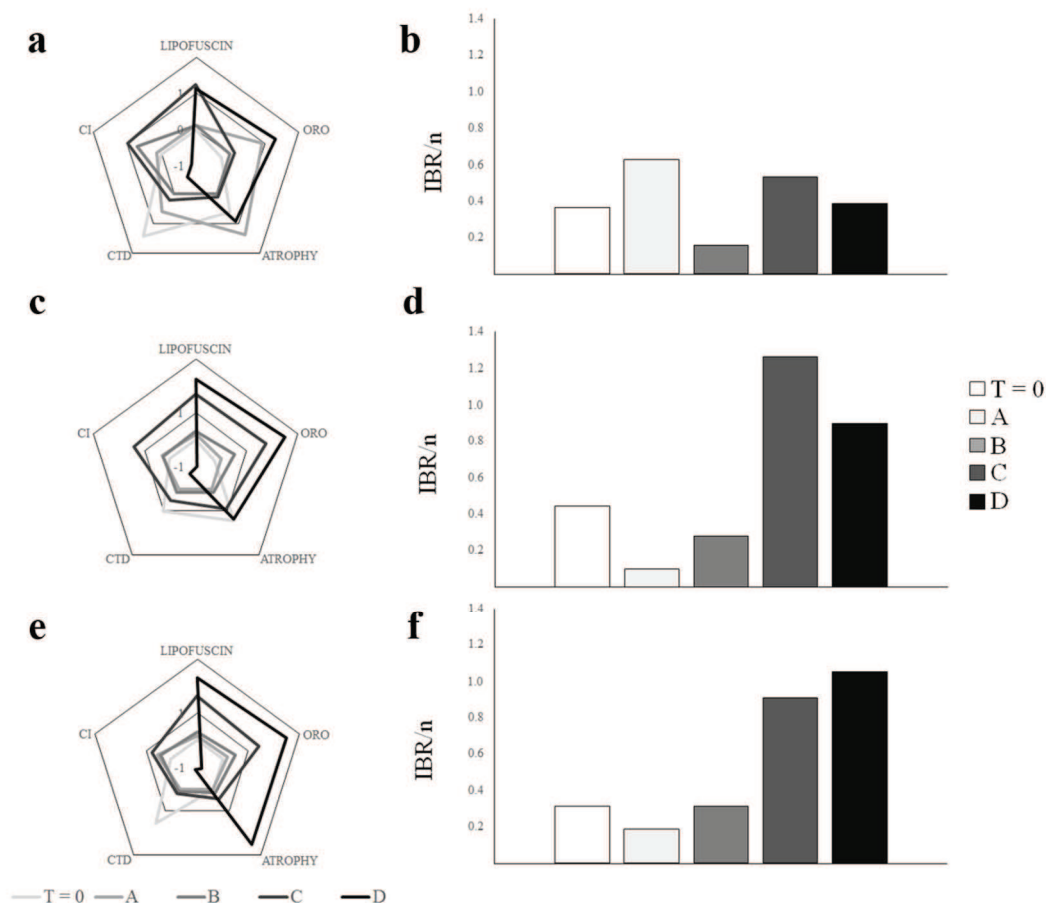


Fig. 5 Integrative Biological Response Index (IBR) star plots and IBR/n values determined at T = 3 (A and B respectively) T = 7 (C and D respectively) and T = 28 (E and F respectively).

The IBR index showed different response patterns after 3, 7 and 28 days of exposure. After 3 days of exposure no clear trends were observed, with all groups presenting similar values in studied responses (Fig. 5a, b). However, after 7 and 28 days of exposure, oysters from tanks C and D showed higher IBR/n levels, mainly due to alterations at the cellular level (lipofuscin and neutral lipids contents) indicating an altered health status (Fig. 5c - f).

4. Discussion

4.1. Platinum accumulation and organ distribution in oysters

During the whole exposure period abiotic factors were closely monitored, showing: i) constant physical/chemical master variables (temperature, pH, salinity, dissolved oxygen concentration), ii) constant Condition Index, iii) “natural”, constant dissolved Pt concentrations in control tanks ($\sim 0.25 \text{ ng.L}^{-1}$), iv) nominal spiked dissolved Pt concentrations in exposure media (Abdou et al., 2018). This setup supports the idea that the observed Pt accumulation and related biomarker responses were due to direct Pt exposure of the test organisms.

Initially, gonads had the lowest Pt load ($\sim 5 \%$), followed by digestive gland and digestive gland + gonads ($\sim 8 - 10 \%$ each), muscle and mantle accounting each for $\sim 20 \%$ and gills with the highest percentage ($\sim 40 \%$; Fig. 2). This distribution pattern in oysters from the Cantabrian coast is somewhat different from that observed in an organotropism study in wild oysters from the Gironde Estuary. In fact, in the Gironde oysters, Pt was evenly distributed between mantle, gills and the visceral mass (including the digestive gland) accounting for $\sim 30 \%$ each. The lowest contribution was reported for muscle ($\sim 10 \%$; Abdou et al., unpublished data). Although Pt quantification in whole oyster individuals from the Cantabrian coast showed similar concentrations ($0.24 \pm 0.07 \text{ ng.g}^{-1}$, $n = 10$) than those from the Gironde Estuary ($0.33 \pm 0.03 \text{ ng.g}^{-1}$, pool of 5 individuals; Abdou et al., unpublished data), an apparent shift towards relatively higher Pt content occur in the digestive gland of the Gironde oysters. This observation is in line with major accumulation of Ag and Cu in wild oysters from the same site, highlighting the importance of this organ in environmental metal exposure (Mikolaczyk et al., 2016). This shift could be explained by differences between both sites in terms of Pt sources, speciation and/or uptake pathway including a possible greater importance of the trophic pathway in the Gironde Estuary.

An increasing trend of Pt concentrations in all organs, both replicate series giving similar values, suggests continuous Pt accumulation in the test-organisms (Fig. 1). Interestingly, both replicate series displayed higher variability in Pt concentrations between the pseudo-replicate individuals from the same tank ($n = 5$) after longer exposure time. This result suggests that inter-individual variability in Pt uptake and distribution may increase with exposure time. This observation is in line with increasing inter-individual variability of several parameters at all biological organization levels following exposure to pollutants including trace metals (e.g. Cd exposure, Depledge and Lundebye, 1996). Such changes in variability of physiological parameters may provide additional useful insights into chemical contamination impact, i.e. serve as a tool to assess the severity of pollution effects on biota (Depledge and Lundebye,

1996), suggesting increasingly adverse effects of Pt exposure during the experiment. However, these variabilities do not change the trend of linear accumulation of Pt in each of the different organs studied (average determination coefficient $\sim 0.97 \pm 0.02$; Table 1). This reflects the accumulation dynamics observed at the whole organism level in a separate set of oyster individuals under the same experimental conditions (Abdou et al., 2018). Accordingly, the obtained results suggest very efficient Pt uptake and accumulation by direct exposure pathway. Accumulation trends close to linearity following Cd exposure were reported for *Corbicula fluminea*, at both the whole organism level and at the organ level (gills, mantle, foot, and visceral mass; Baudrimont et al., 1997). Linear accumulation over time occurs when excretion of metal is negligible or slow compared to the uptake, leading to continuously increasing body burdens during exposure (Langston and Bebianno, 1998). It is assumed that metal-binding ligands do not become saturated or that the growth rate does not exceed the metal assimilation rate, therefore steady-state may take a considerable time to be reached (Langston and Bebianno, 1998).

The Pt distribution between organs, expressed as % Pt content with respect to total organism Pt load, showed a clear shift between the initial conditions and after 3 days of exposure (Fig. 2). This shift is particularly important for gills, containing $\sim 40\%$ of whole soft body Pt at $T = 0$ and $\sim 70\%$ from $T = 3$ to $T = 35$. Relative Pt content in mantle remains constant ($\sim 20\%$) throughout the experiment. Both organs were constantly exposed to ambient Pt in seawater during the exposure periods and may have served as the tissues allowing Pt to enter into the organism. This appears as consistent with the observation that all tissues in fish *Barbus barbus*, exposed to ground automobile catalytic converter material (i.e. particles), showed a clear metal uptake with gills revealing the highest Pt content, although these authors could not exclude a possible artefact due to superficially adsorbed catalyst particles on the gill filaments (Sures et al., 2005). Platinum organotropism in eels *Anguilla anguilla* exposed to PtCl_2 in tap water, did not indicate major accumulation in gills, but the following pattern of Pt distribution: muscle = spleen < liver < gill < kidney < bile < intestine (Zimmermann et al., 2004). These results obtained in fish are not in line with findings in mollusks, such as the marine gastropod *Littorina littorea*, exposed to Pt through the aqueous phase, suggesting Pt ions uptake through gill epithelial cells by passive diffusion and subsequent transport to other tissues via haemolymph (Mulholland and Turner, 2011). In fact, in mollusks bivalves exposed to other elements through direct pathway, trace metal uptake generally occurs mainly via the gills, and to a lesser extent, via the mantle (e.g. *Mytilus edulis* exposed to Cd Marigómez et al., 2002). Accordingly, Pt distribution between oyster organs as observed in the present work is in agreement with previous studies reporting that, in aquatic mollusks, gills constitute a key interface for dissolved metal uptake (Marigómez et al., 2002). This implies that Pt may be re-

distributed between organs, suggesting the possible active response of the organism following Pt exposure.

4.2. Platinum effects on oysters at different levels of biological organization

Tracing direct contamination in digestive gland from bivalves is possible since metal redistribution by haemolymph occurs from the most exposed organs (gills) to less affected tissues (Strady et al., 2011). Furthermore, the digestive gland is the main site for metabolic activity since it is involved in immune defense, detoxification, and in homeostatic regulation of the internal medium (Marigómez et al., 2002). This key organ is also strongly involved in processes of detoxification and elimination of xenobiotics. Digestive gland consists of many blind-ending tubules which are lined by a digestive epithelium constituted of digestive and basophilic cells (Aarab et al., 2011). Histopathological alteration study was therefore focused on the digestive gland, taking in consideration comparison purposes with literature.

In general, few differences were observed for tissue level parameters such as integrity of the digestive gland and digestive tubule atrophy. Yet, higher digestive tubule atrophy was recorded in oysters exposed to highest Pt levels after 28 days (Fig. 3a, b). This tissue alteration has previously been related to general stress conditions including food deprivation (Couch, 1984), and also exposure to pollutants including trace metals (e.g. exposure to Cd; Zaldibar et al., 2007). Although the obtained results did not indicate a clear tissue-level response, they suggest the onset of tissue impairments. In oysters *C.gigas* exposed to trace metals (Ag and Cu), the ratio between digestive and connective tissues (CTD) is a sensitive biomarker increasing together with atrophy of digestive tubules (Rementeria et al., 2016). Other histopathological alterations such as haemocytic infiltration occurred after Ag and Cu exposure and were clearly related to trace metal accumulation. Non-significant results obtained after 35 days of Pt exposure suggest that longer exposure time may be required in order to determine the effects of Pt at the tissue-level.

In contrast, cellular level responses indicated more marked alterations. Lipofuscins are mainly produced due to lipid peroxidation; originating from poly-saturated fatty acids oxidation by Reactive Oxygen Species (ROS); and are accumulated in lysosomes as insoluble lipoprotein granules (Viarengo and Nott, 1993). Oysters exposed to intermediate and relatively high Pt concentrations (tanks C and D) showed significantly higher lipofuscin contents in the digestive epithelium, compared to control conditions after only 7 days of exposure (Fig. 3c, d; Fig. 4a) and this trend was maintained through exposure time. Lipofuscin accumulation throughout the experiment can be attributed to Pt exposure that may increase the presence of ROS in the

digestive gland cells. Similar alterations were already described in bivalves exposed to pollutants, including metals (e.g. Marigómez et al., 2013; Rementeria et al., 2016).

In marine bivalves, neutral lipids contribute to energy resources and help in the gamete development during maturation. Previous studies have indicated that exposure to pollutants (including metals) can lead to a depletion of such reserves in freshwater mussels (e.g. Guerlet et al., 2007; Zorita et al., 2006), and more specifically in oyster *C. gigas* (Séguin et al., 2016). Results of the present study are in agreement with previous works showing a significantly decreasing trend in the accumulation of neutral lipids through time and with increasing exposure concentration. Relatively constant levels of neutral lipids along with conserved tissue integrity in oysters from control tanks reveal that food supply was sufficient for maintenance throughout the exposure period (Rementeria et al., 2016). However, it should be noted that significant differences between tanks C and D oysters, and oysters from tanks B and control occurred only from T = 28 (Fig. 3e, f; Fig. 4b). Accordingly, we can state that similarly to lipofuscin content, Pt has an effect on neutral lipid content for intermediate and relatively high exposure Pt concentrations, while no significant changes occurred for lowest exposure conditions. Consequently, one may assume that Pt contamination as observed in these oysters could deplete energy reserves that were initially intended to growth and which should be allocated to defense mechanisms under stressful conditions.

Black Silver Deposits reveal softly bound metals localized within cellular compartments (Danscher, 1991; Soto et al., 2002). In the present study, all oyster individuals from tanks C and D showed BSD in the intestine and stomach epithelium, gills and haemocytes. The distribution of BSD is in agreement with previous studies performed in oysters (Rementeria et al., 2016) indicating the presence of metals, in those tissues at basal levels. Interestingly, the presence of BSD in the basal layer of the digestive epithelium, a typical zone of metal accumulation (Rodríguez-Iruretagoiena et al., 2016) and in the digestive tubule epithelium increased with exposure time and -concentration. This response indicates an enhanced Pt accumulation in the aforementioned organs (Fig. 3g, h; Fig. 4c). Furthermore, lysosomes in the digestive cells were previously identified as target sites for many contaminants (e.g. Cajaraville et al., 1995; Izagirre and Marigómez, 2009). Quantification of BSD in the digestive epithelium revealed increased intracellular metal levels reflecting Pt exposure concentrations and -time, with significant differences after longer exposure periods. It is conceivable that the accumulation of BSD in the digestive epithelia is related to the presence of metallothioneins that have sequestered Pt ions in the cell cytoplasm and further transferred them to the lysosomes (in many cases together with lipofuscins, Soto et al., 2002).

Overall, the observed responses at the cellular levels reveal a clear toxicological impact of Pt exposure under the present experimental conditions, especially showing significant effects in oysters from tanks D. In these oysters increasing Pt accumulation occurred with a linear pattern, suggesting that oysters are continuously accumulating Pt despite the potential activation of detoxification mechanisms. Platinum exposure induced oxidation reactions as revealed by the increase in lipofuscin content. These organisms also show accumulation of Pt or its metabolites in lysosomes of digestive cells and supposedly intracellular digestion and detoxification (Izagirre and Marigómez, 2009). Activation of these mechanisms is also supported by relatively high Pt contents in faeces of exposed oysters at the different sampling times showing a similar trend in oysters from both, tanks B and C. In fact, average Pt concentration in faeces from oysters exposed to 50 ng.L^{-1} Pt are of $\sim 40 \text{ ng.g}^{-1}$, while it is about twice this value in oysters exposed to 100 ng.L^{-1} at $T = 7$. Those proportions stay approximately constant over the other sampling times, except from a small increase at $T = 21$. Proportions are also kept in faeces content from oysters exposed to $10,000 \text{ ng.L}^{-1}$ at $T = 3$ while a clear increase occurred at $T = 21$, which might suggest intensified excretion mechanism facing the high exposure level.

No adsorption of Pt from seawater on faeces can occur outside the oyster body, since faeces recovery was performed during/after feeding in uncontaminated seawater. This observation clearly suggests that the contamination of faeces by Pt occurs inside the oyster body, either through adsorption of dissolved Pt from seawater in the digestive tract or through active excretion of Pt by digestive gland organelles (e.g. residual bodies) as a detoxification mechanism (similar to Cd exposure, Strady et al., 2011). Mulholland and Turner (2011) studied Pt concentrations in *L. littorea* exposed to Pt-contaminated food source: *Ulva lactuca*. Platinum concentrations in faeces of the marine gastropod were higher than those observed in the macroalgae. These authors suggest that either (i) components of ingested food with relatively low Pt concentration are preferentially digested and/or assimilated by *L. littorea*, or (ii) aqueous Pt adsorbs to faecal material transiting the gut or following egestion (Mulholland and Turner, 2011). Considering our results on cellular Pt toxicology, demonstrating the activation of digestive gland detoxification mechanisms, one may hypothesize that Pt in faeces rather originates from active metal excretion than from passive adsorption, at least in oysters. Decreasing Pt values in faeces of oysters from tanks D at the end of the experiment could suggest the exhaustion of excretion mechanism, although other detoxification mechanisms are still active at least until $T = 28$. This is supported by the fact that oyster tissue accumulation did not reach a steady state at the end of the experiment, contrary to oysters exposed to lower, environmentally relevant concentrations (Abdou et al., 2018).

The Index of Biological Response (IBR) has been successfully applied to pollution sentinel bivalve species in order to assess their health status (Brooks et al., 2011; Cravo et al., 2012; Garmendia et al., 2011; Rementería et al., 2016). Presently, the overall response pattern suggests clear cell level alterations (lipofuscin and neutral lipid accumulation) after only 7 days of exposure. However, no clear increase in tissue-level biomarkers occurred at $T = 28$, except for the atrophy in oysters from tanks D. These results suggest that the overall health status of oysters is not affected at low levels of exposure (tanks B, $50 \text{ ng.L}^{-1} \text{ Pt}$), but that some alterations occur at higher Pt exposure concentrations. These alterations seem to be limited to the cellular level, without significant deleterious effects at tissue (no significant histopathological alterations) or organism (constant Condition Index) levels. However, the decrease of tissue integrity and increased atrophy observed for higher Pt exposure concentration indicate that Pt accumulation may impact the health status of oysters.

In marine organisms, previous studies on Pt contamination mainly focused on aquatic plants such as the marine macroalgae *Ulva lactuca* or microalgae *Chlorella stigmatophora* investigating Pt internalization and extra-cellular accumulation or seawater removal kinetics (Cosden et al., 2003; Shams et al., 2014; Turner et al., 2007). To the best of our knowledge, only two studies report on inorganic Pt accumulation by marine animals, one being the polychaete *Arenicola marina* exposed to Pt in a series of microcosms (French and Turner, 2008), and the second being the study of Mulholland and Turner (2011) exposing Pt to the marine gastropod *Littorina littorea* through direct and trophic pathways. There is therefore a crucial need for more studies on marine organisms, yet, taking into account several recommendations to apply in exposure studies as reviewed in Zimmermann and Sures (2018). Those recommendations include the constant monitoring of several parameters including i) physical-chemical parameters (e.g. toxicity and accumulation of Pt may be temperature-dependent, Veltz et al., 1996), and ii) exposure concentration (nominal exposure concentrations often do not reflect the real exposure conditions considering possible abiotic adsorption, Abdou et al., 2018; Zimmermann and Sures, 2018). Same authors also highly recommend exposure medium renewal, feeding in separate uncontaminated tanks, and the use of environmentally relevant exposure concentrations.

In nature, Pt sources may be diverse and may occur together at the same time. Accordingly, regarding autocatalyst-derived Pt emissions, a recent study proved that the contact with stormwater causes the release of a relatively small fraction ($0.2 - 18 \%$, with 3.3% on average) of the total Pt present in the road dust under the form of nanoparticles (Folens et al., 2018). These authors estimate that, although representing only a minor fraction of the total content in road dust, the nanoparticulate Pt leachate is most susceptible to biological uptake and hence

most relevant in terms of bioavailability. Additionally, apart from inorganic Pt sources, Pt contamination may occur under organic forms due to the presence of Pt-based anticancer drugs, originating especially from hospital effluents (e.g. Kümmerer et al., 1999; Vyas et al., 2014). This Pt source may pose adverse effects on aquatic organisms, including polychaete *Nereis diversicolor* (Fonseca et al., 2017) or marine mussels *Mytilus galloprovincialis* (Trombini et al., 2016). Furthermore, in nature, Pt contamination may occur together with other contamination including other Platinum Group Elements (PGE) for which a synergistic toxicity is observed following exposure to the model aquatic organism *Daphnia magna* (binary metal exposure to Pt and Pd; Zimmermann et al., 2017). Further work considering exposure to different Pt species, to interacting PGE and related responses is therefore required.

5. Conclusions

Overall, this exposure study provides more knowledge on Pt toxicology in marine organisms. Similar features compared to freshwater organism studies were observed, yet, many differences occurred (e.g. organ distribution, accumulation kinetics). Effects of Pt exposure occurred from individual down to molecular levels. The interpretation of biomarkers at different biological organization levels (IBR) suggests the occurrence of a cascade of responses (oxidative stress, intralysosomal metal accumulation, and lipid depletion) and enhanced tissue alterations. Increasing Pt contents in faeces along with Pt exposure levels suggests effective excretion of accumulated Pt but dose-related linear accumulation in tissues and whole organisms over time suggests that excretion of metal is relatively slow compared to the uptake. Although the present work provides clear evidence of adverse effects in oysters exposed to elevated Pt levels, non-significant histopathological observations for the lowest exposure concentration i.e. for the most environmentally relevant level is a valuable information. In fact, generally lower Pt levels are recorded in coastal seawater. However, it should be considered that Pt inputs may occur from several sources and under different forms (dissolved, ground catalyst particles, nanoparticles, organic forms...), which may reach organisms through direct or trophic pathways.

Biomarkers, reflecting the impact of all stressors to which organisms are exposed, provide direct evidence of pollution in surrounding environment (Capela et al., 2016), and may be used as “early” warning signals to anticipate potential impacts at higher levels of biological organization (Allan et al., 2006). Although biomarkers are already part of biological and chemical monitoring requirements in the scope of the Marine Strategy Framework Directive

2008/56/EC (MSFD), their potential responses to emerging metallic contaminants, such as Pt and other TCEs are widely unknown. The present work contributes to improving our understanding of biomarker responses to Pt exposure as integrative tools between chemicals monitoring and their impacts on aquatic organisms.

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This study allowed to gain knowledge on the physiological responses to Pt exposure.

In terms of **Pt bioaccumulation**, organotropism study showed several valuable information:

- ✓ Natural distribution of Pt suggested by our results in non-exposed organisms is the following (from lower to higher Pt content regarding the whole Pt content): gonads < digestive gland << muscle < mantle <<< gills.
- ✓ After only 3 days of exposure, distribution of Pt between organs showed a clear difference compared to initial distribution.
- ✓ Highest Pt accumulation (~ 70 % of total content) occurred in gills, and to a lesser extent in mantle (~ 25 %). Gills might therefore be the key organ of Pt contamination through the dissolved phase.
- ✓ Linear accumulation of Pt was observed in all organs investigated in line with results obtained at the whole individual scale.
- ✓ Time and exposure concentration dependence of Pt content in faeces suggest intensified excretion by oysters.

In terms of **biological effects**, monitoring of various biomarkers allowed to observe that:

- ✓ No clear tissue level response occurs following Pt exposure even though increase in tissue integrity alterations and digestive epithelium atrophy may indicate the onset of tissue alterations.

Oysters exposed to environmentally relevant level of 50 ng.L⁻¹ did not show significant variations in the targeted biomarkers as observed in control organisms. Such result may suggest that no significant deleterious physiological effects of Pt occur in nature with living organisms exposed to lower environmental concentrations. It might also suggest that longer exposure period would be necessary to observe such adverse effects and that our experiment, in this sense, cannot reproduce environmental conditions.

However, in oysters exposed to 100 and 10,000 ng.L⁻¹, significant modifications occurred with:

- ✓ Increasing lipofuscin accumulation suggesting that Pt induces the presence of ROS in the digestive gland.
- ✓ Lower neutral lipid content suggesting that Pt contamination might deplete energy reserves that were initially devoted to growth and/or reproduction and which are thereafter allocated to defense mechanisms to face Pt exposure.
- ✓ Quantification of BSD in the digestive epithelium revealing increasing intracellular metal levels along with increasing Pt exposure concentration and through time.
- ✓ The IBR index suggests a generally decreasing health status of oysters.

Overall, the present study of Pt effects at cellular levels has revealed a clear toxicological impact of Pt exposure, especially showing significant effects in oysters exposed to 10,000 g.L⁻¹. In these oysters, increasing Pt accumulation occurred with a linear pattern suggesting that oysters are continuously accumulating Pt despite the activation of detoxification mechanisms. Platinum exposure provoked in those oysters oxidation reactions reflected by the increase of lipofuscin content and accumulation of Pt or its metabolites in lysosomes of digestive cells and supposedly intracellular digestion and detoxification (Izagirre and Marigómez, 2009). Activation of these mechanisms is also supported by enhanced excretion of faeces with relatively high Pt contents at the different sampling times in oysters exposed to the highest Pt concentration.

To our knowledge, these results are the first to provide information on physiological response following Pt exposure in marine bivalves. Non-significant histopathological and histochemical observations for the lowest exposure concentration i.e. for most environmentally relevant level are promising information on an ecotoxicology basis. Platinum biological adverse effects may indeed be relatively limited considering the fact that natural dissolved Pt concentrations in coastal seawater are generally ~ 100 times lower. However, in the aforementioned laboratory experiment, only pseudo-equilibrium was reached and those conditions might not be fully representative of natural environment, where longer exposure time are reached and many Pt sources may co-occur.

3.4. Conclusion

This chapter reports on a laboratory exposure experiment with oyster *C. gigas* exposed to Pt in seawater. Apart, from analytical development for Pt determination in biological matrices, this study allowed to gain valuable knowledge on Pt uptake by marine biota. Main outcomes are that:

- At environmentally relevant Pt levels: oysters readily accumulate dissolved Pt from seawater, without suffering mortality, until reaching a plateau (after 21 days). A positive linear relation exists between seawater Pt concentrations and oyster tissue Pt concentrations with oysters showing BCF of ~ 500 . When exposed to lowest Pt concentrations of 50 ng.L^{-1} , no significant histopathological nor histochemical alterations occurred.
- At relatively high Pt concentration: linear Pt accumulation occurred in whole organisms and in each organ with no plateau of accumulation at the end of the exposure period, suggesting inefficient detoxification mechanisms. Significant biomarker impairments occurred, especially at cellular levels.

Besides the relevance of laboratory experiments, the knowledge of influencing parameters can also help to understand the complexity of factors affecting the biological availability, bioaccumulation and toxicity of Pt to aquatic animals under field conditions (Zimmermann and Sures, 2018). However, as aforementioned, laboratory experiment allowed to reach an experimental pseudo-equilibrium which does not consider all the environmental parameters that might affect uptake, accumulation and response. Accordingly, in nature one should consider:

- i. The relative importance of the trophic pathway for Pt accumulation as previously investigated in a marine gastropod (Mulholland and Turner, 2011).
- ii. Seasonal physical and chemical gradients affecting biogeochemistry and organism physiology including reproduction cycles and trophic status (de Souza Machado et al., 2016).
- iii. The simultaneous existence of various Pt sources such as nanoparticulate forms (Folens et al., 2018) or organic Pt forms that might also have relevant toxic effects (Trombini et al., 2016).

Thus, some limitations may exist regarding biomonitoring studies (Farrington et al., 2016). They include the fact that it can be difficult to extrapolate observations made with oysters to other species since inter-species differences in bioconcentration of analyte are not well

documented at present. In the same manner, extrapolation to the health of entire ecosystems might be difficult. Authors also report that bivalves are not always found in targeted study area, and if they are present, stable populations are required. This drawback has been offset by bivalves transplantation through caging (Marigómez et al., 2013; Stien et al., 1998), which is a particularly useful approach providing relevant information on chemical contaminants levels, especially trace metals, in seawater.

Overall, the analysis of wild oyster Pt concentrations integrating and increasing the environmental signal has great potential to assess the local dissolved Pt levels in seawater. Oyster *C. gigas* appears as a suitable sentinel species for Pt contamination in coastal environments.

It could be foreseen to apply continuous monitoring of Pt levels for farmed organisms that are devoted to human consumption. Recently, a study conducted in a coastal environment revealed that anthropogenic influences such as sewage wastes from agricultural fields, shrimp culture and domestic sludge govern the concentration patterns of various trace metals including Cu, Cd, Pb, Zn, or As in water, sediments and cultivated *C. gigas* (Jonathan et al., 2017). Given the potential release of Pt from such wastes (de Vos et al., 2002), special care should be given in aquaculture sites for healthy production of seafood. Other sentinel species should be investigated for application in coastal biomonitoring studies. This is for instance the case of the widely used mussels *Mytilus sp.* Its application in biomonitoring programs is suggested as a valid strategy to assess environmental quality of complex environments and drive action planning and management policies (Capolupo et al., 2017).

Cajaraville et al. (2000), suggested the generalized use of biomarkers in biomonitoring field study since they represent “early-warning” indicators that can be used in a predictive way, allowing the initiation of bioremediation strategies before irreversible environmental damage or ecological consequence occur (Cajaraville et al., 2000). Accordingly, the Marine Strategy Framework Directive 2008/56/EC (MSFD) incorporates biomarker responses as early warning signals of pollution (Allan et al., 2006). Following biological response observed after Pt exposure, biomarkers could be considered as integrative tools between Pt level monitoring and its impact on aquatic organisms (Capela et al., 2016).

Chapter 4

Temporal and spatial aspects of biogeochemical platinum cycles in coastal environments

Chapter 4: Temporal and spatial aspects of biogeochemical platinum cycles in coastal environments

4.1. Introduction

There is a crucial need for reliable assessment of Pt environmental distribution and behavior including potential continental/riverine release and potential inputs to the coastal area. Relevant sampling sites should therefore be selected in order to cover different aspects of biogeochemical Pt cycles and, sampling might be performed at appropriate temporal scales.

The sample set constituted in this study consists in a variety of samples:

- Environmental matrices: Pt concentrations were determined in biota samples. Living organisms and more precisely marine bivalves were assessed as suitable sentinel species under laboratory conditions. Another biota sample consisted in plankton material for which, to the best of our knowledge, to date no studies report on Pt levels. Seawater samples were also collected in order to determine environmental dissolved and particulate (Suspended Particulate Matter, SPM) Pt concentrations. Sediment cores were also analyzed.
- Temporal resolution: historical evolution of Pt concentrations were studied through the analysis of environmental archives. They consist in time-resolved sediment cores as well as marine bivalves collected since decades through national biomonitoring programs. Determination of Pt levels in such samples will allow to trace the evolution of Pt contamination in coastal environments in relation with intensified anthropogenic inputs. In addition, studies at high temporal resolution (hourly scale) allow the determination of short-term processes controlling Pt distribution and behavior in coastal systems.
- Spatial resolution: platinum occurrence and distribution were investigated in various contrasting areas. They include the Atlantic coast with samples originating from the Gironde Estuary mouth as well as from the Arcachon Bay, and the northwestern Mediterranean Coast. Those sampling sites allowed to determine historical evolution of Pt inputs, the distribution of Pt levels in contrasting sites including relatively pristine and highly polluted sites, as well as the observation of biogeochemical diurnal / tidal processes.

4.2. Past and present platinum contamination of a major European fluvial–estuarine system: Insights from river sediments and estuarine oysters

First records of Pt anthropogenic inputs in aquatic systems were performed principally in lake or riverine sediment cores. As metals accumulate in sediments, cores may be used as pollutant archives recording change of Pt levels in the study area (Von Gunten et al., 1997). For instance, the analysis of Pt content of sediment cores from the Upper Mystic Lake which is located in a highly urbanized watershed provides an excellent record of industrial and traffic-related pollution in the area. Elevated Pt concentrations in recent (surface) sediments have been attributed to automobile catalysts with accumulation rates 6 to 16 times higher in the period 1992 – 2002, than before the introduction of car catalytic converters (Rauch et al., 2004; Rauch and Hemond, 2003). Riverine sediments are the first sink of traffic-derived Pt emissions in urbanized regions and contemporary riverine sediments may serve as indicators of Pt levels adding that traffic impacted areas could be identified by Pt enrichment (Haus et al., 2007).

In order to assess potential evolution of Pt content in the Gironde fluvial-estuarine system, sediment cores from a historically multi-metal polluted river continuum were studied. The Gironde Estuary (about 170 km in length), draining a 80,000 km² watershed, is a major European estuary considered a model for physical, hydrological and geochemical studies especially on trace element transport and reactivity in impacted watersheds (Lanceleur et al., 2011). Sediment cores collected in the Cajarc and Marcenac sampling sites have revealed the impact of trace metal concentrations originating from a common main point source in a couple of previous studies. This source is a former Zn-ore mining/metallurgic industry located on the Riou-Mort River, a tributary of the Lot River which flows itself into the Garonne River and finally into the Gironde Estuary. Accordingly, previous work reported Cd, Zn, Cu or Pb pollution in the Lot River sediments (Cajarc site), originating from this site (Audry et al., 2004; Sivry et al., 2008). Analysis of such heavily impacted samples might provide valuable knowledge on past environmental Pt level evolution and potential contamination. Another sediment core (Marcenac site) collected upstream the pollution source might allow the determination of regional geochemical background Pt concentrations.

In coastal areas, historical records consisting in estuarine sediments have already evidenced the influence of anthropogenic Pt emissions (e.g. Lérez Estuary, Cobelo-García et al., 2011; Zhong et al., 2012). Platinum in those sampling sites is delivered to the sediment through fluvial inputs including traffic-derived Pt and other sources (e.g. industrial processes). Accordingly, continental runoff is supposed to have a significant influence on the global Pt budget in the oceans, especially considering current anthropogenic emissions (Cobelo-García et al., 2013).

In the same manner, we can wonder if potential Pt riverine inputs may ultimately impact the estuarine compartment. In this sense, living-organisms may be used in order to observe environmental changes of metal inputs. Wild oysters have been annually collected by the French Monitoring Program RNO/ROCCH since the early 1980's. This valuable sample bank already permitted to observe time-resolved trace metal contamination evolution (e.g. Lanceleur et al., 2011). Oysters from the Gironde Estuary mouth were selected in order to gain knowledge on Pt concentrations in wild living biota in a coastal system.

By determining Pt levels in these historical samples, the objective is **to trace back potential past contamination and to observe current Pt levels in the Gironde fluvial-estuarine continuum.**

For this, different endpoints were investigated with:

- The determination of Pt concentrations in sediment core from a riverine system impacted by multi-metal pollution with sediment layers dating from the 1950's to the early 2000's.
- The analysis of Pt levels in estuarine wild oysters obtained from the national sample-bank and collected yearly during the 1980's to the 2010's.
- The assessment of oyster *C. gigas* as a sentinel organism in the field and the observation of environmental bioconcentration factor.

Results from this study have been reported and discussed in a peer-reviewed paper published in the journal *Marine Chemistry*.

Past and present platinum contamination of a major European fluvial–estuarine system: Insights from river sediments and estuarine oysters

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Abstract

Platinum Group Metals (PGM) are modern, technologically relevant elements for which (i) the anthropogenic cycle has outcompeted the natural cycles and (ii) environmental behavior, fate and impact are still widely unknown. Stripping voltammetry was used for accurate determinations of platinum (Pt) in historical records of river sediments and estuarine oysters from the Gironde fluvial–estuarine continuum (SW France) comprising the Lot River. Sediment cores from the Lot River, dated from 1952 to 2001, showed past Pt contamination due to former industrial (smelter) activities in the Lot River watershed. These samples revealed the phasing-out of a historical Pt contamination with Pt/Th (Thorium) values of $11 \times 10^{-5} \pm 0.79 \times 10^{-5}$ for the deepest part of the core which is clearly greater than the regional geochemical background value ($\text{Pt/Th} \sim 2.2 \times 10^{-5} \pm 0.68 \times 10^{-5}$). Wild oyster samples from the mouth of the Gironde Estuary collected from 1981 to 2013 showed Pt concentrations ranging from $0.80 \pm 0.01 \text{ pmol.g}^{-1}$ to $3.10 \pm 0.14 \text{ pmol.g}^{-1}$. Oyster samples have recorded the phasing-out of the smelter-related historical industrial Pt contamination and empirical modeling suggests the recent rise of a new source of Pt to the system. Temporal variations of Pt in oysters attributed to this recent source reflect the exponential increase of Pt demand for car catalytic converters, pointing towards the increasing importance of this emerging source to the aquatic system. Estuarine oysters prove to be suitable bioindicators for Pt contamination providing sensitive monitoring of emission variations over time. Furthermore, oysters may bioconcentrate Pt (bioconcentration factor, BCF $\sim 10^3$) and transfer this metal contamination to the higher food chain. These findings highlight the need for a deeper understanding of environmental Pt contamination, processes and possible adverse effects to biota.

Keywords: Platinum; Sediments; Wild oysters; Bioaccumulation

1. Introduction

Platinum (Pt), one of the rarest elements in the Earth's crust with a typical concentration of 2.6 pmol.g^{-1} (Rudnick and Gao, 2003), is a strategic commodity for industries in many countries (Reith et al., 2014; Cobelo-García et al., 2015) since its physical (density, resistance to corrosion) and chemical (e.g. catalyst) properties serve in many applications such as laboratory and medical equipment or (medical) drugs. The most extended application is the use of Pt-based vehicle catalytic converters which contain from 1 – 2 g for a small car to 12 – 15 g of Pt for a big truck (Tollefson, 2007), representing more than 70% of the total European Pt demand in 2013 (Johnson Matthey, 2014). Therefore these devices are recognized as a major source of Pt to the environment and

together with the burning of fossil fuels, mining and other industrial uses has led to a complete disturbance of the global Pt cycle (Sen and Peucker-Ehrenbrink, 2012). The significant recent increase of Pt concentrations in Antarctic snow (Soyol-Erdene et al., 2011) suggests large-scale atmospheric transport of Pt (Barbante et al., 2001) implying its global contamination. Major concerns on ecosystems and biota contamination arose few years after introducing car catalyst technology in Europe (e.g. Schäfer et al., 1999) and today anthropogenic Pt enrichment is recorded in all Earth compartments: atmosphere, water, sediments, and soils (e.g. Pawlak et al., 2014). Accordingly, Pt is considered an “emerging pollutant” for which it is necessary to develop innovative techniques providing reliable quantitative estimates of environmental pathways, loads, and concentrations (Rodrigues et al., 2009). To date, however, there is still a lack of data on environmental Pt concentrations mostly due to the relative difficulty to measure this element at low ambient levels. Recent work on Pt behavior in the Gironde Estuary has shown that estuarine reactivity along the salinity gradient is crucial for both the Pt distribution in the estuary and dissolved Pt export to the global ocean (Cobelo-García et al., 2014a). The net dissolved Pt addition due to estuarine mixing results in a higher export of dissolved Pt to the ocean than expected from the

riverine input (Cobelo-García et al., 2014a). However, Pt sources and possible contamination of this system are still unknown. The present work aims at identifying past and present Pt contamination and sources by combining the first historical records of Pt in river sediments and estuarine oysters from the Gironde fluvial–estuarine system.

2. Material and methods

2.1. Study area

The Gironde Estuary (about 170 km in length), draining a 80,000 km² watershed, is a major European estuary considered a model for physical, hydrological and geochemical studies especially on trace element transport and reactivity in impacted watersheds (e.g. Lanceleur et al., 2011). The Lot–Garonne–Gironde fluvial–estuarine system is affected by multi-metal pollution originating from a common main point source being a former Zn-ore mining/metallurgic industry (e.g. 1842–1987: Cd, Zn, Cu, Pb; Audry et al., 2004a) on the Riou-Mort River near Decazeville (Fig. 1). After transport, trace metals can settle down and be deposited in river sediments where they accumulate under favorable hydraulic conditions provided by natural and reservoir lakes (Audry et al., 2004a). In 2001, two continuous sediment cores of about 1.4 m-long were collected using a manual corer consisting of a 10 × 10 cm rectangular Plexiglas tube. Sampling was realized in two hydroelectric reservoirs of the Lot River: the reservoirs of Marcenac and Cajarc located respectively upstream and downstream the Riou-Mort River i.e. the hypothetical Pt pollution source (Fig. 1). The sediment cores were sliced in thin horizontal sections at 1 cm resolution from the surface to 25 cm. The lower part was sampled with a 5 cm resolution. Immediately after recovery, each sliced section was centrifuged in order to remove pore waters and then sealed in sampling-bags under nitrogen. Sediment samples were dried at 50 °C to constant weight and then powdered and homogenized with an agate mortar

(Audry et al., 2004a). The second set of samples consisted of a historic series of wild oysters which, as suspension feeders, are exposed to both dissolved metals and metals bound to mineral particles such as phytoplankton and organic matter (Lanceleur et al., 2011) and therefore represent good bioindicators of their environment (Gunther et al., 1999). A time series (1981–2013) of mature (2 year old) wild-growing *Crassostrea gigas* Japanese oyster samples was extracted from the National Network for the Observation of Marine Environment Quality (RNO/ROCCH; i.e. the French Mussel-Watch; Ifremer.fr) specimen bank. Winter (February) oyster samples were chosen for the present work in order to avoid dilution of metal concentration due to variable soft-body weight during reproduction periods (Enríquez-Díaz et al., 2009). Individuals selected for this study originated from the mid-salinity range of the Gironde Estuary, at the La Fosse sampling site (salinity 15–20; Fig. 1), where highest trace metal concentrations are recorded in the same set of oyster samples (e.g. Cd and Ag; Lanceleur et al., 2011). A pool of fifteen individuals for each winter has been selected, lyophilized and grinded.

2.2. Analytical procedure

Samples were ashed in quartz crucibles at 800 °C during 3 h according to the heating scheme described by Nygren et al. (1990) and then digested using a mixture of 5 mL concentrated hydrochloric acid HCl and 3 mL of concentrated nitric acid HNO₃ (both Suprapur®, Merck) at 195 °C for 4 h. Then, samples were allowed to cool down, caps were removed and the acid was evaporated until near dryness. The residue was then redissolved adding 1 mL of concentrated sulfuric acid H₂SO₄ (TraceSELECT®, Fluka), and evaporated again until no fumes were observed (i.e. only H₂SO₄ was present). Cooled contents were then diluted with 0.1 M HCl. Sediment samples were syringe-filtered using PFA syringes (Savillex®) and 25 mm polyethersulfone membranes of 0.45 µm pore size (VWR®) prior to analyses. Platinum measurements

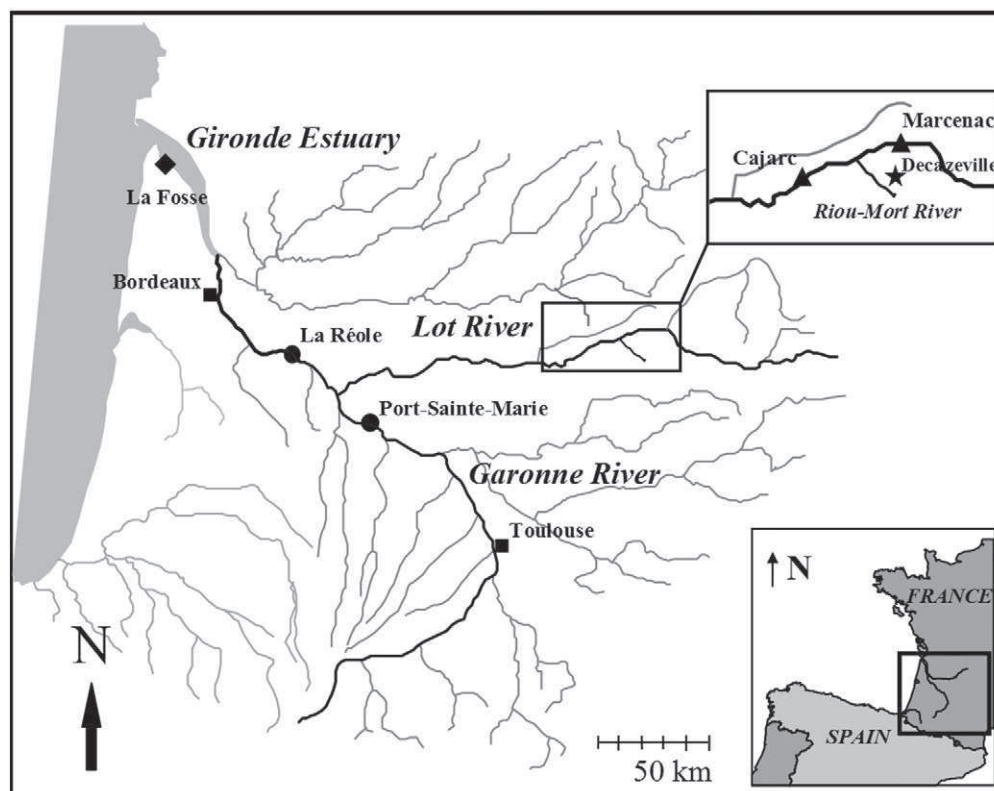


Fig. 1. The Gironde fluvial–estuarine continuum (SW France) with sampling locations: oyster samples were collected in the Gironde Estuary mouth at La Fosse (diamond) and sediment cores were retrieved in the Lot River in Cajarc and Marcenac (triangles). Suspended Particulate Matter (SPM) samples were collected in the Garonne River at the La Réole and Port Sainte Marie sites and analyzed in a previous study (round symbols, Cobelo-García et al., 2014a).

were carried out by means of catalytic adsorptive cathodic stripping voltammetry. Laboratory analyses were performed in a laminar flow bench (ISO-5) housed in an ISO-7 laboratory. Platinum voltammetric determinations were carried out using a μ Autolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm® 663 V.A.) equipped with three electrodes: i) a hanging mercury drop electrode (HMDE; the working electrode), ii) a Ag/AgCl reference electrode, and iii) a glassy carbon auxiliary electrode. A polytetrafluoroethylene (PTFE) voltammetric cell served in all experiments and the potentiostat was controlled using the GPES v. 4.9 software (EcoChemie B.V.). Aliquots of 8 mL of acid-digested sample were pipetted into the voltammetric cell, and two reagents, 3.3 mM formaldehyde (Riedel-Haën), and 0.45 mM hydrazine sulfate (Fluka) were added directly to the solution and allowed for the detection of Pt in the acidic media (see details in Cobelo-García et al., 2014b). Sample solution was first deaerated by 5 min of purging with humidified nitrogen (99.999% ALPHAGAZ™ 1, Air Liquide®). Accumulation on the HMDE was then initiated with a potential set up at -0.3 V for 90 s, while the solution was stirred at 3000 rpm with a PTFE rod. After an equilibration time without stirring, a cathodic stripping scan was carried out by changing the potential from -0.5 V to -1.1 V in the differential pulse mode with a step potential of 4 mV, at a scan rate of 20 mV.s^{-1} , a modulation time of 0.04 s, an interval time of 0.2 s, and a modulation amplitude of 25 mV. Before applying any derivative transformation, the original voltammogram was smoothed, with a smoothing factor of 2 and according to the Savitsky–Golay algorithm. The determination of Pt concentrations was achieved by standard addition method and the signal was processed by second derivative transformation (applied after data smoothing) as described in Cobelo-García et al. (2014b). The improvement in the peak detection and quantification applying the second derivative signal allows for the acquisition of a well-defined peak for Pt at low accumulation times. Second derivative was selected as it is more efficient in eliminating the background interferences than the first derivative, while keeping good peak-shaped transformed signals and better signal-to-noise ratio than higher derivative orders (Cobelo-García et al., 2014b).

For typical masses of 200 mg for sediment and 40 mg for biological samples the detection limits (calculated as three times the standard deviation of blank measurements) were 0.11 pmol.g^{-1} and 0.44 pmol.g^{-1} , respectively. For sediments, accuracy was checked by the analysis of river sediment certified reference material (CRM; JSd-2; Geological Survey of Japan) with recoveries of $\sim 90\%$ and precision (% relative standard deviation; RSD) of about 5% ($n = 3$). Since no CRM existed for Pt in biological matrices, precision of the method was

tested on certified dogfish muscle (DORM) resulting in low RSD between replicates of $\sim 5\%$ ($n = 4$).

Cadmium concentrations in oysters were measured by quadrupole ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and typically differed by less than 15% from the data provided by RNO/ROCCH for aliquots of the same samples analyzed by Graphite Furnace Atomic Absorption Spectrometry (Ifremer.fr). Cadmium and Thorium concentrations in sediment cores were also measured by ICP-MS (Audry et al., 2004a). Accuracy was checked by analysis of CRM (CRM 320; BCR). Recoveries were $\sim 95\%$ and precision was generally better than 5% for concentrations 10 times higher than detection limits.

3. Results

3.1. Platinum and Cd concentrations in sediment cores

Platinum concentrations in the Marcenac core samples were low and constant from the surface (0.3 cm) to the bottom (122 cm) of the core with a mean concentration of $1.07 \pm 0.39 \text{ pmol.g}^{-1}$ ($n = 8$; Fig. 2A). With concentrations ranging from $\sim 1 \text{ pmol.g}^{-1}$ to 7 pmol.g^{-1} , Cajarc sediments exhibit relatively high Pt concentration variations with values clearly higher than the concentrations in the Marcenac core until approximately 40 cm depth (Fig. 2A). The maximum concentration occurred at the very bottom of the core, i.e. 123 cm depth representing about 10 times Marcenac core concentration. After displaying an almost flat profile with rather low and constant Pt concentrations between 40 and 6 cm depth, a positive anomaly can be observed at 4 cm depth. Platinum values finally almost reach Marcenac concentration in the topmost Cajarc sample (2 cm depth) exhibiting a minimal concentration of 0.88 pmol.g^{-1} . Cadmium concentrations were taken from Audry et al. (2004a) and plotted together with Pt for comparison (Fig. 2B). For detailed interpretation and age determination see Audry et al. (2004a). In most areas, hydraulic and mineralogical (chemical) particle fractionations result in increasing heavy metal concentrations with decreasing sediment grain sizes. It is therefore essential to normalize for grain size effects on natural, lithogenic trace metal variability before assessing anthropogenic inputs (Loring and Rantala, 1992). Thorium which is a conservative, lithogenic element in sedimentary cores (Krachler and Shotyk, 2004) and does not originate from any anthropogenic inputs in our study area served as a normalizing element. Several studies have used the particulate Th concentrations for the correction of grain size effects throughout the whole Lot–Garonne–Gironde fluvial–estuarine system (e.g. Coynel et al., 2007; Larrose et al., 2010). The Pt profile is smoothed by Th normalization,

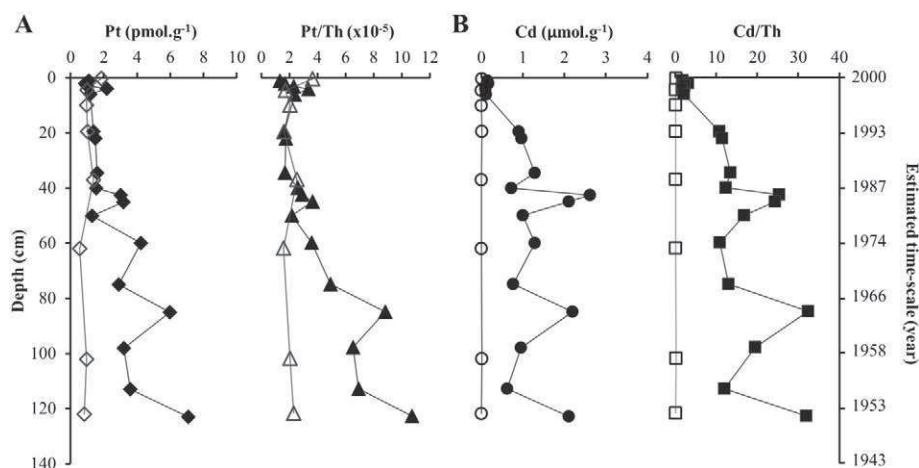


Fig. 2. Depth profiles of Pt and Cd concentrations and normalized concentrations in sediment cores from the Lot River. Sediment cores were collected in the Marcenac (empty symbols; upstream the potential pollution source) and Cajarc (filled symbols; downstream the potential pollution source) sampling sites. Platinum concentrations (A, pmol.g^{-1} , diamonds) and Cd concentrations (B, $\mu\text{mol.g}^{-1}$, round symbols) are represented. Grain size effects were corrected by Thorium concentrations and Pt/Th (A, $\times 10^{-5}$, triangles) and Cd/Th (B, squares) depth profiles give normalized concentrations. Thorium and Cd concentrations, and the estimated timescale (based on ^{137}Cs profile) are from Audry et al. (2004a).

suggesting that grain size effects play a minor, but visible, role in the peak shape of the Pt record in the Cajarc core, i.e. Pt is predominantly enriched in the fine particles (Fig. 2A). However, except from the attenuation of some peaks, normalized Pt profile displays the same positive anomalies as the original Pt concentration profile and confirms the Pt enrichment of Cajarc sediments compared to the Marcenac core.

3.2. Platinum and Cd concentrations in wild oysters

Total Pt concentrations in oyster samples, expressed as pmol.g^{-1} dry weight (dw) of oyster tissue, ranged from 0.80 to 3.1 pmol.g^{-1} (Fig. 3A). From 1981 to 2000, Pt concentrations in oysters were constant with a mean value of $1.8 \pm 0.26 \text{ pmol.g}^{-1}$. Between 2001 and 2008, Pt concentrations increased to a maximum in 2006 being almost 2-fold higher than the previous mean value. Following this peak, Pt concentrations decrease abruptly to a minimum of 0.80 pmol.g^{-1} in

2012. The last measurement corresponding to the year 2013 displays however, a higher value, that reaches again the mean concentration of the 1981–2000 period.

Total Cd concentrations are expressed in $\mu\text{mol.g}^{-1}$ dw of oyster tissue and range from $0.14 \mu\text{mol.g}^{-1}$ to $1.3 \mu\text{mol.g}^{-1}$. The highest values occurred in the early 1980s reflecting the highest industrial activity at the source, followed by an overall decreasing trend after the shutdown of the industrial Zn ore extraction in 1986, from $\sim 0.95 \mu\text{mol.g}^{-1}$ in 1987 to $\sim 0.15 \mu\text{mol.g}^{-1}$ in 2013. These results obtained for pooled oysters from the national sample bank are very similar to those previously published by the RNO/ROCCH (Ifremer.fr) and by Lanceleur et al., 2011.

4. Discussion

4.1. Past industrial Pt contamination in river sediments

Considering the low values and the low variability of Marcenac sediment core Pt concentrations, the Marcenac sampling site may serve as a reference site for Pt pollution of the study area. Located upstream the industrial activity area, this site appears as not contaminated in numerous heavy metals such as Zn, Ag, Cd or Pb, and is thus considered as a reference site for metal pollution of the Lot watershed in several previous studies (e.g. Lanceleur et al., 2011). Therefore, regional background Pt concentration, corresponding to mean Pt concentration of the Marcenac core, is of about 1.07 pmol.g^{-1} . Reservoir sediments from the Lot River appear to be impoverished regarding Upper Continental Crust (UCC) Pt content (2.6 pmol.g^{-1} ; Rudnick and Gao, 2003), likely due to the geological composition of the Lot River watershed which mainly consists of Jurassic marine calcareous sedimentary rocks (Coynel et al., 2007). In such rocks, typical Pt concentrations are rather low, i.e., with typical concentrations lower than 2.6 pmol.g^{-1} and often around 1 pmol.g^{-1} in ordinary chert beds (Hori et al., 2007). Marcenac Pt concentrations were also low compared to other estuarine or coastal systems such as pre-anthropogenic sediments from the Massachusetts Bay (3 pmol.g^{-1} ; Tuit et al., 2000) or unpolluted Portuguese coastal sediments (2.9 pmol.g^{-1} ; Cobelo-García et al., 2011). The regional geochemical background for Pt in the Lot River watershed obtained from mean normalized Pt concentrations in the reference study site of Marcenac is $\text{Pt/Th} \sim 2.2 \times 10^{-5}$.

In order to assess the extent of Pt enrichment of Cajarc sediments compared to background natural values, the enrichment factors (EF) were calculated as follows:

$$\text{EF} = (\text{Pt/Th})_{\text{Cajarc}} / (\text{Pt/Th})_{\text{Marcenac}} \quad (1)$$

Mean Pt/Th ratio of Marcenac sediments was selected as the background value instead of the crustal content in order to estimate the Riou-Mort River pollution impact. Enrichment factors were interpreted according to the scale established by Birth (2003) as detailed in Essien et al. (2009).

When comparing Cd/Th and Pt/Th profiles, positive anomalies occur at the same depths (Fig. 2A and B). This co-variation along the sediment core suggests a common source and transport of Cd and Pt metal inputs to the Lot River. Cadmium input variations in the Lot watershed were related to the history of the treatment of Zn-extraction residuals and were time-resolved according to radiochronology of ^{137}Cs (Audry et al., 2004a). Accordingly, the historical Pt records in Cajarc sediments also closely reflect the evolution of the industrial activity in the Decazeville basin. This includes changes in the treatment process of Zn residuals inducing variations of metal concentrations in river sediments. The bottom part of the core, dated from 1952, displays high Pt concentrations corresponding to moderate Pt enrichment. At that period, residuals were stocked as sludge and subject to leaching during rainfalls. Elevated Pt/Th and Cd/Th values at a depth of 42 cm co-occurred reflecting the accidental seepage of 1986 which was the major pollution event recorded in the reservoir sediments of the Lot River since the 1950s (Audry et al.,

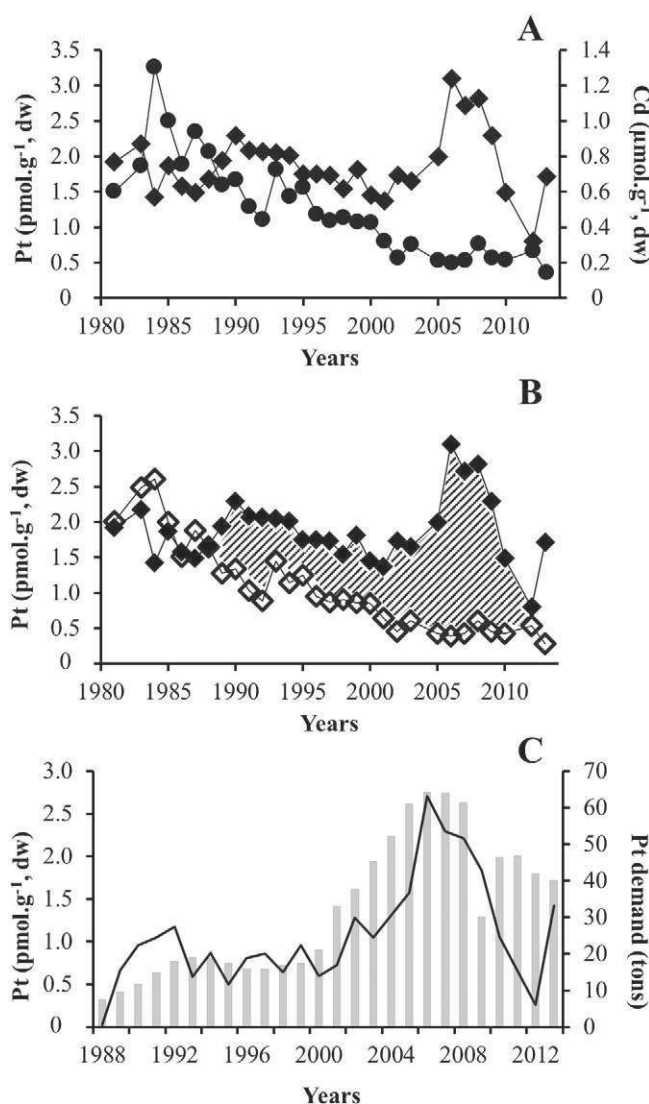


Fig. 3. Time variations of Pt and Cd concentrations in wild oysters from the La Fosse sampling site at the Gironde Estuary mouth. (A) Platinum (pmol.g^{-1} , diamonds) and Cd ($\mu\text{mol.g}^{-1}$, round symbols) concentrations measured in winter oysters collected from 1981 to 2013. (B) Platinum concentrations measured (pmol.g^{-1} , filled diamonds) and estimated (pmol.g^{-1} , empty diamonds) from Cd/Pt molar ratios and Cd concentrations measured in wild oysters. The dashed area corresponds to the lag between measured and estimated Pt concentrations. (C) Platinum concentrations (pmol.g^{-1} , dark line) originating from the difference between measured and estimated Pt concentrations (B, dashed area) and European Pt demand for car catalytic converters (metric tons, bars, after Johnson Matthey, 2014).

2004a). After this accidental event in 1986, Cd and Pt normalized profiles displayed a slight decrease and then constant, relatively low ratios, over ~30 cm with Pt/Th ratios close to the background value at the Marcenac sampling site ($\text{Pt/Th} \sim 1.5 \times 10^{-5}$) and Pt EF showing an overall decreasing trend. For Cd, this decrease was related to the cessation of Zn production in 1986 and subsequent remediation actions: treatment of groundwater tables and the containment of spoil tips by clay covering (Audry et al., 2004a). The recent decrease of both Cd/Th and Pt/Th towards background values suggests that the remediation of the Decazeville site successfully reduced Cd and Pt releases from the former industrial area. In this context, the early 1990's sediments indicate no enrichment in Pt with EF < 1.

Sediment cores already served as indicators of Pt pollution sources in other systems such as an urban lake near Boston, Massachusetts. In these urban sediments the increasing Pt concentrations were clearly related to the introduction of automobile catalysts in the United States with Pt flux being approximately ten times higher than before and recent enrichment factors reaching 100 (calculated in the assumed absence of grain size influence; Rauch and Hemond, 2003). The influence of anthropogenic Pt emissions originating from car catalytic converters can be neglected in the Lot River sediment cores since no important urban area is located upstream from the sediment sampling sites and the population density at the Lot River watershed scale is very low (~ 30 inhabitants/km²; cartesfrance.fr) compared to that of the Bordeaux agglomeration (~ 1000 inhabitants/km², cartesfrance.fr). However, as Pt/Th in the upper sediments of both cores seem to slightly exceed the geochemical background value, we cannot exclude the existence of a slight signal of car converter-derived Pt in the uppermost Lot River sediment layers (Fig. 2A). Such a signal would be consistent with recent works (2013 field campaign) establishing the Pt concentrations of surface Suspended Particulate Matter (SPM) from a Gironde Estuary tributary, the Garonne River, upstream (Port-Sainte-Marie sampling site) and downstream (La Réole sampling site; Fig. 1) the Lot River confluence. Contemporary SPM displayed urban mean Th-normalized Pt values of $\sim 6.5 \times 10^{-5}$ (Cobelo-García et al., 2014a) which are considerably higher than the most recent values in the Cajarc sediment core. At these sampling sites, the Garonne River drains the atmospherically deposited Pt pollution originating from highly urbanized areas such as the Toulouse and Agen urban agglomerations. This diffuse source must account for Pt enrichment of the Garonne watershed. The Garonne River draining a basin comprising two important urban areas Toulouse and Bordeaux agglomerations (both more than 1 million inhabitants; Fig. 1), is the main Gironde Estuary tributary. The Gironde Estuary is therefore submitted to anthropogenic pressure concerning automobile exhaust catalysts and Pt pollution.

4.2. Post-1990 exponential increase of car catalytic converters

As suspension feeder organisms, oysters, and especially the *C. gigas* species, have already served as bioindicators for trace metal contamination (e.g. Claisse, 1989). The Decazeville basin is known as the primary source of Cd in the Gironde watershed (Audry et al., 2004b), where chronic Cd pollution has led to the prohibition of bivalve production and consumption since 1996. Mass balances at the watershed scale showed that even 13–16 years after the termination of the Zn ore treatment (i.e. 1999–2002), the Decazeville basin contributed to more than 61%–92% of total Cd fluxes into the Gironde Estuary (Masson et al., 2006). This is further supported by i) historical records of Cd in estuarine freshwater sediments reflecting the industrial activity in the Decazeville watershed (Grousset et al., 1999) and ii) mass balances at the estuary scale, suggesting that intra-estuarine sources (e.g. the Bordeaux agglomeration) are negligible, compared to the main source (e.g. Schäfer et al., 2002).

Knowing the major source of Cd inputs to the system, the molar ratio between Cd and Pt served to determine Pt source of contamination. Cajarc sediments revealed a characteristic, relatively constant Cd/Pt

value close to 3×10^5 for the Zn-ore treatment period from 1952 to 1978. During the mid-1980s and early 1990s, Cd/Pt molar ratios in Cajarc sediments were clearly higher and reached typical values of $\sim 7 \times 10^5$ suggesting that during the 1986 pollution accident and the following years, Cd release was extremely dominant. Although estuarine transformation and (bio)accumulation processes may be different, similar Cd/Pt variations occurred in wild oysters where Cd/Pt molar ratios were of about 3×10^5 in the early 1980s, and then increased from 1985 to 1989 to $\sim 5 \times 10^5$ which best characterizes the ultimate state of the Decazeville pollution source. These findings support the co-evolution trend of the two trace metals Cd and Pt in the Gironde wild oysters as observed in the Cajarc sediment core, and therefore, their common source and parallel transport along the fluvial–estuarine system, i.e. from the industrial site to the coastal ocean. Given that industrial Pt demand was low before the 1990s (Johnson Matthey, 2014), and in the absence of other reported Pt sources in the watershed, we assume that this ratio reflects the oyster response to the sum of Pt exclusively derived from both the natural sources and historic waste reservoirs of the Decazeville industry.

Based on Cd/Pt molar ratios in oysters and assuming that during the whole observation period nearly all Cd in oysters was derived from the metal point source, we estimated respective theoretical Pt concentrations in oysters over time (Fig. 3B). Before 1988, estimated and measured Pt concentrations were similar, which suggests that Pt in the oysters mostly derived from the industrial site. In contrast, increasing measured Pt concentrations and the gap appearing with estimated values after 1988 were attributed to increasing contributions by other sources (Fig. 3B, dashed area). Platinum demand and use for the majority of application sectors in Europe was constant over time, (Johnson Matthey, 2014), resulting in relatively constant inputs from the watershed. However, recent Pt demand in Europe for autocatalysts is an exception. In fact, from 1981 to 1999, Pt demand for catalytic converters in Europe has increased by 30 times and from 1981 to 2007 even by 100 times (Fig. 3C, Johnson Matthey, 2014). Despite the impossibility to access Pt emission data in France, European Pt demand for autocatalysts seems to be a good proxy for Pt use in catalytic converters and therefore Pt emissions. In this sector, Pt use is almost simultaneous with demand since cars are built and sold in a short period of time in answer to current demand. Furthermore, emission data show that the majority of Pt particle emissions occur during the first thousands kilometers of the car (Artelt et al., 1999) resulting in a short delay between Pt demand and Pt emission. The difference between measured Pt and estimated Pt (using Cd/Pt molar ratios in oysters, Fig. 3B, dashed area) seems to be correlated with the European Pt demand for car catalytic converters (since 1988; Fig. 3C). These simulated Pt concentrations rise in oyster samples until reaching a maximum value in 2006. After which they decrease sharply, probably reflecting a break-down in Pt demand in many sectors due to difficult economic conditions during 2008–2010 (Johnson Matthey, 2010). In fact, the largest fall in automobile Pt demand occurred in Europe, where gross car catalytic converters Pt demand decreased on average by 50% (Johnson Matthey, 2014). Recently, increasing Pt concentrations in oysters might result from a new strong European Pt demand for catalytic converters, which started to increase again since 2010 (Johnson Matthey, 2014). Increase in Pt emissions by cars is recorded by wild oysters, assuming a time-lag of 2–4 years. This response time can be explained by (i) transport time from the source to the estuary mouth (~ 400 km), (ii) estuarine particle residence time (~ 2 years; Jouanneau and Latouche, 1982), (iii) estuarine Pt transformation processes (Cobelo-García et al., 2014a) and (iv) the age of wild oysters at the sampling time (2 year old). As well as wild oysters, time-series analysis of mussels from an urban beach revealed excess (or anthropogenic) Pt related to its use in car catalytic converters and car sales in Spain and Portugal (Neira et al., 2015). Temporal trends of Pt accumulation in both wild oysters and mussels from urbanized aquatic system indicate the change in Pt use over time. Accordingly, the data suggest that, the historical metal pollution source

in the Decazeville area was the major Pt source to the Gironde oysters in the past (before 1990), but that Pt emissions from cars have become increasingly dominant in the two past decades.

4.3. Platinum bioconcentration in wild oysters

Experimental studies showed that traffic-related Pt is uptaken by biota such as plants (e.g. Schäfer et al., 1998) or aquatic animals under different exposure pathways (e.g. water containing road dust; Zimmermann et al., 2002). Field studies analyzed Pt uptake of aquatic organisms such as isopods or amphipods (e.g. Rauch and Morrison, 1999; Haus et al., 2007), and more recently bivalves (e.g. Neira et al., 2015; Ruchter and Sures, 2015). In order to estimate the Pt uptake by wild oysters, bioconcentration factor (BCF) which refers to uptake of a chemical substance from water via respiratory surface and/or skin (Arnot and Gobas, 2006), was estimated as:

$$\text{BCF} = C_B / C_{WD} \quad (2)$$

Where C_B is the chemical concentration in the organism (mmol.kg^{-1}) and C_{WD} the freely dissolved chemical concentration in the water (nmol.L^{-1} ; Arnot and Gobas, 2006). We used dissolved Pt concentrations assuming that dissolved metals are generally potentially more bioavailable than their particulate forms (e.g. Lekhi et al., 2008). This statement is supported by preliminary results on Pt uptake pathways with the study of Pt organ distribution in *C. gigas* revealing higher Pt concentrations in gills (unpublished data) suggesting the dominance of direct contamination pathway, i.e. uptake by the gill epithelium of the dissolved metal from the water column. Maximal dissolved trace metal concentration served to calculate the BCF that is known as being the minimal estimation. This concentration was measured recently in the Gironde estuarine system at 0.62 pmol.L^{-1} in the mid-salinity range (Cobelo-García et al., 2014a). Considering 2013 mean Pt concentration in oysters from the La Fosse site of 1.7 pmol.g^{-1} dw, minimum BCF is of about 3×10^3 . Similar values ($\sim 5 \times 10^3$) were observed in wild mussels from the Samil urban beach (Spain; Neira et al., 2015). In comparison, for Cd and Ag respective minimum BCF were estimated at 2×10^5 and 6×10^6 in the same system (Lanceleur et al., 2011) suggesting that bioconcentration of Pt in wild oysters is lower than for those two trace metals. A study on aquatic macroinvertebrates proved that, although Pt concentrations are relatively low in aquatic habitats, high bioconcentration factors, close to those of essential elements, can occur in biota (Haus et al., 2007). Thus, bioconcentration and uptake of trace metals depend on several factors including the feeding behavior of the organism or the trace metal speciation. For instance, Ruchter and Sures (2015) attributed the low concentrations of traffic related metals in clam tissues to the active avoidance of the soluble metal forms and particles in the water column. Furthermore, uptake mechanisms depend also on the Pt form present in the environment, Pt(IV) being more likely to be taken up at a higher rate than Pt(II) (Rauch and Morrison, 1999). The speciation and partitioning of Pt are highly dependent of the residence time of the metal in the water column (Cobelo-García et al., 2008) and Pt partitioning between the particulate and dissolved phase in the Gironde Estuary suggests complex behavior with no clear salinity dependence (Cobelo-García et al., 2014a). Discrepancies between measurements of Pt uptake in biota and other parameters such as the speciation and phase distribution might be related to a number of oversimplifications. More data are needed on Pt biogeochemical cycle, behavior and speciation since all those parameters vary according to the system. Thus, a recent comparison between current and 20 year old dissolved Pt concentration profiles show that anthropogenic Pt has little effect on dissolved Pt in the open oceans (Suzuki et al., 2014). Our study suggests that coastal waters are more affected by atmospherically-derived anthropogenic Pt.

Even though large Pt inputs must originate from car catalytic converters, other urban sources of Pt contamination should be considered.

In fact surface waters draining hospital wastewaters which contain patient excretion of Pt-based anticancer drugs display Pt concentrations which could exceed natural inputs (Vyas et al., 2014). These drugs may have a significantly higher toxicological impact than catalyst-derived inorganic Pt (Lenz et al., 2007). Furthermore, recent works have revealed that particle adsorption, as well as, the reactivity of their respective degradation products decreased from river water to estuarine water (Turner and Mascorda, 2015), implying the need for further research on Pt species in coastal environments.

5. Conclusion and perspectives

Historical records of Pt in sediment cores and biota samples have allowed the identification of the continental sources that contribute to Pt pressure in the Gironde fluvial–estuarine continuum. The results clearly show the succession of two major sources: a former point source originating from industrial activities in the Lot watershed and a recent diffuse source related to the exponentially increasing Pt use in vehicle catalytic converters. Data on Pt levels in wild oysters suggest that (i) Pt in estuaries is bioavailable to bivalves, (ii) oysters are suitable biomonitors of Pt contamination in the environment, and (iii) oysters from the Gironde Estuary have recorded the succession of different Pt sources in the Gironde watershed, covering the past ~35 years (1981–2013). The recent evolution and dominance of catalytic-converters-derived Pt recorded in wild oysters and the widespread use of this technology all over the world clearly suggest that other watersheds also undergo profound changes in their Pt budget. The forecast increase in population density in coastal areas (UN, 2007) will probably strongly enhance Pt pressure in coastal environments. Future research should account for Pt behavior and speciation in estuaries and other marine systems in order to accurately evaluate its bioavailability to marine organisms and the associated toxicity. All of this information would allow better assessment of potential Pt adverse effects on living organisms and ecosystems.

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This study allowed to gain some knowledge on Pt contamination in the Gironde fluvial-estuarine continuum. Platinum concentrations were determined in historical records of sediment cores and wild oysters. Platinum level evolution occurring in both sample sets suggest environmental change of Pt inputs in the system. Main outcomes of the study are the following:

In sediment cores:

- ✓ Regional geochemical background value of $\sim 0.2 \text{ ng.g}^{-1}$ is determined in a riverine sediment core upstream the pollution source.
- ✓ Downstream the pollution source, sediment showed past Pt contamination related to former industrial (smelter) activities in the Lot River watershed. Samples reflect the phasing-out of a historical Pt contamination with Th-normalized Pt concentrations (corrected for grain size effects) for the older part of the core clearly greater than the regional geochemical background value.
- ✓ Sediment cores prove to be suitable geochemical tools for the assessment of Pt inputs evolution in aquatic system as already stated by previous studies (e.g. Rauch and Hemond, 2003).

In wild oysters:

- ✓ Wild oyster samples have recorded the phasing-out of the smelter-related historical industrial Pt contamination and empirical modeling suggests the recent rise of a new source of Pt to the system.
- ✓ Temporal variations of Pt in oysters attributed to this recent source reflect the exponential increase of Pt demand for car catalytic converters, pointing towards the increasing importance of this emerging source to the aquatic system.
- ✓ Mean Pt concentrations in wild oysters of $\sim 0.3 \text{ ng.g}^{-1}$ as observed in the most recent year (2013) correspond to BCF of $\sim 3 \times 10^3$.

4.3. Platinum in sediments and mussels from the northwestern Mediterranean coast: temporal and spatial aspects

The Mediterranean Sea is a semi-enclosed system with ever growing anthropogenic pressure along its northwestern coast (Martín et al., 2009) that comprises several highly populated cities with extensively urbanized and industrialized areas. Given the progressive urbanization of this coastal zone and the possible changes of seawater chemistry due to additional anthropogenic inputs, previous studies have addressed environmental quality of the Mediterranean Coast and revealed heavy pollution in metals, (Oursel et al., 2013; Tessier et al., 2011), metal-organic components (Pougnnet et al., 2014) and nutrients (Ruggieri et al., 2011), especially in historically exposed sites and major harbors.

The Toulon Bay (southwest, France) is located on the Mediterranean coast. This sampling site has been strongly polluted by anthropogenic wastes originating from both historic (bombardments, fleet scuttling during World War II: one hundred ships destroyed and sunk, mainly in the Small Bay) and ongoing activities (French Navy, commercial traffic, urban raw sewage release, industry, tourism; (Dang et al., 2015; Tessier et al., 2011). Accordingly, high levels of inorganic and organic pollutants are accumulated in sediments of the Toulon Bay (Dang et al., 2015; Pougnnet et al., 2014; Tessier et al., 2011).

The objective of this study is **to provide insights into Pt contamination levels (i) in sediments and mussels from the Toulon Bay at historical timescales and (ii) in recent mussels from different sites along the northwestern Mediterranean coast.**

For this, different items were investigated:

- The Pt concentrations in sediment cores from the Toulon Bay with sediment layers dating from the 1890's to the late 2000's.
- The analysis of Pt levels in farmed mussels *M. galloprovincialis* from the Toulon Bay collected every two years since the 1980's to the 2010's.
- A biomonitoring study using wild mussels from contrasting sites along ~ 300 km of Mediterranean coast.
- The assessment of mussel *M. galloprovincialis* as a sentinel organism of Pt contamination.

Outcomes from this study are ready for submission in the peer-reviewed journal ***Chemosphere***.

Platinum in sediments and mussels from the northwestern Mediterranean coast: temporal and spatial aspects

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Abstract

Platinum (Pt) is considered a Technology Critical Element (TCE) and an emerging metallic contaminant with increasing release into the environment. Gaps in knowledge and understanding of environmental levels, fate and effects of Pt still exist, especially in the marine environment. This work presents Pt concentrations in the northwestern Mediterranean coast including: (i) temporal variability from sediment cores and farmed mussels in the Toulon Bay (historically affected by intense human activities) and (ii) spatial distribution from recent wild mussels collected along ~ 300 km coastline with contrasting ecosystems (including natural reserves), quantified using voltammetry and inductively coupled plasma-mass spectrometry. The historical (> 100 years) record of Pt in sediments from the Toulon Bay suggests the existence of non-negligible Pt sources older than those related to vehicle emission devices, such as petrol industry and coal-fired activities. A strong Pt increase in more recent sediments (from ~ 12 to 16 ng.g⁻¹) and mussels (8-fold increase from ~ 0.12 to 0.80 ng.g⁻¹) covering the past 25 years reflect the overall evolution of Pt demand in Europe (~ 20-fold increase for vehicle catalysts in 20 years). Spatial biomonitoring of Pt in mussels along the northwestern Mediterranean coast is assumed to reflect inter-sites differences of Pt exposure (0.09 to 0.66 ng.g⁻¹) despite seasonal effect on tissue development. This study highlights the need for thorough and regular monitoring of Pt levels in sediments and biota from urbanized coastal areas in order to better assess the environmental impact of this TCE, including potential risks for marine organisms.

Keywords: Platinum; sediments; mussels; biomonitoring; Mediterranean coast

1. Introduction

Platinum is a Technology Critical Element (TCE) and an emerging metallic contaminant for which there is a growing need for accurate assessment of environmental distribution and impacts (Cobelo-García et al., 2015). This element is increasingly used in various applications due to its high chemical resistance and excellent catalytic properties. Anthropogenic activities account for more than 80 % of Pt fluxes at the Earth surface (Sen and Peucker-Ehrenbrink, 2012). Today, the most extended application is the use of Pt in vehicle catalytic converters to control air pollution by cars, trucks, motorcycles and non-road mobile machinery, accounting for more than 70 % of the European Pt demand (Johnson Matthey, 2017).

Increasing Pt emissions from automobile exhaust catalytic converters have led to an increase in Pt concentration in many environmental compartments including atmosphere, soils, sediments, seawater and biota, with anomalies occurring even in very remote, supposedly pristine areas such as the Antarctic snow (e.g. Neira et al., 2015; Obata et al., 2006; Rauch et al., 2004, 2006; Schäfer et al., 1999; Schäfer and Puchelt, 1998; Soyol-Erdene et al., 2011). In spite of growing efforts to improve the understanding of biogeochemical Pt cycles, there is still a lack of relevant environmental data. This is particularly the case in marine environments, mainly due to analytical challenges when analyzing Pt at ultra-trace levels in complex environmental matrices (i.e. seawater, sediments and biota). In fact, only few field studies report on Pt levels, distribution and geochemical behavior in coastal ecosystems. Coastal areas are considered as vulnerable sites due to their anthropogenic socio-economic roles, hosting ~ 70 % of the urban populations and economic activities worldwide (Small and Nicholls, 2003). This pressure implies the risk of serious environmental consequences (McGranahan et al., 2007) such as trace metal contamination and possible transfer to the marine food web. Evidence of measurable anthropogenic Pt inputs into the coastal environment are especially based on the analysis of sediment cores (e.g. Rauch et al., 2004; Tuit et al., 2000). The few papers reporting on Pt in coastal seawater suggest that supposedly uncontaminated surface seawater shows dissolved Pt concentrations in the range of 0.05 - 0.1 ng.L⁻¹ (e.g. Cobelo-García et al., 2014a; Goldberg et al., 1986). The even more limited number of field studies reporting on Pt concentrations in marine organisms prove Pt bioavailability and relatively high potential for Pt bioconcentration, especially in marine bivalves (Abdou et al., 2016; Neira et al., 2015). Accordingly, these organisms appear as suitable biomonitors to assess Pt levels in marine environments (Abdou et al., 2018).

The Mediterranean Sea is a semi-enclosed system with ever growing anthropogenic pressure along its northwestern coast (Durrieu de Madron et al., 2011; Martín et al., 2009) that comprises several highly populated cities with extensively urbanized and industrialized areas. The progressive urbanization of this coastal area and the possible changes of seawater chemistry due to additional anthropogenic inputs have already been reported in previous studies addressing environmental quality of the Mediterranean coast. Indeed, evidences exist on heavy metal pollution (e.g. Oursel et al., 2013; Tessier et al., 2011), metal-organic components (e.g. organotin; Pougnet et al., 2014) and nutrients (Ruggieri et al., 2011), especially at historically exposed sites and major harbors, such as the Toulon Bay.

The present work aims at providing an assessment of Pt levels in: (i) historical samples (sediment cores and farmed mussels) from an industrial and strongly urbanized Mediterranean harbor (Toulon Bay, France), and (ii) wild mussels from selected, contrasting sites along 300 km of the northwestern Mediterranean coast, including industrial sites supposedly influenced by Pt-releasing anthropogenic activities.

2. Material and Methods

2.1. Study area, sampling sites, and sample collection

The northwestern Mediterranean coast is characterized by high urbanization and numerous harbors, with areas that have faced centuries of intense anthropogenic pressure due to urban and industrial emissions, trade, war material spills, navigation, dredging and fishery among other activities. Progressive intense urbanization has led to a general anthropogenically-derived chronic pollution by multiple contaminants of various origins (e.g. trace metals, Oursel et al., 2013). Different sets of samples were collected at contrasting sites in France and Italy, covering a coastline of ~ 300 km in the northwestern Mediterranean (Fig. 1).

The Toulon Bay

Long-term evolution of Pt in a harbor affected by strong multi-component pollution was studied in sediment cores and farmed mussels from the Toulon Bay (TB, Fig. 1). The Toulon Bay is divided into two parts by an artificial embankment, forming the Large Bay and the Small Bay (LB and SB, respectively) and receives urban inputs from Toulon city (~ 600,000 inhabitants, Tessier et al., 2011). The Toulon Bay has been strongly polluted by anthropogenic wastes originating from both historic (bombardments and fleet scuttling during World War II: one hundred ships destroyed and sunk, mainly in the Small Bay) and ongoing activities (French Navy, commercial traffic, urban raw sewage release, industry, tourism; Dang et al., 2015;

Tessier et al., 2011). Accordingly, high levels of inorganic and organic pollutants are accumulated in sediments from the Toulon Bay (e.g. Dang et al., 2015; Pougnet et al., 2014; Tessier et al., 2011). Both sediment cores and mussels were sampled in the SB characterized by a semi-closed shape with a surface of $\sim 9.8 \text{ km}^2$ where the morphology and the artificial barrier limit water circulation favoring accumulation of sediment and associated contaminants (Fig. 1, Tessier et al., 2011).

The sediment cores were sampled by the laboratory PROTEE from November 2008 to June 2009 in the context of a wider survey on sediment quality in the Toulon Bay (Tessier et al., 2011). The two sediment cores analyzed in this study were collected at sampling site TB1 located in the eastern part of SB, close to a former Navy submarine base and in the main navigation channel; and at site TB2 located in the western part of SB, close to a fish/mussel farming area (Fig. 1). The sediment cores were processed as previously described in Tessier et al. (2011). Briefly, 10 cm diameter and 1 m long (Interface Corer, Plexiglas® tube) sediment cores were transported to the laboratory PROTEE directly after sampling and sliced (2 cm resolution) under inert atmosphere (N_2) to preserve redox conditions. The sliced sediment samples were pooled and homogenized (Teflon spoon), placed into pre-cleaned 1 L high-density polyethylene (HDPE) bottles and deep frozen (-18°C). All frozen samples were subsequently freeze-dried (within one month) and sieved (2 mm).

Farmed mussels (35 - 65 mm, *Mytilus galloprovincialis*) were sampled annually since 1984 at the sampling site TB2 (Fig. 1) by the RNO-ROCCH mussel watch program (IFREMER). Freeze-dried soft tissues (pooled total soft bodies of more than 30 individuals) from the National Mussel Watch sample bank (storage at room temperature and in the dark) were selected, covering the period from 1984 to 2014 at 3-years intervals.

Mediterranean coastline

Wild mussels (*M. galloprovincialis*) were sampled at contrasting sites along the northwestern Mediterranean coastline: in Banyuls-sur-Mer, Sète, Saintes-Marie-de-la-Mer, Faraman, and Toulon Bay (respectively BM, ST, SMM, FM, TB; Fig. 1) and in the South of the Corsica Island (Bonifacio, BF, Fig. 1) by the IRSN (French Institute for Radioprotection and Nuclear Safety) within a regular monitoring program (2016-2017). In addition, wild mussels from the Genoa Harbor were collected (GH, Fig. 1) during a field campaign held in April 2016. After manual collection, mussels ($\sim 7 \text{ cm}$) were cleaned, opened, and soft tissue and shells were weighed separately. The Condition Indices ($\text{CI} = \text{Visceral Content, wet weight} / \text{Shell, wet weight}$; Strady et al., 2011) were determined for all the samples. For samples collected along the French

coastline, each sampling corresponded to the collection of ~ 240 individuals that were pooled. For samples collected in the Genoa Harbour, 10 organisms were processed individually. Mussel tissues were then oven-dried at 80 °C to constant weight and dried tissues were kept at room temperature in the dark pending analysis.

Seasonal trace metal level variations have been observed in *M. galloprovincialis* related to variable soft-body weight during reproduction periods and to other environmental factors (Charmasson et al., 1999). In order to observe the seasonality of Pt concentrations, mussel samples from Winter (December – February) were analyzed together with mussels from Summer / Fall (July – October) from the same site, when both sets of samples were available. For the sampling sites BM, BF, and GH, only mussel samples from dates within the reproduction cycle were analyzed (July 2016, October 2016, and April 2016 respectively).

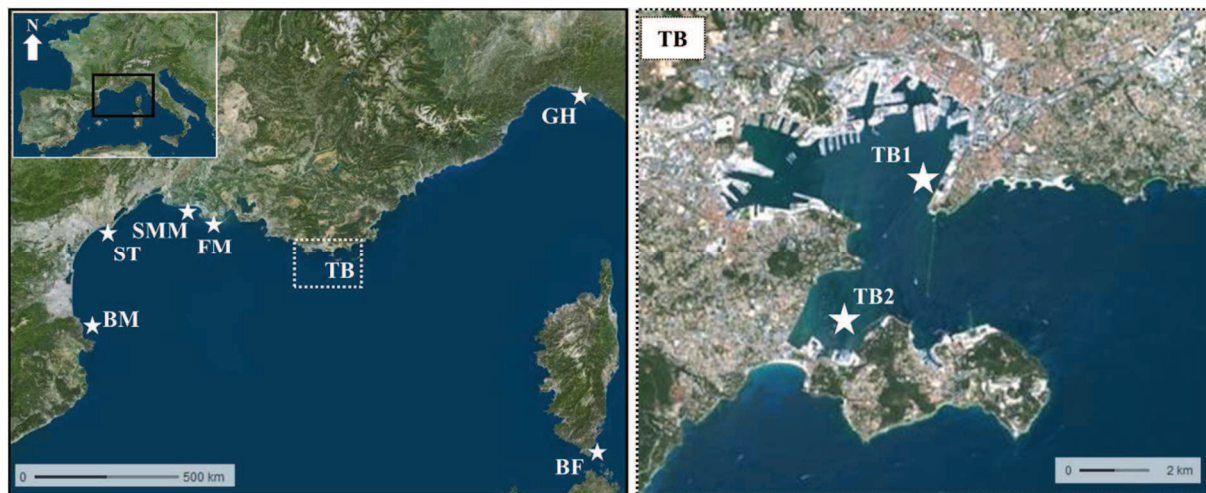


Fig. 1: Sampling sites along the northwestern Mediterranean coast. From east to west BM: Banyuls-sur-Mer, ST: Sète, SMM: Saintes-Maries-de-la-Mer, FM: Faraman, TB: Toulon Bay with the two sampling sites TB1 and TB2 (zoom on the right panel), and GH: Genoa Harbor, Italy.

2.2. Analytical methods

Sediment

Platinum concentrations in sediment core samples were determined by Adsorptive Cathodic Stripping Voltammetry (AdCSV) as described in Cobelo-García et al. (2014b). Measurements were carried out using a μ Autolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm® 663 V.A.) equipped with three electrodes: (i) a hanging mercury drop electrode (HMDE, the working electrode), (ii) a silver/silver-chloride (Ag/AgCl) reference electrode, and (iii) a glassy carbon auxiliary electrode. A polytetrafluoroethylene (PTFE) voltammetric cell served in all experiments and the potentiostat was controlled using the NOVA 2.1 software. About 50 mg of sediment samples were ashed in porcelain crucibles at 800 °C during 3 h (removal of Organic Matter, OM). According to the protocol for sediment treatment described by Cobelo-García et al. (2011), ashed samples were transferred into 30 mL perfluoroalkoxy (PFA) vessels with screw caps (Savillex®) and acid-digested at 195 °C for 4 h using 5 mL HCl and 3 mL HNO₃ (30 % HCl and 65 % HNO₃ Suprapur, Merck®). After this step, vessel caps were removed and the acids evaporated at 195 °C until near dryness. The residue was re-dissolved using 1 mL of sulfuric acid (H₂SO₄, 93 – 98 % Trace metal grade, Fisher Chemical®) at 195 °C. Cooled contents were diluted using 0.1 M HCl, centrifuged at 4000 rpm for 10 min (20 °C), and made up to 25 mL in pre-clean Teflon bottles (Nalgene®).

Aliquots of acid-digested sample were pipetted into the voltammetric cell together with 30 μ L of 3.3 mM formaldehyde (37–41 % Analytical Reagent Grade, Fisher Chemical®), 30 μ L of 0.45 mM hydrazine sulfate (Analytical Reagent Grade, Fisher Chemical®), and 300 μ L of H₂SO₄. Platinum concentrations were determined by standard addition method (adding mono-elementary Pt standard solution 1000 μ g.mL⁻¹ PLASMACAL, SCP Science®) using a deposition time of 180 s and experimental parameters as described elsewhere (Cobelo-García et al., 2014b).

Mussels

A previous inter-method comparison using two completely independent digestion and measurement techniques (Inductively Coupled Plasma-Mass Spectrometry: ICP-MS and AdCSV) has shown that Pt concentrations in natural biological samples (oysters) can be reliably measured by ICP-MS when sufficient sample mass (i.e. \sim 0.25 g dry weight, 0.13 ng Pt) is available (Abdou et al., 2018). Indeed, hafnium-oxides (HfO⁺) interferences that may prevent correct analysis of Pt in biological matrices (Godlewska-Żyłkiewicz, 2004; Pyrzynska, 2015) can be mathematically corrected using the ¹⁹³Ir (iridium) signal that is highly interfered by HfO⁺

(Abdou et al. 2018, Djingova et al., 2003). The ICP-MS method can therefore be an alternative to AdCSV for detection of Pt in biological samples. Due to relatively low sample mass available for farmed mussels from the Toulon Bay, these samples were analyzed by AdCSV applying the same procedure as for sediment cores, using a deposition time of 180 s. In contrast, sufficient sample mass was available for the wild mussel samples from the Mediterranean coastline, allowing for the time saving analysis using ICP-MS (ICP-MS, Thermo, X Series II). About 1.5 g of sample was ashed at 800 °C in porcelain crucibles. After cooling, ashed residues were transferred into acid-cleaned polypropylene (PP) tubes (DigiTUBEs, SCP SCIENCE®), and digested at 110 °C for 3 h with 2 mL HCl and 1 mL HNO₃ (30 % HCl and 65 % HNO₃ Suprapur, Merck®) as described in Abdou et al. (2018). Cooled contents were then diluted in 10 mL MilliQ water, centrifuged at 4000 rpm for 10 min (20 °C). Analyses were performed applying the standard addition method (using mono-elementary Pt standard solution 1000 µg.mL⁻¹ PLASMACAL, SCP Science®).

Quality control

Quality control was performed using the only Certified Reference Materials available for Pt being the BCR®-723 road dust (IRMM) and the Jsd-2 sedimentary rocks (indicative value from GSJ). Platinum quantification by AdCSV provided satisfactory recovery of 89% for BCR®-723 and 98% for Jsd-2 (n = 3) as well as ICP-MS analyses giving recovery values of 87% and 101% respectively. Precision of the analysis, expressed as Relative Standard Deviation (RSD), using both methods were below 10%. The detection limit for particulate (sediment and biota) Pt measured by AdCSV (calculated as 3 x blank standard deviation, n = 5) was estimated to 0.03 ng.g⁻¹ for typical mass of 50 mg. The detection limit for Pt measured in biological samples by ICP-MS (n = 10 blanks) was estimated to 0.003 ng.g⁻¹ for typical biological sample masses of ~ 1.5 g.

3. Results

3.1. Historical record of Pt concentrations in sediments and mussels from the Toulon Bay

Platinum concentrations in the two sediment cores TB1 and TB2 ranged from 6 ng.g⁻¹ to 15 ng.g⁻¹ (Fig. 2A). Samples in the TB1 core showed relatively constant Pt levels of ~ 10 ng.g⁻¹, with only a small variation at 5 cm depth showing Pt concentration of ~ 7 ng.g⁻¹. In contrast, the core TB2 showed a general trend of Pt concentrations decreasing with depth, i.e. the highest concentrations occurred in the top of the core and the lowest values in the bottom (Fig. 2A). Overall, the Pt concentrations tended to be lower in the core TB1 than in TB2. When comparing sediment samples from different depths or different cores, one has to exclude that the observed variations are due to grain size effects because particle size fractionation may lead to increasing trace metal concentrations with decreasing grain size (Loring and Rantala, 1992). Accordingly, grain size effects on natural, lithogenic trace metal variability must be corrected by normalization over a grain size dependent lithogenic element before assessing anthropogenic inputs (Loring and Rantala, 1992). Aluminum (Al) has been chosen as the normalization element for the Toulon Bay and many other systems (e.g. Ho et al., 2012; Pougnnet et al., 2014; Tessier et al., 2011), as Al contents are influenced by natural sedimentation and the effects of enhanced erosion, but not by pollution (Li and Schoonmaker, 2003; Luoma and Rainbow, 2008). In fact, correlation ($R^2 = 0.94$) between potassium (K) and Al concentrations in surface and deep sediment showed the close relationship between Al and clay minerals, supporting the pertinence of Al-normalization (Tessier et al., 2011). In both cores, Al-normalization did not modify the profiles, suggesting that grain size effects on spatial (horizontal and vertical) Pt distribution are negligible (Fig. 2B). These results suggest that in the sediment core TB2 (i) nearly all layers are enriched in Pt compared to the core TB1 and (ii) there is a clear increasing trend in Pt enrichment from the bottom to the top of the core (3-fold; Fig. 2).

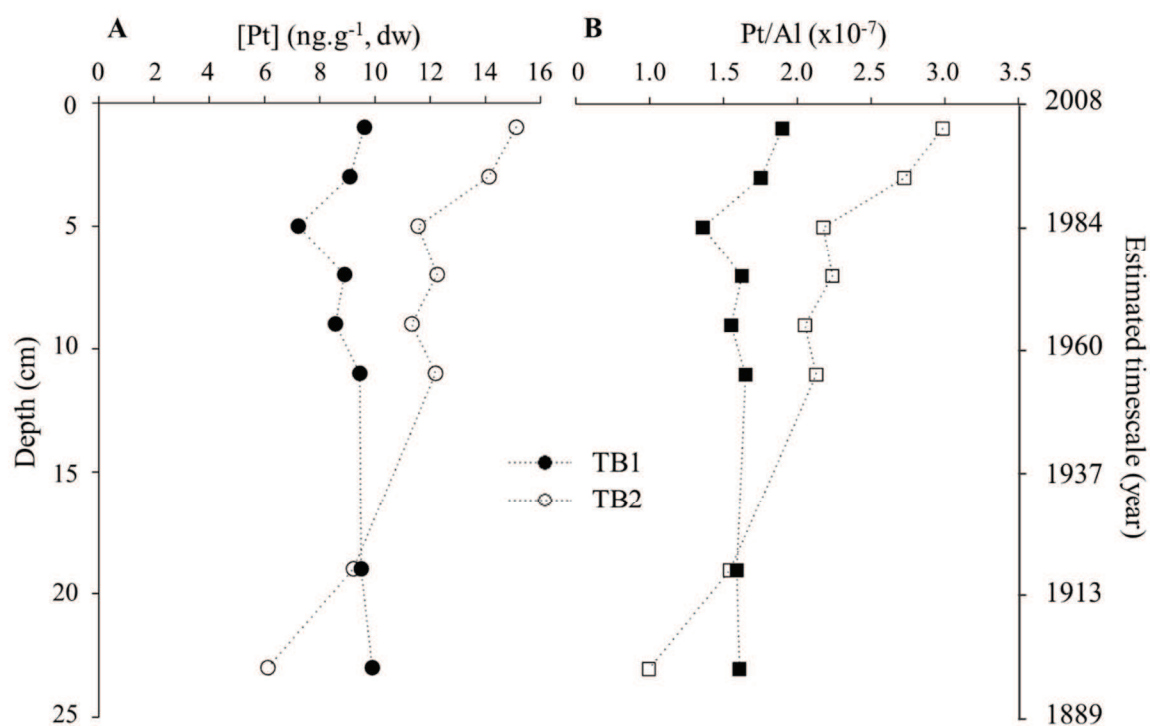


Fig. 2: Depth profiles of Pt concentrations (A) and Al-normalized Pt concentrations (B) in sediment cores from the Toulon Bay. Sediment cores were collected at site TB1 (filled symbols) and TB2 (empty symbols). A: Platinum concentrations (ng.g^{-1} , dw: dry weight, circles), B: Aluminum-corrected Pt concentrations ($\times 10^{-7}$, squares). Aluminum concentrations are from Tessier et al. (2011).

Platinum concentrations in farmed mussels from the Toulon Bay ranged from 0.10 to 0.79 ng.g^{-1} , in the 1984 to 2014 period (Fig. 3). Platinum concentrations were relatively low and constant ($\sim 0.15 \text{ ng.g}^{-1}$) from 1984 to 1990, followed by a clearly increasing trend over time towards a maximum value of 0.79 ng.g^{-1} in 2014, which is ~ 8 times greater than the levels of the 1980's. In the same period, the European Pt gross demand for car catalytic converters evolved from 1-2 tons.y^{-1} in the late 1980s to $\sim 40 \text{ tons.y}^{-1}$ from 2010 to 2014 (Fig. 3).

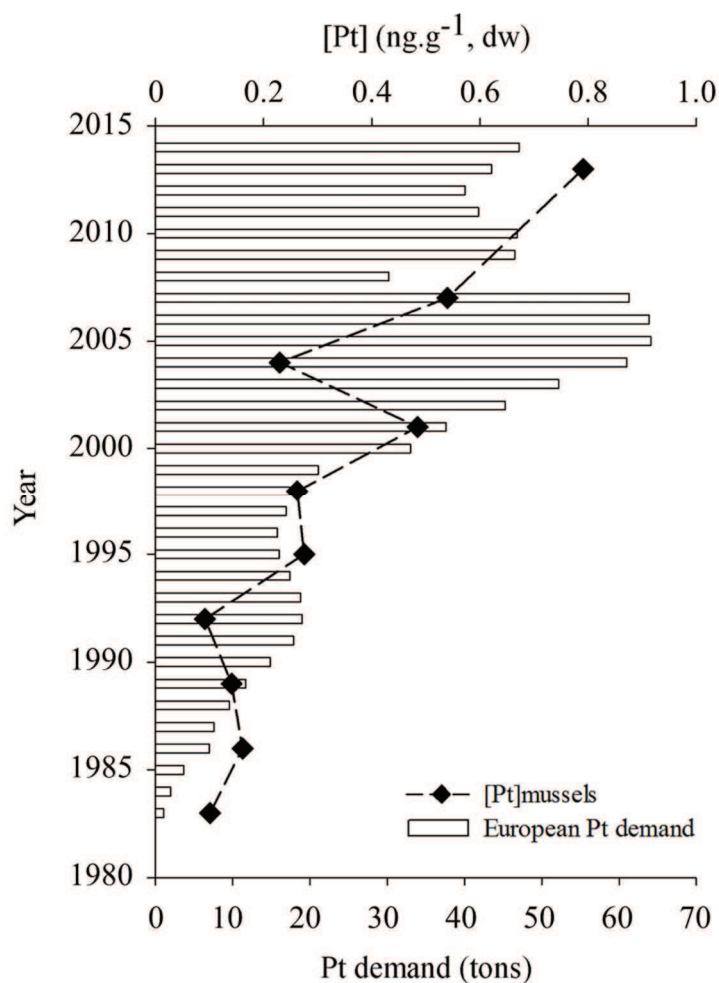


Fig. 3: Platinum concentrations in farmed mussels from the Toulon Bay (ng.g^{-1} , dw; sampling site TB2) and European Pt gross demand (metric tons, after Johnson Matthey, 2017) for car catalytic converters during the 1984-2014 period.

3.2. Platinum concentrations in mussels from the northwestern Mediterranean coast

Platinum concentrations in wild mussels from contrasting sites, expressed in ng.g^{-1} dw ranged from ~ 0.09 to 0.66 ng.g^{-1} (Fig. 4). Winter mussels were compared to Summer / Fall mussels, whenever possible as described in section 2.1.

Winter mussel Pt concentrations varied from ~ 0.33 to 0.55 ng.g^{-1} (Fig. 4). For those mussels, Pt concentrations at the different sites tended to increase from the western to the eastern sites along the coast (ST to TB). Winter mussels from Sète sampling site showed the lowest Pt levels, while highest concentrations occurred in mussels from Faraman site and from the Toulon Bay.

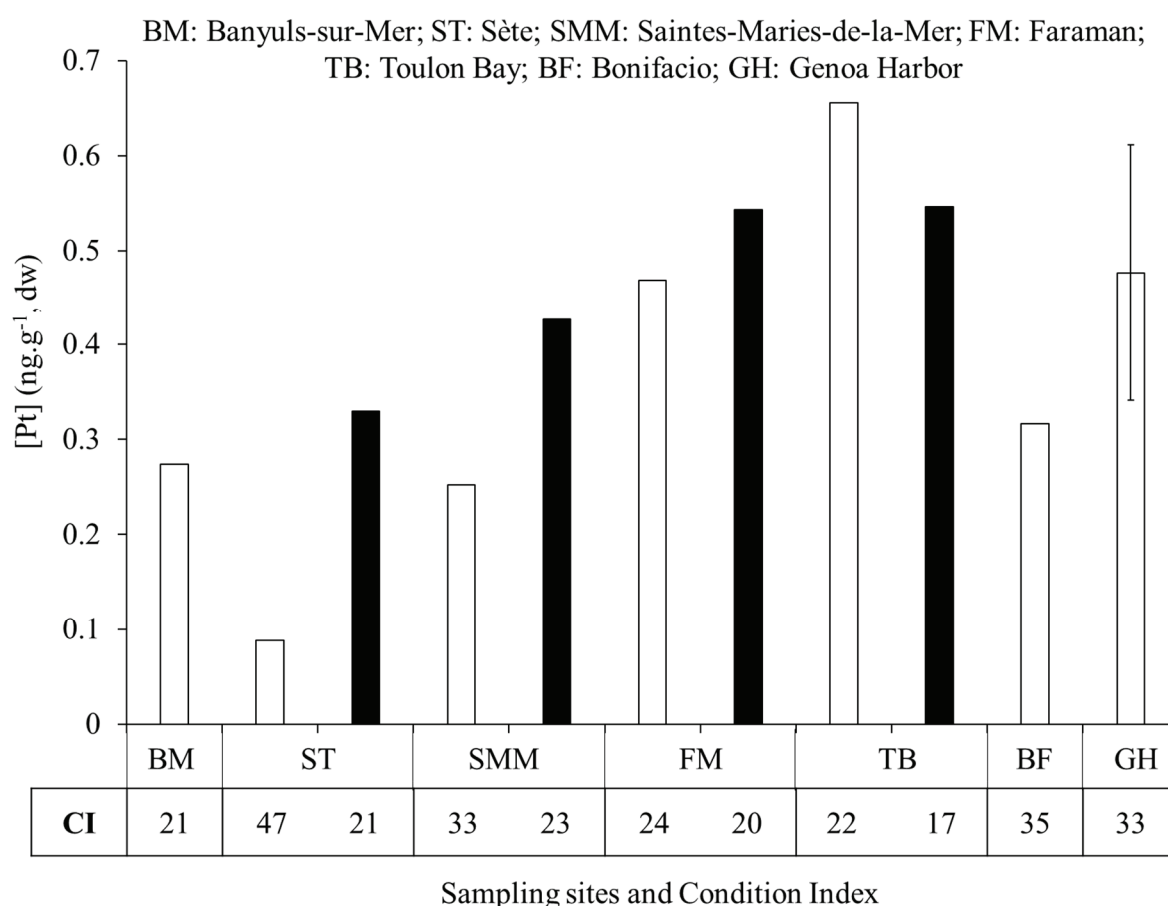


Fig. 4: Platinum concentrations (ng.g^{-1} , dry weight: dw) in wild mussels sampled along the northwestern Mediterranean coast. Winter and Summer / Fall mussels were analyzed for the same sampling sites when possible. Black bars correspond to sample collection during Winter, whereas white bars correspond to sample collection during reproductive season (Summer / Fall); CI: average Condition Index of the mussel pools for the French coastline ($n \sim 240$) and of the 10 individuals from the Genoa Harbor. Error bar represents standard deviation.

Summer / Fall mussel Pt concentrations ranged from ~ 0.09 to 0.66 ng.g^{-1} , with mussels from Sète showing the lowest concentrations at a given sampling period, while highest concentrations occurred in mussels from the Toulon Bay. Platinum concentrations were also determined in wild mussels collected in the Genoa Harbor in April 2016. Ten individuals were analyzed separately, providing an average Pt concentration of $0.48 \pm 0.14 \text{ ng.g}^{-1}$ (GH, Fig. 4). Condition Indices varied from 17 to 47 between sites and sampling periods. Summer / Fall individuals showed CI ranging from 21 to 47 with a mean value of 31, while it varied from 17 to 23 with a mean value of 20 in Winter organisms.

4. Discussion

4.1. Historical records of Pt concentrations in the Toulon Bay

Two complementary approaches to study the temporal evolution of Pt in the Toulon Bay rely on the analysis of environmental archives: sediment cores and mussel samples from a historical sample bank, implying different time scales and resolution.

Historical record in sediments

Average sedimentation rate in the Toulon Bay sediments was estimated from the vertical distribution of ^{210}Pb activity in the sediment cores in previous work (Tessier et al., 2011), suggesting an average sedimentation velocity of $0.21 \pm 0.05 \text{ cm.y}^{-1}$. Accordingly, the studied sediment cores cover the period $\sim 1890 - 2008$ and the top 10 cm layer would reflect ~ 50 years of history (Tessier et al., 2011). As Al-normalization performed to correct for variations in sediment nature and grain size (Sageman and Lyons, 2003) did not modify the Pt profiles in the Toulon Bay sediments (Fig. 2B), the observed variations are attributed to anthropogenic inputs, rather than lithogenic processes, as described elsewhere (e.g. Almécija et al., 2016).

The assessment of the degree of contamination in a given environmental sample requires the establishment of references representing “uncontaminated” conditions in comparable sample types. Classically, authors have compared their results with average values of the Upper Continental Crust (UCC, e.g. Abdou et al., 2016; Cobelo-García et al., 2011; Rauch et al., 2004). This first approach may suggest that the Pt concentrations in the Toulon Bay sediments are clearly higher (up to 30 fold) than the UCC average (0.5 ng.g^{-1} , Rudnick and Gao, 2003). More representative reference samples may be locally identified either in (i) remote areas, presumably not affected by the contaminant (spatial approach) or in (ii) samples representing conditions during periods before the contaminant release (temporal approach). Given the high spatial variability in (i) Pt global contaminant dispersion, measurable even in remote sites (e.g.

polar ice, Soyol-Erdene et al., 2011) and (ii) heterogeneous sediment nature at a large spatial scale, we have considered to apply the temporal approach to reliably determine the contamination degree of Pt in the Toulon Bay.

In fact, Pt concentrations in the bottom layers of the sediment cores, i.e. $\sim 10 \text{ ng.g}^{-1}$ in core TB1 and $\sim 6 \text{ ng.g}^{-1}$ in core TB2, supposedly dating from the early 20th century, could serve as the local reference level for relatively uncontaminated sediment. These values may appear high, compared to background values reported for ancient sediments in the Tagus Estuary ($0.2 - 0.3 \text{ ng.g}^{-1}$, Cobelo-García et al., 2011) or for the UCC Pt content. They are, however, similar in magnitude as Pt records from marine pristine sediment cores (~ 1 up to 5 ng.g^{-1} in Atlantic abyssal pelagic sediment; Colodner et al., 1992) and other pelagic sediments from a variety of locations in the Pacific ocean (0.4 to 22 ng.g^{-1} , Koide et al., 1991), both sampled before the recent emergence of Pt as a global contaminant. Furthermore, pre-anthropogenic coastal sediments from the Massachusetts Bay showed similar Pt levels than bottom layers from the Toulon Bay ($\sim 5 \text{ ng.g}^{-1}$, Tuit et al., 2000). These observations together with high Pt levels in South Pacific pelagic sediment ($\sim 30 \text{ ng.g}^{-1}$, Lee et al., 2003) and in marine manganese nodules (up to 900 ng.g^{-1} , Koide et al., 1991) tend to suggest that relatively high Pt concentrations in coastal sediments may, at least partly, be attributed to the presence of marine particles and not necessarily derive from terrigenous and/or anthropogenic sources. Accordingly, the Toulon Bay bottom sediments may be considered as the local, pre-industrial reference (especially core TB2). In fact, the Toulon Bay receives water from the open Mediterranean Sea via the Ligurian Current flowing from east to west including deep water areas (Duffa et al., 2011), transporting particles of marine origin into the bay. These findings highlight the need for better knowledge of marine particle baseline Pt concentrations in the Mediterranean Sea and worldwide, as a prerequisite to accurately evaluate anthropogenic contamination in coastal particles and sediments by distinguishing geochemical background from anthropogenic signals.

The Pt/Al values suggest increasing concentrations from the bottom to the top in the core TB2 (~ 3 -fold increase from the bottom to the top layers; Fig. 2B). However, the relatively small recent increase in Pt/Al values ($\sim 20 \%$ in the period ~ 1984 -2008) combined with the relatively high values in the bottom of the core TB1 may be due to its position in the eastern part of the SB, more exposed to the marine incoming water than the site TB2 (counterclockwise circulation in the Toulon Bay; Duffa et al., 2011). In addition, the site TB1 is located close to a main navigation channel, thus, one cannot fully exclude post-depositional Pt redistribution of the sediment. Post-depositional Pt redistribution processes might include both physical mixing (e.g. navigation, dredging...) and chemical redistribution related to diagenesis processes (Tuit et al.,

2000). Chemically-driven Pt mobility was already observed in other coastal sediments showing maximum Pt enrichment at the base of the surface mixed layer after scavenging at the oxic/anoxic boundary (Cobelo-García et al., 2011). Furthermore, a sediment core from the Boston Harbor also showed chemical mobility of Pt, implying Pt remobilization in sediments at short time scales (Tuit et al., 2000). According to Colodner et al. (1992), a fraction of Pt in marine sediments is labile, suggesting that Pt may be scavenged from seawater into both Fe-Mn oxyhydroxide minerals and organic-rich sediments, so that these phases might dominate the Pt distribution in sediments. Previous work has compared trace metal (Cu, Hg, Pb, and Zn) distributions in the same cores from the Toulon Bay showing some evidence of partial mixing in the core TB1 (Dang et al., 2015; Tessier et al., 2011).

In contrast, the position of the core TB2 in a shallow zone with low water regeneration (Tessier et al., 2011) and less impacted by currents, ship traffic and/or dredging, suggests negligible sediment reworking. In this core, the continuous increase of Pt/Al over the past century before the mid-1980s cannot be explained by the relatively new technology of car catalytic converters, implying additional, yet less reported anthropogenic Pt sources to the marine environment. In fact, Pt concentrations in surface sediments from the Boston Harbor dating from 1978 (i.e. integrating Pt emissions before the introduction of car catalysts) showed a 17-fold enrichment of Pt concentrations relative to background values (i.e. up to 12.5 ng.g^{-1} , Tuit et al., 2000). This observation suggests that, despite the higher Pt release rates of the early catalytic converters and considering the relatively low number of catalyst-equipped cars at that time, other, probably industrial sources may have affected the Pt levels in harbor sediments (Tuit et al., 2000). Older Pt emissions might be related to the use of Pt in several industrial applications, since Pt played an important role in various catalytic processes (e.g. catalytic cracking of crude petroleum) applied for the production of the very high octane fuels required for aircraft and automobile engines during World War II (Peavy, 1958). After the war, Pt catalyst reforming processes, introduced to the refining industry in 1949, produced huge amounts of lower octane fuels for automobile engines, and industrial / home heating fuels. Accordingly, the years 1950 to 1960 were considered as the industry's "platinum period" (Peavy, 1958). In addition, emissions from intense coal industry during the first half of the 20th century, may have contributed to elevated Pt concentrations, since high Pt concentrations were reported for coal samples ($\sim 5 \text{ ng.g}^{-1}$, Finkelman and Aruscavage, 1981; up to 70 ng.g^{-1} , Dai et al., 2003). As a result of the scuttling of the French Navy fleet during WWII (November 1942, Tessier et al., 2011), huge amounts of industrial coal are still present in the surface sediments inside the Toulon Bay (Garnier et al., unpublished data). Although the Pt concentrations in this coal are unknown, given the range of values reported in the literature, industrial coal and/or fly ashes could partly explain the Pt

accumulation in the core TB2, with a stronger increasing trend before ~ 1960, and a period of weaker increase during ~ 1960 – 1990.

Recent increase of Pt concentrations (~ 50 % increase in ~ 24 years in core TB2; Fig. 2) is consistent with observations in other coastal environments, such as the Tagus Estuary. In this system, Pt values at the top of the sediment core (up to 9.5 ng.g⁻¹, Cobelo-García et al., 2011) representing ~ 30 - 40 times the local background are observed. Platinum concentrations in saltmarsh sediments impacted by high traffic in the same estuary showed a surface peak value exceeding 40 ng.g⁻¹ (Almécija et al., 2015). This recent contamination has been clearly attributed to increasing Pt demand, use and emission related to the introduction of car exhaust catalytic converters since the early 1990's (Rauch and Morrison, 2008). Considering Pt concentration originating from catalysts of ~ 4 ng.g⁻¹ for the topmost samples in sediment core TB2, Pt deposition rates are estimated to ~ 15 g Pt.y⁻¹ based on an annual sedimentation rate of ~ 0.2 cm.y⁻¹, a density of wet sediment of 0.64 g.cm⁻³ and a porosity of 0.7, as well as a SB surface of 9.8 km² (Tessier et al., 2011). A rough estimation of Toulon Bay catalyst emission of ~ 10 to 20 g Pt.y⁻¹ is obtained assuming ~ 200,000 vehicles equipped with catalysts with an average yearly mileage of 1,000 km.vehicle⁻¹ and an average emission rate ranging between 50 and 100 ng Pt.km⁻¹ (based on values from Rauch and Peucker-Ehrenbrink, 2015). As observed on a global scale in Rauch et al. (2005), the difference between emission and deposition rates may originate from several factors including over-/underestimated emission rates which is influenced by the age and the type of vehicle (e.g. Moldovan et al., 2002) or the occurrence of recent additional sources (e.g. wastewaters and Pt from anticancer-drugs, Vyas et al., 2014). Exportation of fine-grained urban particles towards the outer bay or the open sea by currents and/or potential partial Pt dissolution from urban particles in contact with seawater may also occur (Cobelo-García et al., 2014a). In addition, dissolved Pt will probably be diluted and exported rather than accumulated in sediments. Despite potential errors in the estimations, calculated Pt emission rates from car catalysts in the SB are generally in good agreement with recent Pt deposition rates in sediment core TB2 and might therefore explain a large fraction of total Pt deposition in the area.

Historical record in mussels

The temporal evolution of Pt concentrations in mussels farmed close to the site of core TB2 (Fig. 1) reflected a ~ 8 -fold increase over the 1984 to 2014 period, especially since the mid-1990s (Fig. 3). Together with the ~ 20 -fold increase in European Pt demand for car catalysts over the same period, this observation suggests that vehicle-emitted Pt has been introduced into the coastal ecosystem of the Toulon Bay with an effective transfer to living marine organisms. These first results for Pt in Mediterranean bivalves are in line with increasing Pt/Al values in the TB2 core from the same site and with previous work on wild oysters and mussels from the French and the Spanish (Galician) Atlantic coast (oysters, Abdou et al., 2016; mussels, Neira et al., 2015). Given the fact, that biomonitoring organisms are sampled on a regular basis, sediment-specific uncertainties due to physical or chemical disturbance of the historical record are excluded and, therefore, the use of sentinel organisms from sample banks may provide reliable information on trends in ambient contaminant pressure over time. In fact, the recent Pt increase in mussels goes along with a decrease in Pb as observed in the same mussel samples from the Toulon Bay (Dang et al., 2015) and in Galician Mussels (Neira et al., 2015). These opposite trends may be explained by the successive ban of leaded gasoline (leading to decreasing Pb emissions) in parallel to the growing deployment of catalyst-equipped vehicles, since leaded gasoline damages the catalysts and thus is not compatible with this modern technology (Schäfer and Puchelt, 1998). The apparent baseline Pt concentration (0.1 ng g^{-1} ; Fig. 3) in Toulon Bay mussels could reflect either natural Pt background although one cannot exclude that these concentrations partly result from other historic Pt contamination as recorded in the sediment.

4.2. Biomonitoring of Pt in mussels along the northwestern Mediterranean coast

Spatial distribution of Pt concentrations in mussels from contrasting sites along the Mediterranean coast seemed to roughly follow an increasing gradient from western to eastern sites, reflecting inter-site differences in terms of urbanization and supposed Pt inputs. Generally, in order to avoid trace metal variations related to seasonality, including reproduction, leading to dilution effects (Enríquez-Díaz et al., 2009), Winter organisms are used for comparison. In those mussels, the lowest Pt concentrations occurred at the sampling site Sète. Low Pt concentrations also occurred in mussels available from sites Banyuls-sur-Mer (Natural Reserve of Cerbère-Banyuls) and Bonifacio (Summer / Fall mussels). All those three sites are located in supposedly less exposed areas, i.e. far from major urban areas. Slightly higher Pt concentrations ($\sim 0.4 \text{ ng.g}^{-1}$) measured in Winter mussels from sampling site Saintes-Maries-

de-la-Mer cannot be explained by intense urban pressure, yet, mussel sampling was performed inside the leisure harbor. Even though the sampling site Faraman is located in a non-urbanized area (Natural Regional Park of Camargue), relatively high Pt concentrations ($\sim 0.5 \text{ ng.g}^{-1}$) in wild Winter mussels suggest contamination of this site, possibly by seawater draining pollution from La-Fos-sur-Mer city. In fact, in the late 1980's, the Gulf of Fos was considered as one of the most polluted neritic areas in Europe with important metal pollution (Cu, Pb, Cd, Zn; Benon et al., 1978). This area hosts important oil industries that might be a source of Pt to the marine environment as previously discussed (section 4.1.). Other studies report high trace metal contamination in native and caged mussels *M. galloprovincialis* from areas close to major urban and industrial centers such as La-Fos-sur-Mer (Andral et al., 2004), with a battery of biomarkers indicating disturbed health in mussels (Zorita et al., 2007) from the most impacted zones including the La-Fos-sur-Mer Harbor. In addition the sampling site Faraman is directly under the influence of the Rhône outflow (Charmasson et al., 1999) which may drain all the pollution originating from the urbanized watershed. Similar Pt concentrations occurred in mussels from sampling site TB2 in the Toulon Bay (Seyne sur Mer), showing the highest Pt level (0.55 ng.g^{-1}) of the studied sites. This value is somewhat lower than the 2014 value ($\sim 0.8 \text{ ng.g}^{-1}$) in farmed mussels from the same area in the Toulon Bay. Platinum concentration range in mussels from the Genoa Harbor, marked by industrial / urban water pollution (Ruggieri et al., 2011), is similar to levels observed in the Toulon Bay (Fig. 4). These observations suggest that Pt accumulation in mussels from the different sites may reflect the supposed exposure from anthropogenic pressure, similarly to other trace metals, showing strong gradients between mussels from less populated areas or regions with no major river inputs (e.g. Sète) and those from heavily populated and industrialized port areas (Fowler and Oregioni, 1976).

Few field studies report on Pt levels in biota from coastal marine environments. Recent samples of wild mussels *M. galloprovincialis* from an urban beach in Vigo (Spain) revealed Pt concentrations of $\sim 0.45 \text{ ng.g}^{-1}$ (2011 value, Neira et al., 2015), while wild oysters (*Crassostrea gigas*) from the moderately contaminated Gironde Estuary mouth contained $\sim 0.33 \text{ ng.g}^{-1}$ (2013 value, Abdou et al., 2016). Accordingly, Pt levels in mussels from the Mediterranean sites are similar in magnitude to those in wild bivalves from the Atlantic coast, but with clearly higher values in confined harbor sites with relatively high urban/industrial pressure. Bioconcentration factors (BCF), comparing Pt accumulation in tissues to ambient seawater concentrations were determined as $\text{BCF} = C_B / C_{WD}$, where C_B is the Pt concentration in the organism (ng.g^{-1} , dw) and C_{WD} the dissolved Pt concentration (Pt_D) in seawater (ng.L^{-1} , Arnot and Gobas, 2006) for the Genoa Harbor mussels (no Pt_D data available for the other sites). Average BCF in the Genoa Harbor mussels was $\sim 4 \times 10^3$, i.e. similar to values reported for wild mussels *M. galloprovincialis* from an urban beach ($\sim 5 \times 10^3$; Neira et al., 2015) and for wild oysters from

the Gironde Estuary ($\sim 3 \times 10^3$, Abdou et al., 2016). These values show that marine bivalves bioconcentrate Pt ambient signal reflecting: (i) the presence of natural bioavailable Pt species and (ii) their suitable use for Pt monitoring in coastal systems. Although it is assumed that dissolved metals are potentially more bioavailable than their particulate forms (e.g. Lekhi et al., 2008), filter-feeders such as mussels are able to absorb metals, and more particularly PGEs, in dissolved and particulate forms (Zimmermann and Sures, 2018). Given that Pt emissions from car exhaust systems mainly occur as very small particles (down to nanometric size; Folens et al., 2018), more work is necessary to characterize the behavior of such particles in the marine environment and understand their role in Pt transfer to living organisms.

Physiological status including growth and reproductive state may impact metal concentrations in bivalves, due to ‘dilution’ effects by rapid tissue growth or weight increase during gonad development (Casas et al., 2008; Couture et al., 2010). Therefore, contaminant concentrations in tissues reflect ambient concentrations, but also food availability or seasonal reproduction cycles (Andral et al., 2004; Charmasson et al., 1999; Dang et al., 2015). Inter-site comparison may require adjustment of raw concentration data from different sites with reference to a standard Condition Index to limit the risk of miss-interpretation, as shown from a mussel caging study, where site-dependent growth differences occurred at relatively short timescales (Andral et al., 2004). Comparison of Pt concentrations in the soft bodies of mussels sampled in Winter and in Summer/Fall from four of the seven studied sites clearly suggests that Pt concentrations undergo seasonal fluctuations (Fig. 4). The Condition Indices in the different mussel samples ranged from 17 to 47 with higher CI for Summer/Fall samples than for Winter samples, when comparison was available (Fig. 4). This result is expected supposing higher soft tissue mass during the reproduction season than during Winter. At three sites, the Winter concentrations are greater than the respective Summer values, as expected, whereas at the Toulon Bay site, Pt concentrations were higher in Summer, despite higher CI (Fig. 4). The latter observation might reflect environmental Pt exposure related with either site-specific changes in bio-transfer or temporarily higher local Pt inputs (e.g. tourism related). However, these hypotheses cannot be validated with the available dataset. The observed seasonal fluctuations do not change the order of supposed Pt exposure between the studied sites (Sète to Toulon Bay; Fig. 1), suggesting that the uncertainties discussed above do not invalidate the spatial biomonitoring approach, yet warranting an optimized sampling strategy to limit potential biases.

5. Conclusions

Historical records of both sediment cores and mussels collected in a severely polluted site, the Toulon Bay, suggest the existence of former (non-vehicle related) sources of Pt in the system. They also revealed the impact of recent contamination related to increasing Pt emissions through car catalytic converters that may be of major importance in this area under high anthropogenic pressure. Biomonitoring in mussels from contrasting areas along the northwestern Mediterranean coastline suggests a relation between the degree of exposure and the Pt concentrations in mussel tissues. Mussels could therefore be considered as a potential good bioindicator of Pt levels in marine environments. Considering the relatively high Pt concentrations in some sampling sites, systematic (bio-)monitoring programs should therefore be run in such coastal environments marked by historical and current Pt contamination. Continuous metal inputs to the coastal zone may lead to increasing pollution in these already highly contaminated areas. Further investigations are therefore of major importance to accurately determine biogeochemical Pt distribution, reactivity and fate in marine waters.

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This study allowed to assess Pt distribution in a strongly urbanized Mediterranean harbor, the Toulon Bay, and along a key European coastal area being the northwestern Mediterranean coast. Main outcomes from this study are the following:

In sediment cores:

- ✓ The Toulon Bay bottom sediments representing the local, pre-industrial reference show relatively high Pt concentrations ($\sim 6 - 10 \text{ ng.g}^{-1}$) that may reflect the marine origin of particles and/or chemically-driven Pt mobility.
- ✓ Grain-size corrected (Pt/Al) values suggest increasing concentrations from the bottom to the top in both sediment cores.
- ✓ Catalytic converter-derived and other urban Pt emissions may cause increasing Pt levels in sediment cores together with a former source which is assumed to be related to coal industry.

In farmed mussels:

- ✓ Temporal evolution of Pt concentrations in mussels revealed a ~ 8 - fold increase over the 1984 to 2014 period, especially since the mid-1990s.
- ✓ The recent Pt increase in mussels goes along with the growing deployment of catalyst-equipped vehicles.

In wild mussels:

- ✓ Spatial distribution of Pt concentrations in mussels seemed to reflect inter-site differences in terms of urbanization and supposed Pt inputs.
- ✓ Observed seasonal fluctuations do not change the order of supposed Pt exposure between the sites studied.
- ✓ The Toulon Bay and the Genoa Harbor (northwest Italy) showed the highest Pt concentrations.
- ✓ Mussel *M. galloprovincialis* appears as a promising sentinel for Pt contamination monitoring in coastal areas. This species already served to evidence increasing Pt contamination in an urban beach in Spain (Neira et al., 2015).

4.4. Spatial variability and sources of platinum in the Genoa Harbor – from geochemical background to urban levels

The Genoa Harbor is an artificial harbor located on the northwest coast of Italy and belongs to the most important ports in the Mediterranean Sea, known for its contamination due to a variety of activities. Port infrastructures extend over approximately 7 million m² along about 20 km of coastline, protected by wave breakers. For many years, industrial effluents were the major cause of water pollution in the Genoa Harbor before the shut-down of main heavy industries adjacent to the harbor during the 1990s. Nowadays, discharges from sewage treatment plants and from creeks have led to a general elevation in nutrients, organic matter and faecal pollution in the harbor waters (Ruggieri et al., 2011).

In order to better understand the geochemical behavior of Pt in seawater and interactions with suspended particulate matter (SPM) in an industrialized site, mapping of dissolved and particulate Pt levels was performed in the Genoa Harbor. Considering the potential various anthropogenic inputs occurring in the Genoa Harbor, and the open sea influence on the more external sites, the study of Pt partitioning might provide knowledge on Pt geochemistry in this anthropogenically impacted system.

The objective is to **provide first information on (i) Pt levels, (ii) spatial distribution, and (iii) factors controlling Pt partitioning between seawater and particles in a major Mediterranean harbor.**

For this, two main actions were performed:

- The determination of Pt concentrations in seawater and SPM samples, together with physical/chemical master variables, collected and measured along two profiles in the Genoa Harbor.
- The examination of Pt partitioning (distribution coefficient K_D) in relation with potential Pt inputs originating from various sources.

Outcomes from this study are ready for submission in the peer-reviewed scientific journal *Estuarine, Coastal and Shelf Science*.

Spatial variability and sources of platinum in a contaminated harbor – from geochemical background to urban levels

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Abstract

Biogeochemical cycles including processes controlling platinum (Pt) distribution remain widely unknown in aquatic environments, especially in coastal systems. Platinum concentrations in seawater (Pt_D) and in Suspended Particulate Matter (SPM, Pt_P) were measured, together with master variables comprising dissolved oxygen, dissolved and particulate organic carbon, chlorophyll-a, turbidity, and ammonium levels, along two longitudinal profiles in the industrial Genoa Harbor (northwest Italy). Concentrations and spatial distribution of Pt_D and Pt_P levels reflect distinct concentration gradients that were attributed to different Pt sources such as hospital, domestic and industrial wastewater and/or road run off. These first information suggest considerable anthropogenic contamination in the confined harbor compared to the proposed reference value for the western Mediterranean surface seawater measured at external sites ($\sim 0.08 \text{ ng.L}^{-1}$). Biogeochemical processes inducing changes in partitioning and fate of Pt in coastal seawater reflect spatial variability, highlighting the need for comprehensive environmental monitoring at appropriate spatial scale.

Keywords: Platinum; Technology Critical Element; seawater; suspended particulate matter; distribution coefficient; coastal environment

1. Introduction

Platinum (Pt) is a Technology Critical Element (TCE) and an emerging contaminant of growing concern according to evidences of anthropogenic disturbance on its global biogeochemical cycle (Rudnick and Gao, 2003; Cobelo-García et al., 2015). Among its specific properties, Pt is an excellent catalyst used in industrial processes, such as petrol chemistry, but more importantly in vehicle catalytic converters to reduce gaseous pollutants in exhaust fumes. Strongly increasing Pt demand for this technology results in growing anthropogenic Pt inputs into the environment, leading to a global increase of Pt concentrations in all environmental compartments including soils, atmosphere and aquatic systems (e.g. Rauch et al., 2006; Rauch and Morrison, 1999; Schäfer et al., 1999). However other anthropogenic sources of Pt may occur in aquatic environments including Pt-derived anti-cancer drugs (e.g. Vyas et al., 2014).

Coastal areas are facing high anthropogenic pressure due to the increasing density of human population in such attractive zones (Small and Nicholls, 2003). Monitoring of trace metal concentrations in these sites is therefore essential to detect potential contamination and prevent associated adverse consequences on the ecosystem, especially for emerging contaminants. Platinum concentration in seawater is difficult to determine precisely, especially in less contaminated sites, because of extremely low environmental levels (sub ng.L^{-1} ; Mashio et al., 2017). In fact, the existing studies on marine coastal areas, including saline estuaries, report dissolved Pt concentrations ranging from 0.05 to 0.3 ng.g^{-1} (Cobelo-García et al., 2013, 2014a; Goldberg et al., 1986; Mashio et al., 2016, 2017; Obata et al., 2006; Turetta et al., 2003; van den Berg and Jacinto, 1988). An even smaller number of studies report Pt concentrations in Suspended Particulate Matter (SPM) ranging from ~ 2 to 10 ng.g^{-1} in the Lérez Estuary and from ~ 0.2 to 1 ng.g^{-1} in the Gironde Estuary (Cobelo-García et al., 2013, 2014a). Variations in Pt concentrations and possible Pt enrichment in those coastal sites have been attributed to anthropogenic Pt inputs, especially in systems receiving anthropogenically-impacted freshwater inputs, as observed in the Tokyo Bay (e.g. Obata et al., 2006). Several studies have addressed bioaccumulation of Pt in wild marine bivalves, such as oysters and mussels (Abdou et al., 2016; Neira et al., 2015). However, there is lack of data on the comprehensive characterization of the factors controlling the behavior of Pt in coastal seawater, including its particle–water interactions, and therefore huge uncertainties remain concerning its transport, bioavailability and fate in the coastal ocean (Cobelo-García et al., 2013).

The Mediterranean Sea is a semi-enclosed system under ever-growing anthropogenic pressure along its northwestern coast, comprising several highly populated cities with extensively urbanized and industrialized areas (Martín et al., 2009). Considering the possible changes in

seawater chemistry due to additional anthropogenic inputs, previous studies have addressed environmental quality in historical and industrial harbors such as the Toulon Bay or the Genoa Harbor, reflecting intense pollution by metals, (e.g. Oursel et al., 2013; Tessier et al., 2011), metal-organic components (Pougnet et al., 2014) and nutrients (Ruggieri et al., 2011). The Genoa Harbor on the northwest coast of Italy (Ligurian Sea), is one of the most important ports of the Mediterranean Sea, known for heavy contamination due to a variety of different activities. For many years, industrial effluents were the major cause of water pollution in this site, before the shut-down of main heavy industries adjacent to the harbor during the 1990s. Nowadays, discharges from sewage treatment plants and from creeks account for generally high levels of nutrients, organic matter and faecal pollution in the harbor waters (Ruggieri et al., 2011).

The present work provides first information on Pt levels and spatial distribution in this major Mediterranean harbor combined with water quality master variables. Such findings may help to improve our understanding on Pt sources and cycling in an anthropogenically impacted harbor. The objectives are therefore: (i) to determine Pt levels and spatial distribution in a marine system under strong anthropogenic influence, involving different Pt sources, and (ii) to characterize the factors controlling Pt distribution and partitioning between seawater and suspended particles considered a prerequisite to better understand its biogeochemical cycle in coastal environments.

2. Material and methods

2.1. Study area and sampling strategy

The Genoa Harbor is an artificial harbor on the northwest coast of Italy (44°24'39.8"N 8°55'44.8"E). The port infrastructures extend over approximately 7 million m² along about 20 km of coastline, protected by artificial wave breakers. The innermost, natural part of the Genoa Harbor is the so-called Old Port hosting a ferry terminal, marinas and receiving urban treated sewage inputs (Ruggieri et al., 2011). Outside the Old Port a wide, less confined area protected by artificial wave breakers extends to the east and to the west, hosting diverse industrial activities and receiving discharges from a coal-fired power plant, treated urban wastewater and untreated surface run-off (Ruggieri et al., 2011). In addition, the area has been subjected to important dredging in the last decade (Cutroneo et al., 2012). Two rivers discharge freshwater and treated wastewater into the harbor area: the Polcevera River to the west and the Bisagno River to the east (Fig. 1). Both rivers run through the city of Genoa and are surrounded by dense urban structures hosting a variety of commercial and industrial activities.

Sampling

Two longitudinal profiles were performed to collect surface seawater and SPM samples inside and outside the Genoa Harbor on-board the Research Vessel MASO (3rd – 6th April 2017; Fig. 1). The first profile was carried out from the inner part of the port towards the eastern entrance (sampling sites E1 to E6; Fig. 1), i.e. along a supposed decreasing contamination gradient covering the strongly confined Old Port (sites E1 and E2), a less confined zone inside the wave breakers (site E3), the eastern Inlet (site E4) close to the mouth of the Bisagno River and two sites outside the Harbor, directly in contact with open Mediterranean water, i.e. the Ligurian Current (sites E5 and E6).

The second profile addressed the western part of the Genoa Harbor (sampling sites W1 to W6, Fig. 1) hosting coal deposits, a terminal for oil products (no storage tanks in the harbor), and receiving power plant discharges and inputs from the Polcevera River transporting sewage treatment plant effluents (Ruggieri et al., 2011).

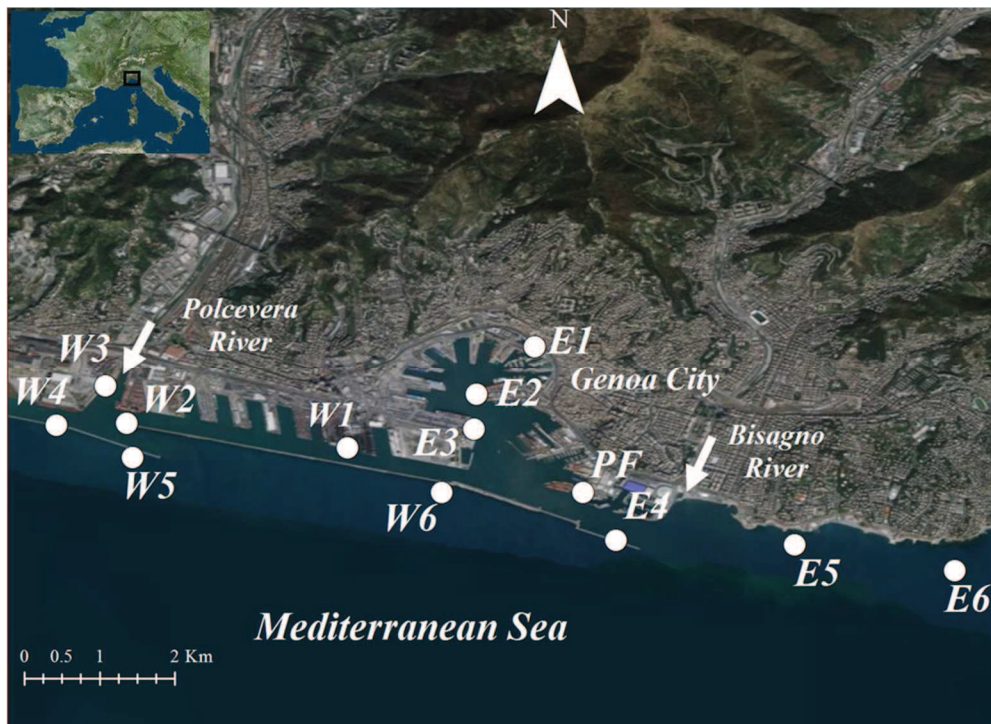


Fig. 1: Sampling sites in the Genoa Harbor. Two sampling profiles were performed with an eastward profile (sites E1 to E6) and a westward profile (sites W1 to W6). Sampling site PF corresponds to the CNR Platform. White arrows indicate river mouths.

Additional samples were taken at the CNR (National Research Council) Observation Platform (site PF, Fig. 1) on April 6th 2017 at three different times of the day (10 am, 12 pm, 2 pm). This sampling site is located at the limit of the confined area of the harbor, supporting diverse harbor activities such as navigation, ship construction and wrecking industries. The major hospitals of Genoa are located rather close to this area, including a relevant Center for Medical Oncology.

Seawater and SPM samples were collected at all sites (i.e., on-board the R/V MASO or from the CNR Platform) by pumping surface water (~ 5 m depth) with an electric pumping system connected to Teflon tubing into acid-washed 500 mL Teflon bottles (Nalgene®), previously rinsed with pumped water at each site. Water samples for analyses of dissolved Pt (Pt_D) were filtered immediately through 0.2 µm polycarbonate filters (Nucleopore®) with a filter-syringe (Sartorius®) into acid-washed 60 mL Teflon bottles (Nalgene®), previously rinsed with an aliquot of the filtrate, then acidified to pH = 1 (36.5–38 % HCl Baker Instra) and stored in the dark at 4 °C pending analysis. Water aliquots from the 500 mL Teflon bottle were collected to quantify ammonium (NH₄⁺) and dissolved organic carbon (DOC) concentrations. The former was filtered as aforementioned into pre-cleaned 15 mL polypropylene tubes (Kartell®), and stored frozen pending analysis. Aliquots for DOC were filtered through pre-combusted (500 °C, 4 h) glass-fiber filters (0.7 µm, Whatman® GF/F) using a pre-cleaned (RBS detergent, thoroughly rinsed with ultra-pure deionised water, Milli-Q® water) and pre-combusted glass syringe. Filtrates were collected in pre-cleaned and pre-combusted glass bottles, acidified (HCl 0.3 % v/v; Baker, Analyzed) and then stored in the dark at 4 °C until analyses.

Particulate samples were collected for analyses of particulate platinum (Pt_P) and particulate organic carbon (POC). For Pt_P, SPM were collected on pre-weighed Teflon filters (47 mm, 0.45 µm FHLC filter, Merck Millipore®), whereas for POC, SPM were collected on pre-combusted and pre-weighed glass-fiber filters (0.7 µm, Whatman® GF/F). In both cases, filters were then oven-dried (48 h at 50 °C) to constant weight, re-weighed (to determine SPM content) and kept in the dark at room temperature pending analysis.

Parallel to sampling, other master variables (i.e., temperature, dissolved oxygen, salinity, pH and turbidity) as well as chlorophyll-a (Chl-a) concentration were measured *in situ* using a submersible probe (OS316Plus, Idronaut®), previously calibrated on-board the R/V MASO or in an on-site laboratory on the CNR Platform. Salinity was measured using the Practical Salinity Scale.

2.2. Analytical methods

Platinum in seawater

Seawater samples were analyzed by Adsorptive Cathodic Stripping Voltammetry (AdCSV) as described in Cobelo-García et al. (2014b). Measurements were carried out using a μ Autolab Type III potentiostat (Metrohm®) connected to a polarographic stand (Metrohm® 663 VA Stand) equipped with three electrodes: (i) a hanging mercury drop electrode (HMDE, the working electrode), (ii) a silver/silver-chloride (Ag/AgCl) reference electrode, and (iii) a glassy carbon auxiliary electrode. A polytetrafluoroethylene (PTFE) voltammetric cell served in all experiments and the potentiostat was controlled using the NOVA 2.1 software. Elimination of organic matter by UV oxidation was performed by placing sample aliquots in capped Teflon bottles with overnight irradiation using two 64 W UV lamps (NIQ 60/35 XL, Heraeus) under a fume hood. Aliquots (10 mL) of UV-digested sample were pipetted into the voltammetric cell together with 30 μ L of 3.3 mM formaldehyde (37–41 % Analytical Reagent Grade, Fisher Chemical®), 30 μ L of 0.45 mM hydrazine sulfate (Analytical Reagent Grade, Fisher Chemical®), and 300 μ L of sulfuric acid (H_2SO_4 , 93 – 98 % Trace metal grade, Fisher Chemical®). Platinum concentrations were determined by standard addition method (adding mono-elementary Pt standard solution 1000 $\mu\text{g}.\text{mL}^{-1}$ PLASMACAL, SCP Science®) using a deposition time of 300 s and experimental parameters as described elsewhere (Cobelo-García et al., 2014b). In the absence of Certified Reference Material (CRM) for dissolved Pt in seawater, analytical quality of the voltammetric procedure was evaluated by means of determination of Pt spiked CRM seawater (CASS-6, NRCC) giving recoveries > 80% and precision expressed as Relative Standard Deviation (RSD) lower than 10 % ($n = 5$) at the 0.5 $\text{ng}.\text{L}^{-1}$ range. The detection limit (D.L.) for dissolved Pt measured by AdCSV (calculated as 3 x blank standard deviation, $n = 20$) was estimated to 0.03 $\text{ng}.\text{L}^{-1}$.

Platinum in SPM

Suspended Particulate Matter samples were analyzed by Triple Quadrupole-Inductively Coupled Plasma Mass Spectrometry (TQ-ICP-MS, Thermo® iCAP TQ). Teflon filters with SPM (typical mass of ~ 3 - 5 mg) were acid digested as described in Abdou et al. (2018). Briefly, 2 mL HCl and 1 mL HNO_3 (30 % HCl and 65 % HNO_3 Suprapur, Merck®) were added to the samples in polypropylene (PP) tubes (DigiTUBEs, SCP SCIENCE®) with caps. Samples were placed in a Teflon-coated heating block, and digested at 110 °C for 3 h. Cooled contents were then diluted in 10 mL MilliQ water, centrifuged at 4000 rpm for 10 min (20 °C) and filters were discarded. Analyses were performed applying the standard addition method (using mono-

elementary Pt standard solution 1000 $\mu\text{g.mL}^{-1}$ PLASMACAL, SCP Science®). After obtaining the raw signal, Hafnium-oxide (HfO^+) interferences were monitored using the ^{193}Ir signal that is highly interfered by HfO^+ (Djingova et al., 2003). Raw signal was thereafter mathematically corrected using $^{194}\text{Pt} / ^{195}\text{Pt}$ natural ratio and assuming that HfO^+ is the only/dominant spectral interference on those selected isotopes. Analytical quality control was performed using the BCR®-723 road dust (Institute for Reference Materials and Measurements) and Jsd-2 sedimentary rocks (indicative value from Geological Survey of Japan) as available CRMs for Pt concentrations. Analyses of these CRMs by ICP-MS gave satisfactory recovery values of 87% and 101% respectively ($n = 3$) and precision expressed as RSD of $\sim 5\%$. The detection limit (D.L.) for particulate Pt (calculated as 3 x blank standard deviation, $n = 10$) was estimated to 0.34 ng.g^{-1} for typical sediment sample mass of 4 mg.

Complementary measurements

Dissolved organic carbon (DOC) was analyzed with an automated analyzer (TOC-L, Shimadzu®) as described in Sharp et al. (1993). Accuracy of DOC measurements was tested using reference material (Deep Sea Reference, DSR, University of Miami) providing recovery results $> 90\%$. Analytical precision expressed as %RSD is of 5% at the 0.5 mg.L^{-1} range. The D.L. (calculated as 3 x blank standard deviation, $n = 10$) was estimated to 0.06 mg.L^{-1} . Particulate organic carbon (POC) was analyzed with a LECO® CS-125 after carbonate elimination as described elsewhere (e.g. Schäfer et al., 2002). Analytical quality was checked by measuring CRMs (e.g. LECO 501–503). Accuracy was within 5% of the certified values and the analytical error generally better than 5% (% RSD). Ammonium concentrations were determined by colorimetric procedure (visible-UV spectrophotometer, Thermo®, Genesys 20, Aminot et al., 1997). Analytical error of the method is lower than 5% (% RSD), accuracy within 5% of the reference values, and the D.L. of $18 \mu\text{g.L}^{-1}$. The correlation matrix for the different parameters was produced using the software PAST® applying the linear r (Pearson) parametric correlation coefficient.

Sediment–water distribution coefficients, K_D , defining the concentration ratios of Pt_P over Pt_D were calculated according to the equation (1)

$$K_D = [\text{Pt}_\text{P}] / [\text{Pt}_\text{D}] \times 10^3 \quad (1)$$

3. Results

The results obtained are represented for two distinct profiles, both starting from inside the Genoa harbor and going towards the open Mediterranean Sea along the east and west part of the coastline. The eastward profile (sites E1 to E6) extends from the innermost Old Port (site E1) to the Ligurian Current (E6), and the westward profile (sites W1 to W6) from inside the wave breakers to sites outside. Salinity (S) of ~ 37.7 in the inner harbor, compared to $S = 38.0$ in the outmost sites, suggests desalinization corresponding to a freshwater contribution of $\sim 1\%$. The spatial distribution of master variables in both profiles showed O_2 saturation between $\sim 70\%$ and 98% , Chl-a between 0.2 and $1.6 \mu\text{g.L}^{-1}$, NH_4^+ between 25 and $140 \mu\text{g.L}^{-1}$ and POC ranging from 1% to 5% (Fig. 2A). Despite common ranges in master variables among profiles, there were differences between the profiles. The eastward profile shows clear concentration gradients, with O_2 saturation increasing towards the open sea, and decreasing Chl-a, NH_4^+ and POC % (except for one higher value at site E5; Fig. 2A). Ammonium values at sites E5 and E6 were below the D.L. ($18 \mu\text{g.L}^{-1}$). In contrast, the westward profile showed no clear trend for O_2 saturation levels (relatively constant $\sim 90\%$). Increasing Chl-a and POC % gradients seem to occur for stations inside the wave breakers, while most NH_4^+ concentrations are below the D.L. (Fig. 2A).

The observed PtD concentrations ranged from 0.08 ng.L^{-1} to 0.18 ng.L^{-1} and showed clear concentration differences between sites inside the harbor and sites outside the wave breakers (Fig. 2B). Note that the highest PtD concentrations in both profiles occurred close to the river mouths (W3 and E4). However, the westward profile showed somewhat similar PtD concentrations at the sites W1 to W3, all inside the wave breakers and a decrease towards the most distant site of this profile ($\sim 0.10 \text{ ng.L}^{-1}$ at W6). The eastward profile displayed an increasing PtD gradient from $\sim 0.10 \text{ ng.L}^{-1}$ at E1 to 0.18 ng.L^{-1} at E4 and a sharp drop towards lower values at sites E5 and E6 (0.08 ng.L^{-1} ; Fig. 2B).

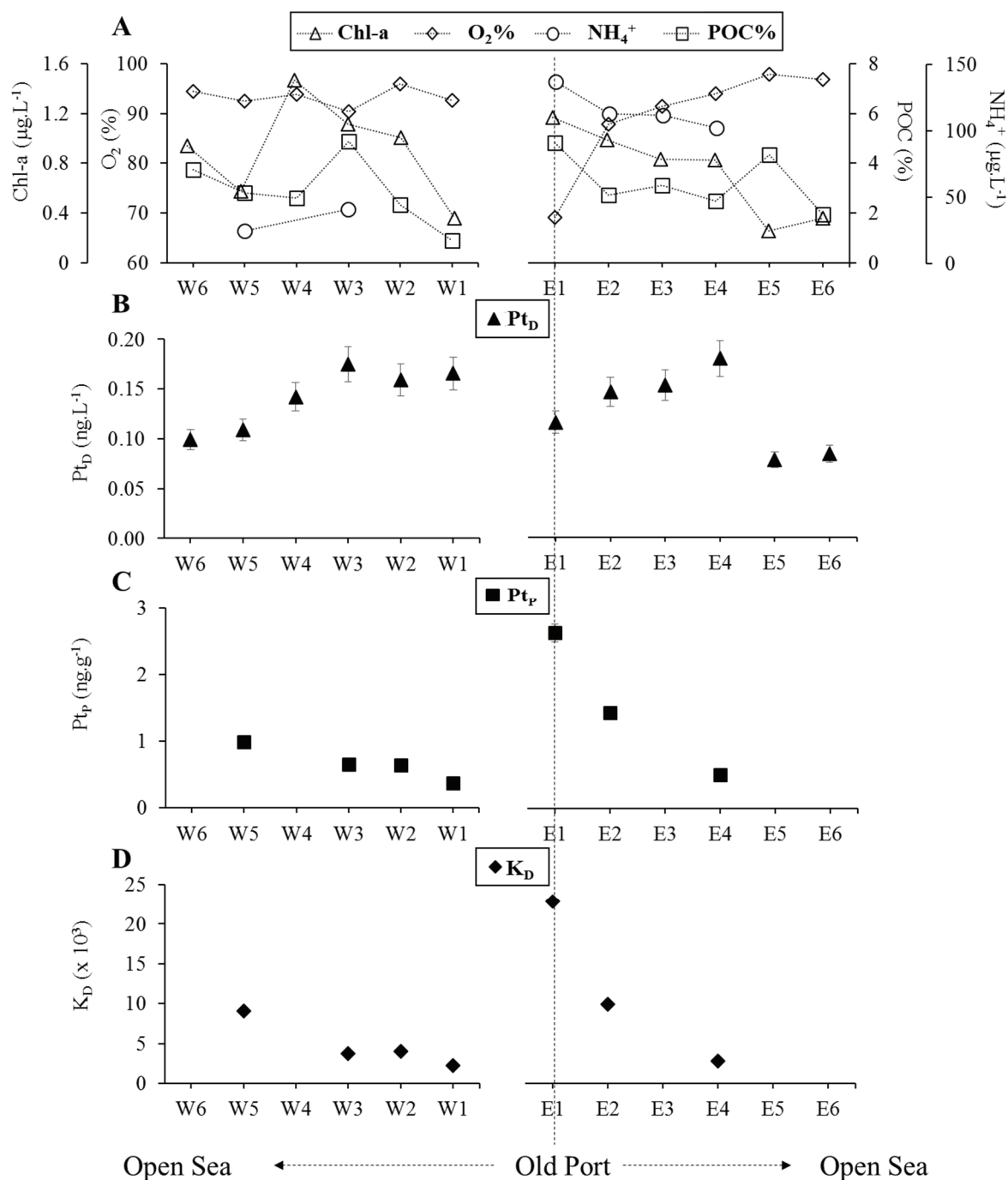


Fig. 2: Spatial distribution of dissolved and particulate Pt concentrations and master variables along two profiles in the Genoa Harbor. The eastward profile corresponds to sites E1 to E6, with E1 being located in the Old Port, while the westward profile corresponds to sites W1 to W6. With A: Master variables chlorophyll-a (Chl-a), dissolved oxygen saturation ($O_2\%$), particulate organic carbon (POC%) and ammonium (NH_4^+); B: dissolved Pt concentrations (Pt_D); C: particulate Pt concentrations (Pt_P); and D: distribution coefficient K_D ($\times 10^3$). Error bars correspond to Relative Standard Deviation (% RSD).

The observed P_{TP} concentrations ranged from below D.L. (i.e. $< 0.34 \text{ ng.g}^{-1}$) up to $\sim 3 \text{ ng.g}^{-1}$ (Fig. 2C). Along the westward profile, P_{TP} concentrations were relatively constant and low ($< 1 \text{ ng.g}^{-1}$) with values below D.L. in sites W4 and W6. In the eastward profile, P_{TP} were highest in the Old Port (site E1), and showed an overall decreasing trend towards the outer sites reaching values below D.L. at sites E5 and E6 (P_{TP} level at site E3 was also below the D.L.).

Sediment–water distribution coefficients varied from 2×10^3 to 23×10^3 and showed eastward and westward gradients similar to those observed for P_{TP} concentration variations (Fig. 2C).

The relationships between spatial variations of P_{TD} and P_{TP} concentrations and the other parameters measured are displayed separately for each profile only for the sites within the wave breakers, assuming that external site parameters reflect marine influence rather than harbor-related processes (Table 1). Despite the low number of samples, results of the correlation matrix suggest that correlations exist between selected master variables and Pt levels in both the dissolved and particulate phases along the eastward profile (Table 1). Accordingly, P_{TD} concentrations seem to be positively correlated with dissolved oxygen levels ($r = 0.90$) and negatively correlated with Chl-a ($r \sim 0.9$), and with NH_4^+ , DOC, POC, and turbidity levels ($r > 0.9$). Opposite behavior occurred for P_{TP} concentrations. In the westward profile, weak correlations are found for Pt levels when related to the selected variables, except for P_{TD} concentrations that were positively correlated with POC ($r \sim 0.7$), and P_{TP} concentrations negatively correlated with DOC values ($r \sim -0.9$).

Table 1: Correlation table of Pt_D and Pt_P concentrations with master variables. Values correspond to Pearson's r coefficient. n.d.: not determined. Note that only the sites inside the harbor are considered, because the parameters in the outside sites were assumed to reflect open sea controls.

		O₂%	Chl-a	NH₄⁺	DOC	POC	Turbidity
<i>Eastward profile</i> (E1 to E4)	Pt_D	0.90	-0.86	-0.96	-0.98	-0.92	-0.91
	Pt_P	-0.92	0.98	0.85	0.87	0.95	0.96
<i>Westward profile</i> (W1 to W4)	Pt_D	-0.48	0.12	n.d.	-0.31	0.73	0.69
	Pt_P	0.12	0.11	n.d.	-0.88	-0.36	-0.14

Platinum concentrations measured at the CNR Platform (fixed site PF; Fig. 1) at three different times of the day showed variations for both Pt_D (0.09 to 0.15 ng.L⁻¹) and Pt_P (3.1 to 14 ng.g⁻¹; Table 2). Dissolved Pt concentrations were in the same range as those measured along the profiles, whereas relatively higher Pt_P levels occurred, which led to higher K_D values. The master variables at this sampling site were in the same range as levels observed in other internal sites (Table 2; Fig. 2A). Only dissolved O₂ saturation values were somewhat lower than those measured along the profiles and were close to those in the Old Port site (E1; Fig. 2A).

Table 2: Platinum concentrations and master variables at the CNR Platform (site PF) at 10 am, 12 pm, and 2 pm. Turb.: turbidity.

PF site	Pt_D (ng.L⁻¹)	Pt_P (ng.g⁻¹)	K_D (x10³)	O₂%	Chl-a (µg.L⁻¹)	NH₄⁺ (µg.L⁻¹)	DOC (mg.L⁻¹)	POC%	Turb. (NTU)
10 am	0.09	7.7	82	82	1.0	47	1.0	2.5	4.0
12 pm	0.15	14	94	83	1.4	48	0.68	5.2	2.6
2 pm	0.11	3.1	27	87	1.6	45	0.63	2.0	4.6

4. Discussion

4.1. Platinum concentration and spatial distribution in seawater and particles along the two longitudinal profiles

The two profiles sampled westward and eastward from the Old Port show elevated Pt_D concentrations within the whole harbor area, compared to the external sites (E5, E6 and W5, W6). The lowest Pt_D values occurred at the sites E5 and E6 (Fig. 2B) mainly influenced by open Mediterranean water transported with the east-west oriented northern Ligurian Current. Therefore, the Pt_D concentrations in seawater from these sites (0.08 ng.L^{-1}) may be considered as representative of surface water in the northwestern Mediterranean Sea. This assumption is supported by identical Pt_D levels of 0.08 ng.L^{-1} in surface water from an open-sea site, south from the Balearic Islands (Abdou et al., unpublished data). Accordingly, open Mediterranean surface water Pt_D concentrations appear somewhat higher than average Pt_D in the North Atlantic seawater ($\sim 0.05 \text{ ng.L}^{-1}$, Colodner, 1991), and Pt_D in northwest Pacific Ocean ($\sim 0.04 \text{ ng.L}^{-1}$; Suzuki et al., 2014). Furthermore, the supposed Mediterranean surface water Pt_D is similar to values in the coastal North Atlantic Ocean off the slightly Pt-contaminated Gironde Estuary (0.08 ng.L^{-1} ; Cobelo-García et al., 2014a) and lies within the range of values reported for the Lérez Estuary seawater end-member (from 0.08 to 0.10 g.L^{-1} , Cobelo-García et al., 2013). These observations are consistent with expected relatively higher Pt inputs into the semi-enclosed Mediterranean Sea due to the strong urbanization of its coastline. Similarly, the western profile shows the lowest Pt_D values (i.e. $\sim 0.10 \text{ ng.L}^{-1}$; Fig. 2B) at the sites located outside the wave breakers (W5 and W6). These concentrations are slightly higher than open Mediterranean Pt_D , suggesting minor local contamination off the harbor and ‘downstream’ from the sites E5 and E6 (with respect to the dominant Ligurian Current). Conversely, inside the wave breakers, contamination is consistently higher with Pt_D concentrations of up to more than 2-fold the open Mediterranean value (Fig. 2B).

Samples collected at the CNR Platform (site PF), show temporal variations of Pt_D concentrations from 0.09 to 0.15 ng.L^{-1} (Table 2), i.e. covering the whole range from the open sea level to typical inside harbor values within few hours. Along the eastern profile, from the inner to the outer part of the harbor, Pt_D concentrations tended to increase with a maximum which seem to occur at site E4, close to the mouth of the Bisagno River (Fig. 1) while the highest Pt_D in the westward profile is observed inside the harbor at site W3, i.e. near the Polcevera River mouth. According to Ruggieri et al. (2011) this part of the harbor hosts coal deposits and a terminal for oil products. Coal industry may contribute to local Pt concentrations, since high Pt concentrations have been reported for coal samples ($\sim 5 \text{ ng.g}^{-1}$, Finkelman and Aruscavage, 1981; up to 70 ng.g^{-1} , Dai et al., 2003). Historical intense coal-based industrial

activities in the Genoa Harbor (Ruggieri et al., 2011) and erosion/leaching of coal deposits stored uncovered against wind and/or rain may suggest the presence of industrial coal particles in the surface sediments around the Polcevera River mouth. Although the Pt concentrations in this coal are unknown, one cannot exclude that industrial coal and/or fly ashes could contribute to the Pt enrichment at this sampling site given the range of values reported in the literature. Additionally, Pt inputs from sewage treatment plants may reach the river mouth, adding to the potential Pt sources impacting site W3. Consequently, the present dataset suggests that such anthropogenic inputs supplied to rivers or directly in the harbor by urban and/or industrial wastewater discharges may represent important local Pt_D point sources to the coastal waters, although the Genoa Harbor Pt_D is far below levels reported for the highly polluted Tokyo Bay estuaries with concentrations up to $\sim 7 \text{ ng.L}^{-1}$ (Obata et al., 2006).

Particulate Pt concentrations in suspended particles partly reflect the general concentration gradient from inside the harbor towards the open sea. In fact, Pt_P concentrations were measurable (~ 0.4 to 3 ng.g^{-1}) at all internal sites and behind the wave breakers (except site E3), whereas for the external sites from outside the wave breakers Pt_P were below detection limit ($< 0.34 \text{ ng.g}^{-1}$; Fig. 2C). The Pt_P levels inside the Genoa Harbor were similar to those reported for the Lérez Estuary (~ 1 to 15 ng.g^{-1}), interpreted as anthropogenic Pt enrichment (Cobelo-García et al., 2013). In fact, the highest Pt_P concentrations occurred at the CNR Platform (reaching 14 ng.g^{-1} , Table 2). This site is in direct vicinity of several ship construction and wrecking sites, but also at $< 5 \text{ km}$ distance from the urban highway and very close to urban heavy traffic roads, a likely source for Pt bearing particles. Furthermore, the CNR platform and surrounding waters are quite close to the urban area hosting the major hospitals and the relevant Center for Medical Oncology. Given the importance of Pt-based anti-cancer drugs (Vyas et al., 2014), further work should examine the role of potential Pt release through hospital effluents in this area. All these observations clearly support the idea that different anthropogenic activities in the urban area of Genoa represent overlapping potential Pt sources contributing to local Pt contamination of the marine environment.

4.2. Factors controlling platinum distribution and partitioning inside the Genoa Harbor

Platinum concentrations in both dissolved and particulate phases might be controlled by several factors implying various biogeochemical processes. Assuming that Pt originates from multiple potential point sources, including urban and industrial wastewater, hospital effluents and surface run-off (e.g. Pawlak et al., 2014), prevailing at different sites, it is possible that Pt speciation differs from site to site. Indeed, hospital effluents may transport dissolved Pt ranging from $< 10 \text{ ng.L}^{-1}$ to $3.5 \text{ }\mu\text{g.L}^{-1}$ (Rauch and Peucker-Ehrenbrink, 2015), mainly excreted from anticancer drugs and/or derived metabolites. Such excretion also continues outside the hospital, as more and more patients leave for home directly after the treatment. In fact, Vyas et al. (2014) suggest that the majority of Pt is emitted from outpatients at home. If Pt supposedly is excreted mainly in the dissolved phase, dissolved/particulate partitioning in sewage and the related removal efficiency in wastewater treatment plants remain widely unknown.

Similarly, both run-off and sewage may contain PGE-bearing catalytic converter fragments with intact PGE-load (Prichard and Fisher, 2012), which may undergo partial dissolution in contact with seawater (forming the stable $\text{PtCl}_5(\text{OH})^{2-}$ complex as evidenced in Cobelo-García et al., 2014a). However, biogeochemical processes, inherent to anthropogenic discharges, can widely modify the original Pt phase. For instance, urban wastewaters released into the Genoa Harbor strongly influence the local water quality, as revealed by high nutrient (NH_4^+ levels), high turbidity, low dissolved oxygen percentage, the presence of faecal coliforms and high phytoplankton biomass (Ruggieri et al., 2011). Accordingly, one would expect inputs of different co-existing forms of Pt from different sources into the marine environment with wastewater and run off. Considering only the sites E1 to E4 located inside the relatively stagnant harbor waterbody (due to confinement and low tidal amplitudes; Cutroneo et al., 2012; Cutroneo et al., 2017), the eastward gradients of Pt concentrations have been correlated with parameters that could reflect sewage effluent signature. Inside the harbor, Pt_D concentrations were positively correlated with dissolved oxygen ($r = 0.90$), and negatively correlated with turbidity, Chl-a, % POC, DOC, and NH_4^+ ($r \sim 0.9$). The opposite trends occurred for Pt_P concentrations, which were positively correlated with Chl-a, % POC, DOC, and NH_4^+ inside the harbor. Assuming that these major variables may serve as a proxy for wastewater inputs and degradation, this observation suggest Pt dissolution from solid carrier phases, either road dust (Sebek et al., 2011) or small sewage particles, and subsequent dilution of Pt_D by seawater. In fact, high levels of Pt_P and relatively low levels of Pt_D , where sewage inputs are at maximum (site E1) might be explained by: (i) the presence of originally high Pt_P concentrations in wastewaters or run off, and/or (ii) the adsorption of dissolved Pt on organic-rich ligands present in wastewaters (high concentrations of dissolved and particulate organic carbon in the turbid

sewage plume), also considering sediment resuspension which may occur in this shallower sampling site. Previous work has shown that relatively high dissolved metal loads in wastewater inputs equilibrate in the recipient waters. Accordingly, efficient adsorption on high complexation site particles induces profound changes in element partitioning between the dissolved and particulate phases (e.g. Deycard et al., 2014). Indeed, the residual POC in effluent wastewater mainly consists of reactive (degradable) organic matter, once exposed to natural micro-organism communities in the receiving waterbody (Deycard et al., 2017). Organic matter, especially thiols and reduced sulfur groups present in wastewaters may strongly binds soft metals and potentially compete with inorganic ligands such as Cl^- (Levard et al., 2013; Wood et al., 1990). Platinum partitioning between the two phases might be partly influenced by interactions with POC although the degree of surface complexation with organic matter remains unknown (Couceiro et al., 2007). Experiments proved that Pt adsorption kinetics onto estuarine sediments is greatest for cohesive sediments with relatively high total carbon contents (Couceiro et al., 2007). Another experimental study showed that complexation with organic ligands may be critical to particle-water fractionation of Pt discharged into aquatic system, removal from the dissolved phase being most favorable when entering a turbid water course (Turner et al., 2006). Accordingly, highest Pt_p levels co-occurring with high turbidity sewage inputs in the Genoa Harbor seem in line with those observations. A rough mass balance for this eastern profile inside the harbor suggests that Pt release from suspended particles would account for a Pt_D addition of $\sim 0.02 \text{ ng.L}^{-1}$, whereas the observed Pt_D values increased by $\sim 0.06 \text{ ng.L}^{-1}$. Accordingly, the amount of Pt presumably transferred from the particulate to the dissolved phase is not contradictory to the field data but does not explain the observed Pt_D addition, supporting the idea of additional Pt_D inputs by the Bisagno River. Outside the wave breakers, such biogeochemical equilibration processes and the resulting higher Pt_D would be masked by massive dilution with open Mediterranean water.

If such processes and changes in particle nature influence the spatial distribution of Pt_p , they also may impact Pt_p at the temporal scale. In fact, at the CNR Platform (site PF) important variations occurred within the same day for both, the dissolved and particulate phases. At 12 pm both Pt_D and Pt_p reached maximum values of 0.15 ng.L^{-1} and 14 ng.g^{-1} , respectively, corresponding to maximum POC% values, which were similar to those observed at the innermost station (site E1; i.e. $\sim 5 \%$, Table 2). Since treated effluent wastewater may contain nearly 45 % POC (Deycard et al., 2017), sewage waters might already be diluted at this sampling site. In fact, the generally low dissolved oxygen levels observed at the CNR Platform may confirm the influence of sewage as a Pt source. Accordingly, spatial and temporal variations in Pt levels inside the Genoa Harbor could reflect both the distance from the source and the release dynamics. In fact, a clear diurnal cycle has been reported for element

concentrations and fluxes in urban wastewater and in hospital effluents, reflecting inhabitants' daily life and the variable contaminant residence times in the treatment plants (Deycard et al., 2014; Kümmerer and Helmers, 1997). Such temporal variations could explain why Pt_P varied by a factor of ~ 5 (i.e. from 3 to 14 ng.g⁻¹) within few hours. The related daily variations in K_D values may suggest that the observed K_D might not describe equilibrium conditions. However, the average K_D values obtained for the Genoa Harbor ($\sim 8 \times 10^3$) are consistent with values obtained for the seawater end-member of the Gironde Estuary ($\sim 4 \times 10^3$, Cobelo-García et al., 2014a), and the maximum K_D value recorded at the CNR Platform ($\sim 1 \times 10^5$) is similar to those found in the seawater end-member of the Lérez Estuary (Cobelo-García et al., 2013). Estuarine particle-water interactions are likely controlled by decreasing proportion of positively charged reactive species along the salinity gradient (Cobelo-García et al., 2008). Increasing repulsion between SPM surface and Pt species might explain decreasing K_D with increasing salinity (Cobelo-García et al., 2014a). Outside the Old Port but still inside the harbor, the growing influence of open seawater with a decrease in the turbidity, as well as the change in the nature of SPM surfaces (decrease in %POC) might explain low Pt_P concentrations (below detection limit), going along with increasing Pt_D .

5. Conclusions

This work provides the first information on Pt distribution and reactivity in an industrialized and urbanized Mediterranean harbor. Measured concentrations for Pt_D (~ 0.08 to 0.18 ng.L⁻¹) and Pt_P (< 0.34 to 14 ng.g⁻¹) allowed proposing $Pt_D \sim 0.08$ ng.L⁻¹ (as measured in the Ligurian Current and south from the Balearic Islands) as a reference value for western Mediterranean surface seawater. Consequently, there is a clear evidence of anthropogenic Pt contamination at all sites studied within the Genoa Harbor showing distinct concentration gradients, attributed to different overlapping local sources such as industry, run-off, hospital effluents, domestic wastewater as well as dust and atmospheric deposition. Gradients in both Pt_D and Pt_P as well as major variables attributed to input and degradation of urban sewage inside the relatively stagnant harbor waters reflect biogeochemical processes modifying the nature, surface properties and abundance of suspended particles, leading to profound changes in Pt partitioning and carrier phases. Such mobilization of anthropogenic Pt may enhance its bioavailability, bio-uptake and induce potential effects on marine organisms. The role of biological and biogeochemical processes and responses from marine wildlife to contamination, including potential Pt complexation to biologically-derived organic ligands such as algae exudates, are still unknown and need further investigation.

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Results from this study provide first information on Pt distribution and reactivity in an industrialized and urbanized Mediterranean harbor. Mapping performed in the Genoa Harbor and its surroundings allows the assessment of different Pt sources affecting both the dissolved and particulate phases. The main outcomes from this study are the following:

- ✓ Dissolved Pt concentration of $\sim 0.08 \text{ ng.L}^{-1}$ (as measured in the Ligurian Current and south from the Balears Islands) is proposed as a reference value for western Mediterranean surface seawater.
- ✓ Clear evidence of anthropogenic Pt contamination occurred in all sites within the Genoa Harbor.
- ✓ Platinum inputs and degradation of urban sewage inside the relatively stagnant harbor waters lead to gradients of dissolved and particulate Pt implying profound changes in Pt partitioning and carrier phases.

Overall, the spatial distribution showed clearly higher Pt levels at the inner stations, evidencing the importance of Pt contamination sources in modifying the coastal water quality as already reported for other pollution parameters inside the Genoa Harbor (Ruggieri et al., 2011). Platinum behavior following “organic” contamination should be closely monitored especially considering its potential enrichment and the supposed species (anti-cancer drugs and their metabolites) in sewage.

4.5. Short-term variations in platinum partitioning in contrasting European coastal environments: the role of primary production and local point sources

Plankton material, comprising phytoplankton is an assemblage of key organisms at the base of the marine trophic chain. The most important role of trace metals in algal physiology is that of essential micronutrients (Sunda, 1989). In addition to their nutritional role, trace metals may also inhibit growth due to their toxicity at elevated concentrations (Sunda, 1989).

Interactions between trace metals and the marine biological communities are reciprocal; that is, not only trace metals affect the biota, but the biota, in turn, may have a profound effect on trace metal chemistry (Sunda, 1989). The incorporation of trace elements by phytoplankton can regulate trace element forms and availability. In fact, uptake by algal cells may reduce the amount of dissolved metal in the surrounding seawater therefore reducing availability to other organisms and especially to higher trophic levels. Furthermore, number of direct and indirect mechanisms enables phytoplankton to modify, capture and sequester trace elements.

Assessment of Pt content in such samples constitutes a valuable approach to better understand the aquatic biogeochemical cycles of Pt that would include primary producers.

The objective of this study is to **provide first information on phytoplankton controls on Pt biogeochemical cycles in coastal environments.**

The following points were addressed:

- Platinum concentrations in seawater and plankton material, together with physical/chemical master variables related to primary production, were measured along a diurnal cycle performed in the Gironde Estuary mouth.
- The determination of Pt concentrations in the next higher links of the marine trophic chain, being zooplankton and bivalves from the same sampling site.
- The comparison with Pt levels in seawater, plankton material and bivalves from the moderately contaminated Arcachon Bay and the strongly impacted Genoa Harbor.

Outcomes from this study are ready for submission to the peer-reviewed scientific journal ***Marine Pollution Bulletin***.

Short-term variations in platinum partitioning in contrasting European coastal environments: the role of primary production and local point sources

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Abstract

Short-term Pt dynamics in three contrasting European coastal sites were compared: (i) the highly turbid Gironde Estuary (SW France), (ii) the Arcachon Bay known for intensive seafood production (SW France), and (iii) the strongly polluted Genoa Harbor (NW Italy). At all sites, hourly sampling and continued in-situ measurements at fixed sampling points were combined, to study diurnal cycles (> 24 h) of dissolved Pt concentrations in seawater (Pt_D) and master variables reflecting primary production activity (chlorophyll-a, phaeopigments and solar irradiation). Plankton nets were used to sample phyto- and zoo-plankton over the whole diel cycle in the Gironde Estuary mouth, while in the Arcachon Bay and the Genoa Harbor discrete plankton sampling was performed. Bivalves (wild oysters or mussels), reflecting higher trophic level, were sampled at all sites. Results from the Gironde Estuary suggest that in periods of intense primary production, phytoplankton activity controls cycling and solid/liquid partitioning of Pt concentration during the daytime. During the night, zooplankton grazing may release Pt into the dissolved phase. These processes are partly masked by fluctuations due to external factors such as tide or local sources, especially in more confined and/or urbanized coastal water bodies such as the Arcachon Bay and the Genoa Harbor. Platinum levels in plankton and bivalves from contrasting sites along the Mediterranean and Atlantic coasts reflected the general Pt exposure in seawater, but there was no evidence of Pt biomagnification along the trophic chain. These results clearly suggest that Pt contamination of coastal waters and marine organisms is now a common feature in urbanized coastal sites and that Pt transfer to the marine food chain depends on several biogeochemical controls.

Keywords: Platinum, seawater, plankton, diurnal cycle, biomagnification

1. Introduction

Platinum is a Technology Critical Element (TCE) and an emerging metallic contaminant, for which there is an increasing need for accurate assessment of environmental impacts and natural dynamics (Cobelo-García et al., 2015). This element is increasingly used in various applications, the most important being vehicle catalytic converters. Increasing Pt emissions from automobile exhaust catalytic converters have led to an increase in Pt concentration in all Earth compartments with anthropogenic activities accounting for more than 80 % of Pt fluxes at the Earth surface (Sen and Peucker-Ehrenbrink, 2012). Dissolved Pt concentrations in seawater from open oceans (Colodner et al., 1993; Suzuki et al., 2014) are generally far below seawater concentrations near the coast such as in estuaries and at the continental shelf (Cobelo-García et al., 2013; Mashio et al., 2016, 2017; Obata et al., 2006). Coastal environments facing ever-growing human pressure (~ 70 % of worldwide urban populations and economic activities, Small and Nicholls, 2003) receive increasing anthropogenic Pt inputs from various sources (Ruchter et al., 2015) and Pt bioavailability to living organisms and especially to marine biota is non-negligible. In fact, the limited environmentally relevant information in the literature reports on Pt concentrations in marine macroalgae (Hodge et al., 1986) and in wild marine bivalves (Abdou et al., 2016; Neira et al., 2015), suggesting bioconcentration factors of $\sim 10^3$ in bivalves. There is even less information on the possible transfer mechanisms controlling Pt accumulation throughout the aquatic trophic chain, although phytoplankton communities may play a key role in the geochemical cycling of trace metals in coastal ecosystems (Cloern, 1996; Caetano and Vale, 2003).

In order to determine the potential relevance of phytoplankton in marine Pt biogeochemical cycles, sampling at ~ 1 h resolution covering diurnal cycles was performed in contrasting coastal environments in the Gironde Estuary mouth, the Arcachon Bay and the Genoa Harbor. The objectives of the present work are (i) to identify potential relations in short-term (hourly timescale) Pt concentrations with phytoplankton production/degradation cycles, (ii) to assess possible transfer of Pt through the aquatic trophic chain, and (iii) to investigate the potential use of phytoplankton material as a geochemical tool for biomonitoring Pt contamination in coastal systems.

2. Material and Methods

2.1. Study area and sample collection

The Gironde Estuary (~170 km in length; 80,000 km² watershed area) is a major European estuary located in southwest France (Figure 1A), considered a model for physical, hydrological and geochemical studies, especially on trace element transport and reactivity in anthropogenically impacted watersheds (Lanceleur et al., 2011). Its most important hydrodynamic characteristics are the semi-diurnal meso- to macro-tidal regime and the maximum turbidity zone ($> 1 \text{ g.L}^{-1}$ of suspended particulate matter), exported occasionally to the Atlantic Ocean (Doxaran et al., 2009). Sampling in the Gironde Estuary mouth was performed on-board the Research Vessel (R/V) *Thalia* at a fixed sampling site (45°40'00.2"N, 1°10'33.0"W, Figure 1A) during low discharge conditions ($\sim 235 \text{ m}^3.\text{s}^{-1}$, DIREN, public agency; 22nd/23rd June 2017), over 29 h on a close-to-hourly basis (i.e., night samples every two hours). In addition, wild oyster samples (*Crassostrea gigas*; 15 individuals, $\sim 9 \text{ cm}$ shell length) were collected at the La Fosse sampling site (45°28'23.4"N, 0°59'50.9"W; Figure 1A).

The Arcachon Bay is a meso-tidal lagoon in southwest France, $\sim 100 \text{ km}$ to the south of the Gironde Estuary mouth (Figure 1B) and represents an important breeding ecosystem for regional seafood production, especially oysters. This bay, exposed to urban and riverine inputs, on the Atlantic coast was selected for comparison with the Gironde Estuary. In fact, the sampling site (Comprian) in a main current channel of the bay receives inputs from the Leyre River and the city of Arcachon and seawater from the Atlantic Ocean at high tide. In May 2015 (21st – 22nd), hourly sampling over a diurnal cycle of 31 hours was performed on board R/V *Planula IV* (Comprian site, 44°40.823'N, 1°05.902'W, Figure 1B). Wild oysters (*C. Gigas*; 10 individuals, $\sim 9 \text{ cm}$ shell length) were collected manually on a bank near the Comprian site during low tide.

The Genoa Harbor is an artificial harbor of the northwest coast of Italy (Ligurian Sea, Figure 1C) and belongs to the most important ports of the Mediterranean Sea, known for contamination due to various human activities. Formerly, industrial effluents were the major cause of water pollution, while nowadays, discharges from sewage and creeks account for high levels in nutrients, organic matter and faecal pollution in the harbor waters (Ruggieri et al., 2011). This sampling site may serve to study biogeochemical cycles of Pt in a heavily industrialized area. In April 2016 (19th – 20th), hourly sampling covering diurnal cycle of 25 hours was performed inside the Genoa Harbor at the CNR platform (National Research Council, 44°23'44"N, 08°55'53"E, Figure 1C). At the platform, wild mussels (*Mytilus galloprovincialis*, 10 individuals, $\sim 7 \text{ cm}$ shell length) were also collected manually.

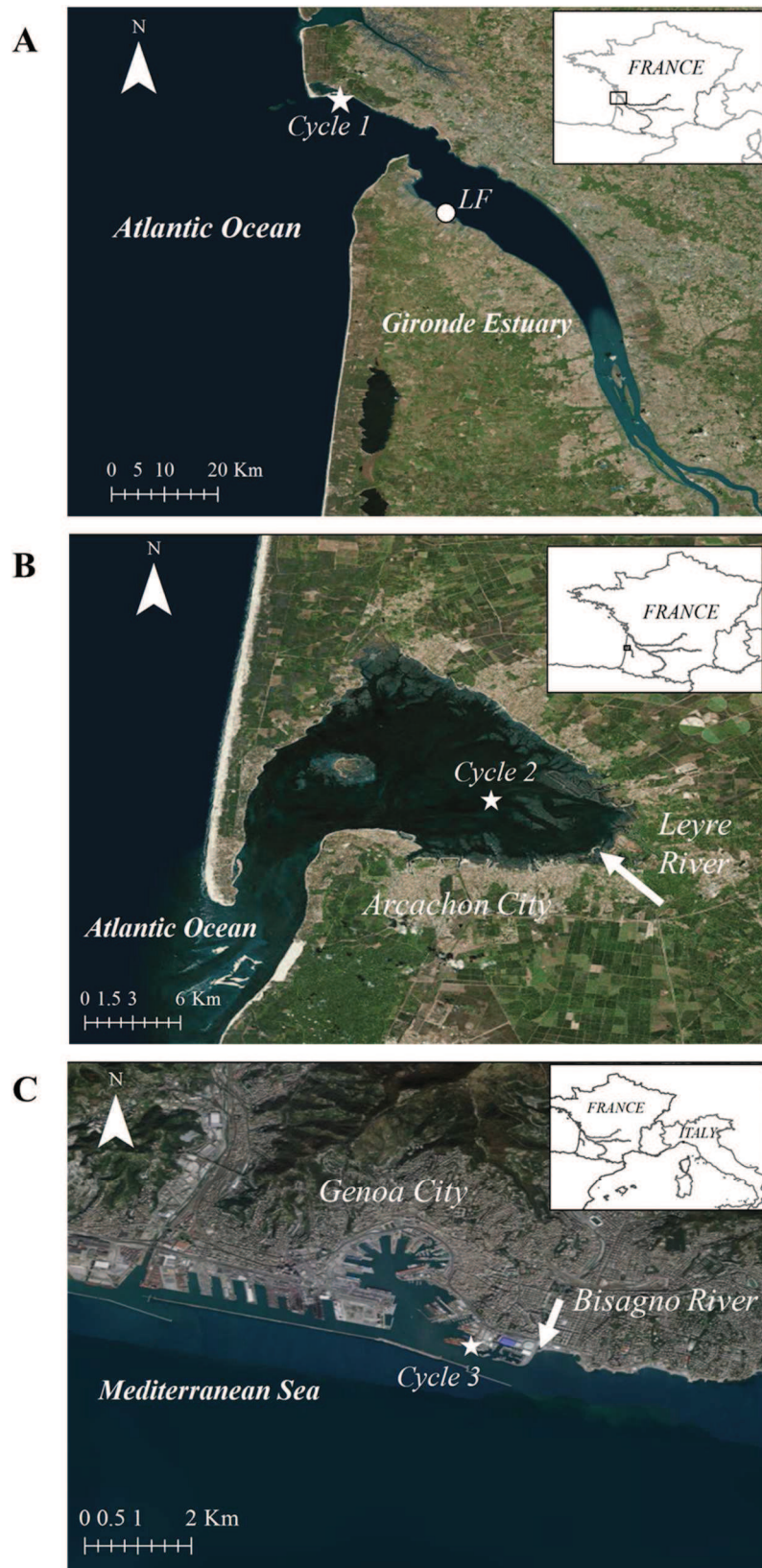


Figure 1: Study areas with diurnal cycles' sampling site (white stars). A: Gironde Estuary (Cycle 1), B: Arcachon Bay (Cycle 2, white arrow: Leyre River mouth), C: Genoa Harbor (Cycle 3, white arrow: Bisagno River mouth). Wild bivalve samples were collected at La Fosse (LF; oysters) sampling site in the Gironde Estuary and at the “cycle” sampling site for the Arcachon Bay (Comprian; oysters) and the Genoa Harbor (mussels).

At all sites, surface seawater was pumped using an electric pumping system connected to Teflon tubing. Seawater samples were immediately filtered through 0.2 µm polycarbonate filters (Nucleopore®) using a filter-syringe (Sartorius®) and placed in pre-cleaned 60 mL Teflon bottles (Nalgene®), previously rinsed with filtrate. Samples were acidified to pH = 1 (36.5 – 38 % HCl Baker Instra) and stored in the dark at 4 °C pending analysis.

Suspended Particulate Matter (SPM) concentrations in surface water were determined by filtration of precise water volumes through pre-weighed filters (Whatman® GF/F, 0.45 µm). The filters were then dried to constant weight at 45 °C, and weighed again. Furthermore, particulate samples were collected for chlorophyll-a and phaeopigments determination by filtering seawater (Whatman® GF/F, 0.7 µm) and filters were deep-frozen (- 80 °C) pending analysis.

All plankton material (i.e., both plankton organisms and other tripton or SPM) was collected by surface horizontal (~ 1m depth) sampling at the fix sampling station during the diurnal cycle of the Gironde Estuary and at variable times during daytime in the Arcachon Bay (n = 8; Comprian station) and the Genoa Harbor (n = 5). Given the strong tidal currents and the related suspension of silts and fine-grained sand in the Gironde Estuary we used sampling nets with nominal cut-off of 200 µm for the Gironde Estuary mouth, whereas the cut-off for the other sampling sites was 20 µm. Seawater containing concentrated plankton cells was retrieved from the collector and centrifuged at 1,500 rpm. The supernatant was removed and the collected residue was rinsed with seawater, deep-frozen, freeze-dried, and kept in the dark at room temperature pending analysis.

After manual collection of wild bivalves, samples were cleaned and depurated with oxygenation bubbling in seawater for 48 hours. Samples were dissected and soft tissues were placed in acid-cleaned polypropylene (PP) tubes (DigiTUBEs, SCP SCIENCE®). Samples were then deep-frozen, freeze dried, and kept in the dark at room temperature pending analysis.

At all sites, physical-chemical parameters such as temperature, salinity and oxygen saturation (O₂%) were measured *in situ* with a portable TetraCon 96® probe (PROFILINE, WTW). Information on solar radiation (insolation) was obtained from the “Meteo France” and “Infoclimat” databases for the SW of France and from “DIAM” (Dipartimento di Ingegneria Ambientale, University of Genoa) for the NW of Italy.

2.2. Analytical methods

Seawater

Seawater samples were analyzed by Adsorptive Cathodic Stripping Voltammetry (AdCSV) as described in Cobelo-García et al. (2014b). Measurements were carried out using a μ Autolab Type III potentiostat (Metrohm® Autolab B.V.) connected to a polarographic stand (Metrohm® 663 V.A.) equipped with three electrodes: (i) a hanging mercury drop electrode (HMDE, the working electrode), (ii) a silver/silver-chloride (Ag/AgCl) reference electrode, and (iii) a glassy carbon auxiliary electrode. A polytetrafluoroethylene (PTFE) voltammetric cell served in all experiments and the potentiostat was controlled using the NOVA 2.1 software. Elimination of organic matter by UV oxidation was performed by placing sample aliquots in capped Teflon tubes with overnight irradiation using two 64 W UV lamps (NIQ 60/35 XL, Heraeus) under a fume hood. Aliquots (10 mL) of UV-digested sample were pipetted into the voltammetric cell together with 30 μ L of 3.3 mM formaldehyde (37–41 % Analytical Reagent Grade, Fisher Chemical®), and 30 μ L of 0.45 mM hydrazine sulfate (Analytical Reagent Grade, Fisher Chemical®). Platinum concentrations were determined by standard addition method (adding mono-elementary Pt standard solution 1000 μ g.mL⁻¹ PLASMACAL, SCP Science®) using a deposition time of 300 s and experimental parameters described elsewhere (Cobelo-García et al., 2014b).

The detection limit for dissolved Pt measured by AdCSV (calculated as 3 x blank standard deviation, n = 20) was estimated to 0.03 ng.L⁻¹. In the absence of CRM for dissolved Pt in seawater, analytical quality of the voltammetric procedure was evaluated by determination of Pt spiked CRM seawater (CASS-6, NRCC) giving recoveries > 80% and precision expressed as Relative Standard Deviation (RSD) better than 10 % (n = 3) at the 0.5 ng.L⁻¹ range.

Biological samples: plankton material and bivalves

Ashing was applied as a pre-concentration method for Pt content in biological material (e.g. Schäfer et al., 1998). About 0.8 g of dry plankton material was ashed in porcelain crucibles at 800 °C during 3 h according to the heating scheme described by Nygren et al. (1990). After ashing and homogenization in an agate mortar, ~ 50 mg aliquots were then transferred into PP tubes, and digested at 110 °C for 3 h with 2 mL HCl and 1 mL HNO₃ (30 % HCl and 65 % HNO₃ Suprapur, Merck®) as described in Abdou et al. (2018). Cooled contents were then diluted in 10 mL MilliQ® water, centrifuged at 4000 rpm for 10 min (20 °C). Same procedure was applied for whole dried bivalve tissues.

Platinum concentrations were determined by Triple Quadrupole-Inductively Coupled Plasma Mass Spectrometry (TQ-ICP-MS, Thermo® iCAP TQ). Hafnium-oxide (HfO^+) interferences were monitored using the ^{193}Ir signal that is highly interfered by HfO^+ . Raw signal was thereafter corrected through the application of mathematical calculation using $^{194}\text{Pt} / ^{195}\text{Pt}$ natural ratio assuming that HfO^+ is the only dominant spectral interference on those selected isotopes as described in Abdou et al. (2018). Analyses were performed applying the standard addition method (using mono-elementary Pt standard solution $1000 \mu\text{g.mL}^{-1}$ PLASMACAL, SCP Science®).

The detection limit for Pt levels in biota measured by TQ-ICP-MS (calculated as 3 x blank standard deviation, $n = 10$) was estimated to 0.03 ng.g^{-1} for a nominal ashed sample mass of 50 mg (representative of plankton dried material) and 0.001 ng.g^{-1} for typical biological sample mass of 1.5 g (representative of bivalve dried material). Since no Certified Reference Material (CRM) for Pt in biological matrices is available, quality control was performed using available CRMs for Pt concentrations in solid matrices: the BCR®-723 road dust (IRMM) and Jsd-2 sedimentary rocks (indicative value from GSJ). Analyses of these CRMs by ICP-MS gave satisfactory recovery values of 87% and 101% respectively ($n = 3$). Moreover, a previous inter-method comparison using two completely independent digestion and measurement techniques (TQ-ICP-MS and AdCSV) has shown that Pt concentrations in natural biological samples (oysters) can be reliably measured by the aforementioned TQ-ICP-MS technique when sufficient sample mass and/or Pt content are available (Abdou et al., 2018). Aliquots of plankton net material were analyzed in parallel, using both ICP-MS and AdCSV providing, as observed for bivalve samples, similar results between both methods ($\text{RSD} < 10 \%$, $n = 2$).

Visual examination confirmed that silt and clay particles were absent in the plankton net material, but some minerals belonging to middle/coarse sand fraction were present. The major volume of these samples was planktonic material, mainly phytoplankton during the day and zooplankton during the night. This is consistent with results of phytoplankton studies on the Portuguese shelf (Caetano and Vale, 2003). Nevertheless, assuming that Pt concentrations in sand are lower than those in fine-grained silt and clay materials (i.e., surface/volume sorption-relation) and based on the particulate Pt (Pt_p) values reported for SPM sampled in the Gironde Estuary mouth without grain-size cut-off range $\sim 0.25 - 0.3 \text{ ng.g}^{-1}$ (November 2012; Cobelo-García et al., 2014a), we have estimated the maximum Pt_p in suspended sand at 0.2 ng.g^{-1} . Furthermore, using the loss on ignition (8 hours at 800°C) as a proxy of the amount of organic material present in the samples, we have estimated minimum Pt_p concentrations in the organic fraction as follows (Equation 1):

$$Pt_{PK} = \frac{[Pt_{tot} - Pt_{ss} \times (1 - \%OM)]}{\%OM} \quad (1)$$

With Pt_{PK} : the Pt concentration in the organic fraction (the planktonic fraction), Pt_{tot} : the Pt concentration in the total plankton net material sample, Pt_{ss} : the Pt concentration in suspended sand estimated at 0.2 ng.g^{-1} from data in Cobelo-García et al. (2014a), and $\%OM$: the percentage of organic matter determined using the loss on ignition. Despite the potential influences of lithogenic material such as mineral or Fe-oxide particles, as well as non-living organic aggregates and phytodetritus (Twining et al., 2011), other field studies analyzing bulk particulate matter assumed low lithogenic and detrital contributions for a range of trace metals (Twining and Baines, 2013). In contrast to the highly dynamic Gironde Estuary mouth and the Arcachon Bay, where strong tidal currents occur, visual examination of Genoa Harbor plankton material (20 μm mesh) showed that samples were composed of phytoplankton cells. No sandy particles were detected, in line with the weak currents, suggesting that the mineral fraction (Pt_{ss}) in plankton nets was negligible in Genoa Harbor.

Chlorophyll-a and phaeopigments

Chlorophyll a (Chl-a) was extracted in 90 % acetone and determined by spectrofluorometry (Trilogy, Turner, Strickland and Parsons, 1972). Dilutions of a solution of Chl-a (Anacystis nidulans, 1 mg.L^{-1} ; C-6144; Sigma-Aldrich®) was used to calibrate the fluorimeter. Phaeopigments (phaeo) were also determined using the same analysis (after acidification, HCl 3N). Due to sampling logistics, Chl-a and phaeo data at the Genoa Harbor are only available for daytime period.

2.3. Data normalization: the effect of turbidity

Assuming a positive correlation of chlorophyll-a and phaeopigment concentrations with SPM concentrations during daytime (determination coefficient $R^2 \sim 0.7$), as frequently observed in various systems including the Gironde Estuary (Irigoien and Castel, 1997), the normalized phytoplankton pigments/SPM ratio could be a better SPM-independent indicator of potential phytoplankton production than pigment concentration alone (Irigoien and Castel, 1997). Therefore, in this study, phytoplankton pigments Chl-a and phaeo data are SPM-normalized values. Likewise, in order to represent the effective solar radiation penetrating the water column, solar radiation values were also normalized by SPM concentrations (rad/SPM, Castelle et al., 2009).

3. Results

3.1. Physical-chemical variables and Pt concentrations in seawater and plankton material

Gironde Estuary

Master physical/chemical parameters monitored over the Cycle 1 (Figure 2A) showed salinity variations from 30 to 32.5, depending on tidal variations at the estuary mouth with increasing salinity showing seawater entrance. The SPM concentration varied from ~ 5 to 50 mg.L^{-1} . Effective solar radiation ratio (i.e., SPM-normalized solar radiation: rad/SPM) varied from 0 to 30, showing two maxima (i.e., at 5 pm and 12 am of the following day). Oxygen saturation levels varying from ~ 100 to 110% are coincident with high tide at 5 pm on the first day. During the night, $\text{O}_2\%$ levels are at minimum, coincident with low salinity (ebb flow). During the following flood event, when salinity increased in the middle of the nighttime (5 am), $\text{O}_2\%$ increased only slightly, fully displaying the second $\text{O}_2\%$ maximum during the second day at 12 am during low tide. During the cycle, the lower $\text{O}_2\%$ values corresponded to $\sim 100 \%$ saturation, implying in all cases well oxygenated waters.

Phytoplankton pigment/SPM ratios along the diurnal cycle showed similar variations for Chl-a/SPM and phaeo/SPM, but at different levels (variations from ~ 0.1 to 0.7×10^{-3} ; Figure 2B). Accordingly, on the first day, increasing Chl-a/SPM and phaeo/SPM ratios co-occurred with the aforementioned first peak in rad/SPM , $\text{O}_2\%$ and salinity at 5 pm. This maximum was of 0.7×10^{-3} for Chl-a/SPM while it was of 0.25×10^{-3} for phaeo/SPM. Decreasing trend occurred thereafter and minimum values were reached for both parameters in the middle of the night. Two peaks occurred during the second day, coincident with the high tide input at 5 am for the former and high rad/SPM and $\text{O}_2\%$ at 12 am for the latter.

Hourly measured Ptd concentrations ranged from ~ 0.05 to 0.11 ng.L^{-1} showing alternating trends (Figure 2C). Three main peaks occurred: (i) one on the first day at 2 pm (0.11 ng.L^{-1}), followed by a decrease down to 0.06 ng.L^{-1} , (ii) a small peak during the night at 9 pm and (iii) a third peak of $\sim 0.11 \text{ ng.L}^{-1}$ at 9 am of the second day. During the Cycle 1, PtPK concentrations ranged from 0.01 to 2.9 ng.g^{-1} (Figure 2D). During the nighttime, minimum values occurred and the following day, PtPK showed increasing levels from the early morning to a maximum of $\sim 3 \text{ ng.g}^{-1}$ at 12 am.

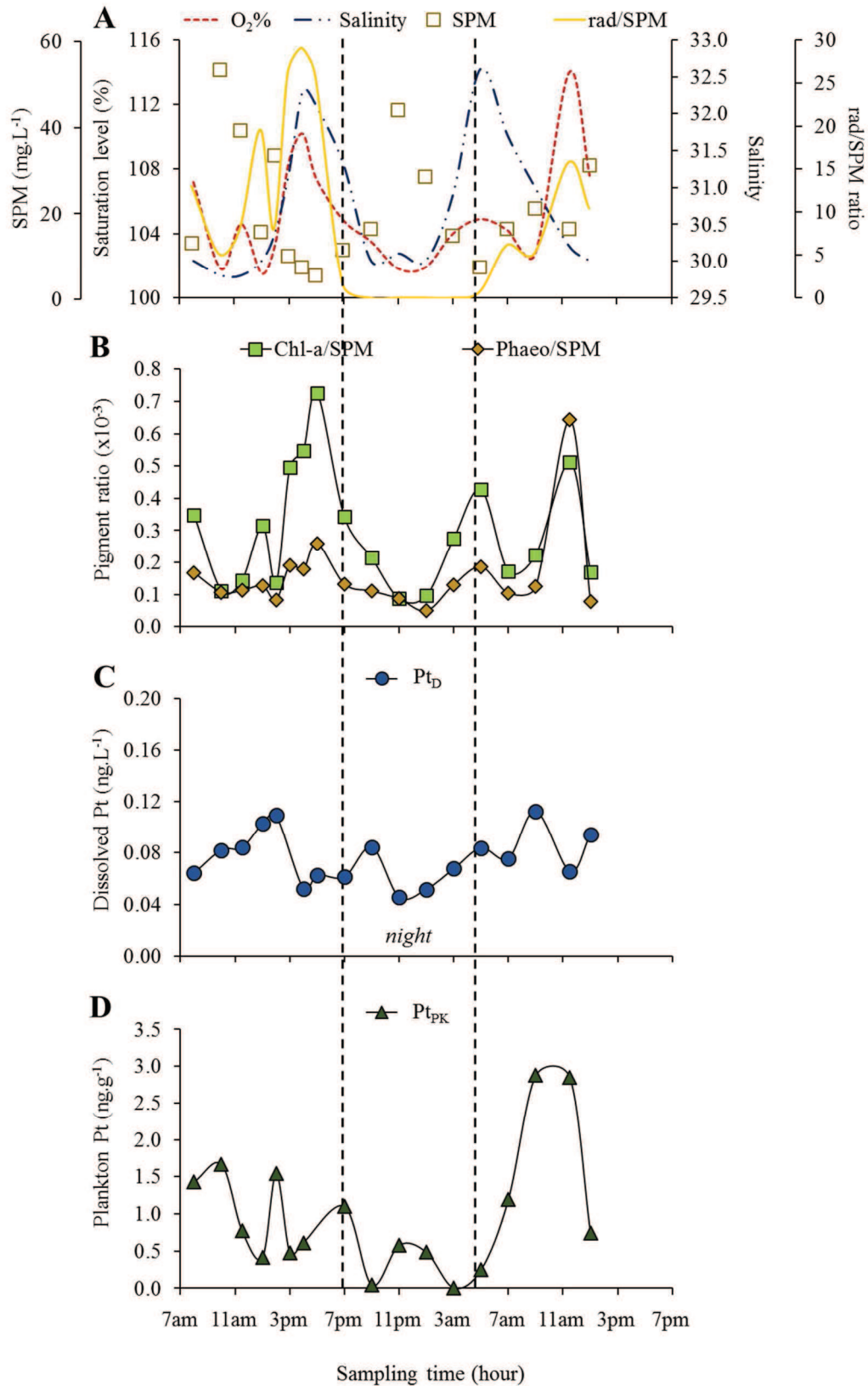


Figure 2: Short-term variations in physical/chemical variables and Pt concentrations in seawater and plankton material from the Gironde Estuary in June 2017. A: SPM (squares), O₂% (dashed line), salinity (dashed-dotted line), rad/SPM (solid line); B: Chl-a/SPM ratio (x 10⁻³, squares) and phaeo/SPM (x 10⁻³, diamonds); C: Pt_D (ng.L⁻¹, circles); D: Pt_{PK} (ng.g⁻¹, triangles).

Arcachon Bay

Results from Cycle 2 (Arcachon Bay; Figure 3) showed consistent physical-chemical parameters, but no clear diurnal P_{TD} trends. Salinity ranged from 22 to 32 and $O_2\%$ from 75 to 85 % (i.e., always below 100% saturation observed in the Gironde Estuary mouth), with both parameters showing similar patterns (maxima at 9 pm and 9 am; Figure 3A). In general, SPM concentrations ranged from ~ 6 to 48 mg.L^{-1} and followed tidal dynamics. Effective radiation ratio reached maximum values (~ 25) at 3 pm on the first day and 11 am on the following day. Phaeo/SPM values were constant (0.1×10^{-3}) over the cycle, whereas Chl-a/SPM showed higher levels during the day ($\sim 0.2 \times 10^{-3}$) with maxima at 9 pm and ~ 11 am and lower values during the night ($\sim 0.1 \times 10^{-3}$; Figure 3B). Dissolved P_{TD} concentrations varied from 0.05 to 0.15 ng.L^{-1} (Figure 3C) on the first day and during nighttime. In the morning of the second day (from 8 am onwards), P_{TD} levels suggest an increase matching high tide maximum at ~ 11 am and thereafter a clear decrease towards 0.05 ng.L^{-1} at 6 pm.

Genoa Harbor

Results from the CNR Platform in the Genoa Harbor were very different to those in the previous sites. Salinity was constantly at ~ 38 as no tidal variation occurred at this site (not shown) and SPM concentrations varied little from ~ 2 to 8 mg.L^{-1} and were 6-fold lower than in the Atlantic sites; (Figure 4A).

Oxygen saturation levels ($O_2\%$) were between 85 % and 100 %, i.e. between those observed in the Gironde Estuary mouth (minimum 100%) and in the Arcachon Bay, showing lower values during the night (Figure 4A). Maximum rad/SPM occurred at 11 am.

In addition, Chl-a/SPM and phaeo/SPM ratios were almost double those in the Arcachon Bay and correspond to ranges observed in the Gironde Estuary mouth (around 0.45×10^{-3} and 0.15×10^{-3} , respectively, Figure 4B). However, no clear trend occurred in both cases though a concomitant minimum may be supposed at ~ 5 pm. Likewise, no clear day / night trends in P_{TD} concentrations ($\sim 0.08 \text{ ng.L}^{-1}$ to 0.16 ng.L^{-1}) occurred, although there might be a slight variation opposite to that of $O_2\%$; (Figure 4C).

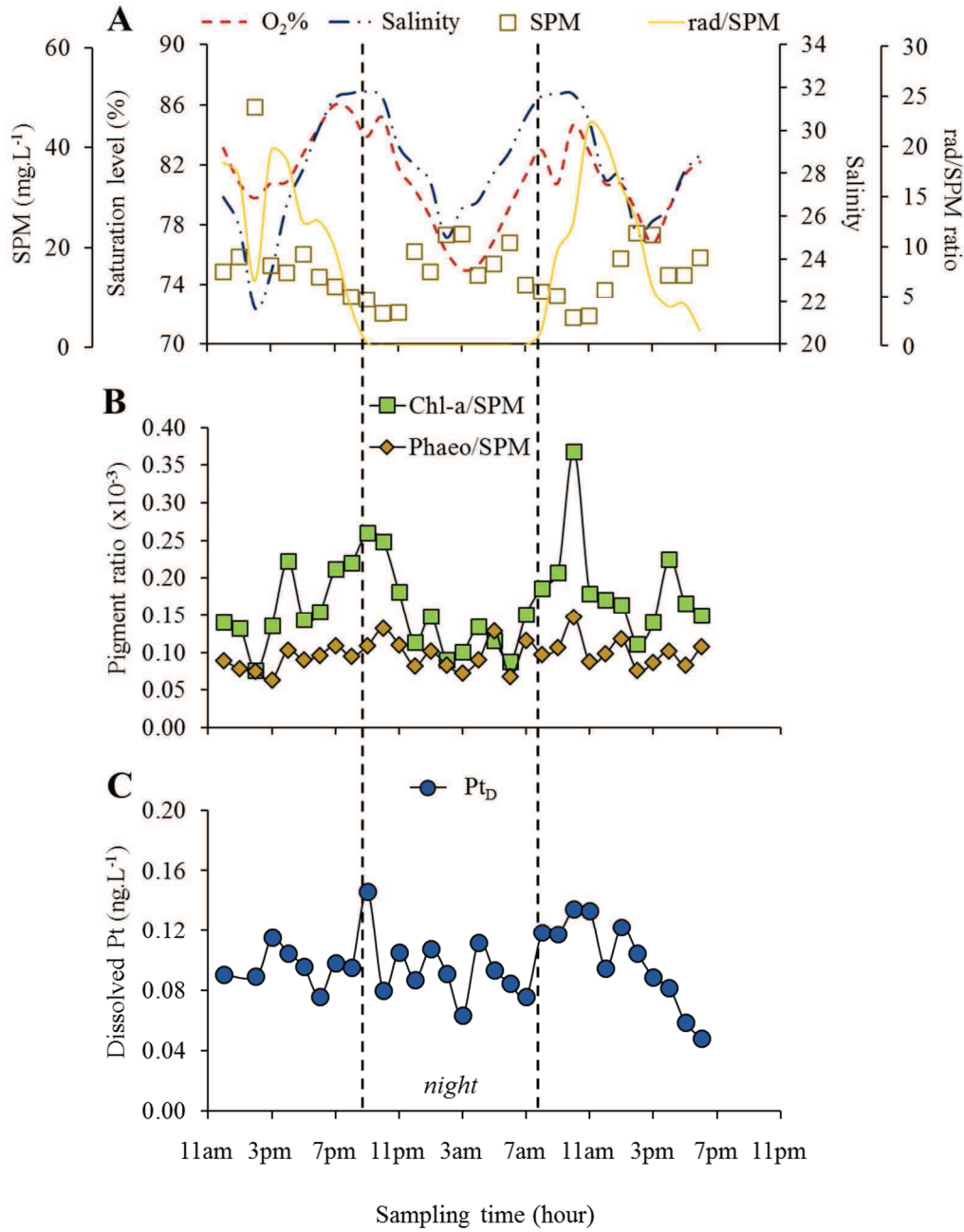


Figure 3: Short-term variations in physical/chemical variables and Pt_D concentrations in the Arcachon Bay in May 2015. A: SPM (squares), O₂% (dashed line), salinity (dashed-dotted line), rad/SPM (solid line); B: Chl-a/SPM ratio (x 10⁻³, squares) and phaeo/SPM (x 10⁻³, diamonds); C: Pt_D (ng.L⁻¹, circles).

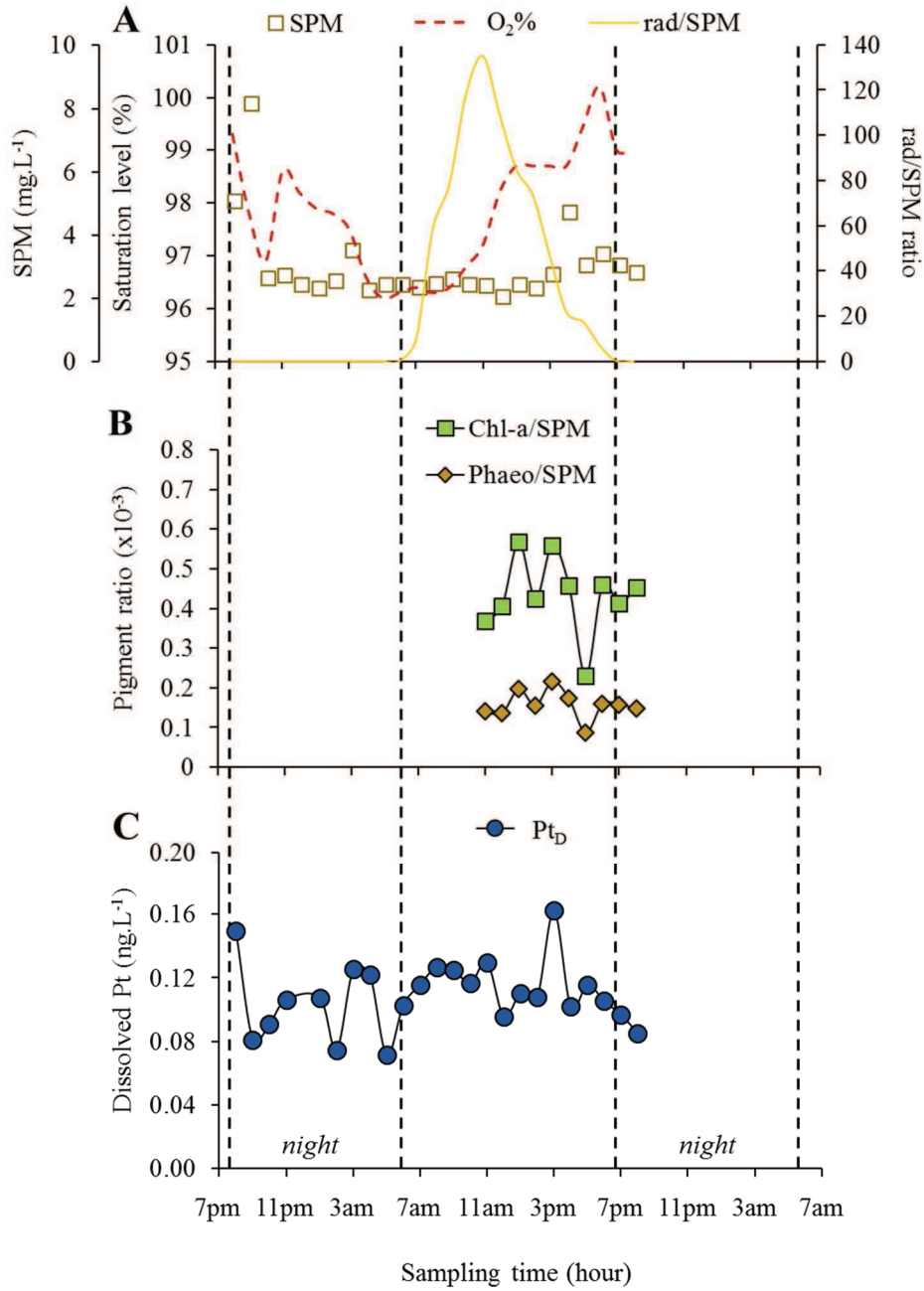


Figure 4: Short-term variations of physical/chemical variables and Pt_D in the Genoa Harbor in April 2016. A: SPM (squares), $O_2\%$ (dashed line), rad/SPM (solid line); B: Chl-a/SPM ratio ($\times 10^{-3}$, squares) and phaeo/SPM ($\times 10^{-3}$, diamonds); C: Pt_D (ng.L⁻¹, circles).

3.2. Platinum concentrations in plankton and bivalves from the three contrasting sites

In order to compare Pt_{PK} in plankton material from the three sites collected in the Arcachon Bay and the Genoa Harbor only daytime Pt_{PK} concentrations are reported, because these samples are mainly composed of phytoplankton cells. Bioconcentration factors (BCF; Arnot and Gobas, 2006) were determined according to the following Equation 2:

$$BCF = \frac{Pt_{biota}}{Pt_D} \times 10^3 \quad (2)$$

With Pt_{biota} : the Pt concentration in biota sample (corresponding to Pt_{PK} in this study), Pt_D : the dissolved Pt concentration and 10^3 a correcting factor for units.

In the Gironde Estuary mouth average daytime Pt_{PK} was $\sim 1.3 \text{ ng.g}^{-1}$ (Table 1), which is somewhat higher than Pt_{PK} of $\sim 0.99 \text{ ng.g}^{-1}$ measured during the whole day/night cycle (data not shown). In the Arcachon Bay and the Genoa Harbor, Pt_{PK} were clearly higher, i.e. 2.3 and 8.0 ng.g^{-1} (Table 1).

Platinum concentration in bivalves (Pt_{BV}) was $\sim 0.33 \text{ ng.g}^{-1}$ in wild oysters (*Crassostrea gigas*) from the La Fosse site i.e. greater than in the Arcachon Bay (0.20 ng.g^{-1} ; Table 1). Wild mussels (*Mytilus galloprovincialis*) from the Genoa Harbor had $Pt_{BV} \sim 0.48 \text{ ng.g}^{-1}$. The resulting BCF for plankton (BCF_{PK}) ranged from 1.6×10^4 to 7.3×10^4 at the different sites, whereas BCF for bivalves (BCF_{BV}) varied from 2.4×10^3 to 4.2×10^3 , i.e. were lower by \sim one order of magnitude.

Table 1: Platinum concentrations in seawater (Pt_D), plankton (Pt_{PK}) and bivalves (Pt_{BV}) from the three sites and associated bioconcentration factors (BCF; BCF_{PK} and BCF_{BV}).

Sampling site	[Pt] _D (ng.L ⁻¹)			[Pt] _{PK} (ng.g ⁻¹)			[Pt] _{BV} (ng.g ⁻¹)			BCF _{PK}	BCF _{BV}
	Mean	SD	n	Mean	SD	n	Mean	SD	n		
Gironde Estuary	0.08	0.02	18	1.3	0.78	13	0.33	0.03	5	1.6×10^4	3.0×10^3
Arcachon Bay	0.10	0.02	30	2.3	0.66	3	0.20	0.06	10	3.1×10^4	2.4×10^3
Genoa Harbor	0.11	0.02	25	8.0	3.2	5	0.48	0.14	10	7.3×10^4	4.2×10^3

4. Discussion

4.1. Controls on diurnal Pt cycles in the water column of the three contrasting sites

Diurnal cycles of the different parameters observed in the Gironde Estuary suggest that their variations are related with plankton production/degradation activities. Accordingly, different patterns prevail during daytime and nighttime.

Visual examination showed that plankton net material collected during the day mainly consisted in phytoplankton cells. This is in accordance with daily maxima in Chl-a/SPM ratio observed on both days (Figure 2B). On both days, high Chl-a/SPM occurred under conditions favorable for primary production, as high rad/SPM (rad/SPM) during high tide allows for optimum primary production, although one cannot exclude direct transport of coastal phytoplankton to the sampling site. Note that highest SPM generally occurred during the low tide and reflect the downstream position of the Maximum Turbidity Zone oscillating with the tide (Figure 2A). In the Gironde Estuary mouth, primary production seems to be light-driven rather than nutrient-limited, as Chl-a/SPM ratio shows a positive linear correlation ($R^2 \sim 0.7$) against rad/SPM during the daytime, which is in accordance with predictions on light-limited phytoplankton growth (Goosen et al., 1999). These conditions go along with a general O₂% oversaturation of the water mass, while no major change in phaeo/SPM ratio occurred, suggesting low phytoplankton degradation associated with low SPM conditions (Figure 2A, 2B).

The variations of P_{TD} from 0.06 ng.L⁻¹ to 0.11 ng.L⁻¹ over the cycle may be partly due to mixing of estuarine waters with ocean water in line with P_{TD} addition along the Gironde Estuary salinity gradient under moderate discharge conditions, resulting in P_{TD} \sim 0.08 ng.L⁻¹ at S = 28.1 (Cobelo-García et al., 2014a). Furthermore, P_{TD} level in the coastal northeastern Atlantic Ocean is \sim 0.05 ng.L⁻¹, as observed during the same field campaign at a more external sampling site (45°32'16.8", 1°08'49.2"W; unpublished data). This value completes and confirms previous North Atlantic Ocean P_{TD} values (\sim 0.02 - 0.06 ng.L⁻¹, Colodner, 1991), whereas inside the Gironde Estuary, P_{TD} levels may reach 0.16 ng.L⁻¹ in the low salinity range (S = 5.4, Cobelo-García et al., 2014a).

Various biogeochemical processes in coastal waters (e.g. desorption, adsorption, dissolution/precipitation, bio-uptake or –release) may transfer metals between the dissolved and the particulate phases; e.g. Turner, 2007). Although exact transfer mechanisms explaining exchanges of Pt between different carrier phases are widely unknown, the observed variations in P_{TD} and P_{PK} may be either due to (i) internal transfer processes or (ii) external source/sinks releasing or sequestering Pt from the estuarine water column. Internal transfer processes imply

equilibrated mass balance, whereas non-equilibrated mass balances would suggest an open system involving external sources or sinks. In the present work, such mass balances do not take into account the fine-grained particulate phase, including silt and clay, also containing Fe and Mn oxy/hydroxides, potentially important Pt carrier phases (Colodner et al., 1992).

Mass balance for the first day (hours) suggests that the overall increase in Pt_D is similar in magnitude as the decrease in particulate Pt (calculated from Pt_{PK} and SPM concentrations), i.e. the increase in Pt_D would be explained by transfer of Pt from the particulate to the dissolved phase. This supposed transfer occurs under low rad/SPM conditions, suggesting minimum phytoplankton activity as supported by minimum Chla/SPM (Figure 2B). During the afternoon of the first day, raising tide triggers upstream movement of the estuarine turbidity zone, resulting in maximum rad/SPM and, consequently, maximum primary production as reflected by Chla/SPM. At the same time, a strong decline in Pt_D and concomitant increase in Pt_{PK} suggests sorption of dissolved Pt by phytoplankton. Consequently, the opposite temporal trends in Pt_D and Pt_{PK} on the first day (Figure 2D), supported by mass balance estimates, clearly supports Pt transfer to primary producers, i.e. the basis of the marine food chain.

During the night and low tide, decreasing Chla/SPM, phaeo/SPM and Pt_{PK} suggest, together with low salinity, that in the downward part of the estuarine maximum turbidity zone degradation of phytoplankton occurs, resulting in release of Pt_D into the water column (Figure 2D). In fact, during the night, the plankton material is no longer composed of phytoplankton cells but rather of zooplankton organisms that perform a vertical migration for feeding (Daro, 1988). Thus, Pt_{PK} (0.5 ng.g^{-1}) observed during nighttime may reflect Pt levels in zooplankton organisms.

The strong increase in Pt_D concentrations during the second half of the night might reflect Pt release due to the fact that trace elements incorporated or adsorbed by phytoplankton will remain in the particulate phase until cell lyses and remineralization occur (Sanders and Abbe, 1987, Sanders and Riedel, 1998). The Pt_D (0.08 ng.L^{-1}) at the next high tide (5 am) is higher than the observed Atlantic Ocean value and suggests local addition of Pt_D supporting the hypothesis of Pt_D release by phytoplankton grazing.

Mass balance for the second day suggests a clear concomitant increase in both, Pt_D and particulate Pt, implying an additional Pt source. Interestingly, the maximum Pt_{PK} coincides with maximum phaeo/SPM ratio, serving as a proxy for phytoplankton degradation. The hypothesis of higher recycling of organic material on the second day is supported by phosphate concentrations below detection limit ($< 0.05 \text{ } \mu\text{M}$) on the first day, whereas on the second day measurable phosphate occurred ($\sim 0.05 \text{ } \mu\text{M}$; data not shown). Cell sinking may intensively

occur during the night, following zooplankton grazing activity (Luoma et al., 1998). Biogenic particles can sink more or less rapidly (depending on algal species) and oscillate in the water column due to sediment resuspension cycles, especially in high-energy systems such as the Gironde Estuary. Accordingly, physical mechanisms may mask biogeochemical exchanges of Pt between the dissolved and the particulate phases by (i) sequestering Pt-bearing particles in sediments or (ii) releasing Pt_{PK} and/or Pt_D from temporary sinks/sources. Given tidal coefficients of 81 on the first day and 92 on the second, one would expect a strong increase in tidal amplitude and resulting currents, especially at mid-tide, inducing both sediment resuspension and erosion of micro-phytobenthic mattes which form extended layers on the sediment surface in the intertidal areas (Strady et al., 2011). These phytobenthic layers are potential metal carriers and their tidal suspension may influence metal cycles in coastal systems (Strady et al., 2011). One may hypothesize that the increasing of Pt_{PK} on the second day may result from erosion of sediment/phytobenthic layers due to increasing currents at mid-tide. The concomitant increase of Pt_D would then reflect ongoing degradation of dead cells as supported by strongly increasing phaeo/SPM (Figure 2B). At the end of the morning, maximum rad/SPM, Chl-a/SPM and decreasing Pt_D may be again attributed to Pt sorption by phytoplankton under optimum primary production conditions, inducing maximum Pt_{PK} . Inversely, the subsequent strong decrease in Pt_{PK} and higher Pt_D at the end of the cycle coincide with a sharp decrease in rad/SPM due to doubled SPM. Both observations clearly suggest alternated exchange of Pt between the dissolved and the particulate phases, supported by equilibrated mass balances.

In the Arcachon Bay (Cycle 2), SPM concentrations and rad/SPM ranges were similar or only slightly lower than those in the Gironde Estuary mouth (Cycle 1). Accordingly, a clear diurnal cycle of primary production occurred, yet maximum Chl-a/SPM were clearly lower in the Arcachon Bay, suggesting lower primary production (Figure 3B). Accordingly, lower production and/or higher consumption in the riverine channels of the Arcachon Bay, showing globally higher SPM coinciding with lower salinity may explain lower O_2 levels. At the same time, Phaeo/SPM values were similar in magnitude for both cycles, but lower variations in the Arcachon cycle suggest that phytoplankton degradation processes also were less intense.

The Pt_D did not show a clear diurnal cycle, especially during the first day (Figure 3C), but a clear decreasing trend on the second day. Since no Pt_{PK} data are available for this cycle, in contrast to the Gironde Estuary mouth, the variations in Pt_D cannot be clearly attributed to diurnal cycles in relation with phytoplankton production and degradation. This observation may suggest additional influences in this semi-enclosed bay, such as Pt inputs from the surrounding urban areas, including surface run off and sewage from the urban agglomerations around the Arcachon Bay. Note that the major part of sewage from Arcachon City itself and the closest

surroundings is released into the open ocean at ~ 40 km to the south of the Bay inlet. In fact, relatively high P_{TD} levels of 0.08 to 0.15 ng.L^{-1} most probably reflect contamination.

In the Genoa Harbor, SPM concentrations were clearly lower than in the Atlantic coast sites and rad/SPM ranges reached the highest values of all sites. Given the generally high nutrient load (Ruggieri et al., 2011), this may explain the highest Chl-a/SPM , i.e. the highest phytoplankton production in this site (Figure 4B). Phaeo/SPM similar to those in the Gironde Estuary may suggest moderate degradation processes. Similar to the Arcachon Bay, in the semi-enclosed Genoa Harbor, no clear trends in P_{TD} occurred, suggesting together with rather constant salinity that the observed variations probably reflect changing inputs and biogeochemical recycling of anthropogenic Pt in this heavily urbanized and industrialized harbor. In fact, the P_{TD} levels in the Genoa Harbor (average 0.12 ng.L^{-1}) were mostly higher than 0.08 ng.L^{-1} , i.e. the typical value of the open Mediterranean Sea surface water (Ligurian Current; unpublished). The fact that the lowest P_D concentration occurred during the night may support Pt inputs by urban sewage, typically showing greater values during the day due to excretion of anticancer drugs (Kümmerer and Helmers, 1997), especially as the sampling site is located near the Bisagno River mouth (Figure 1C), draining the center of Genoa with nearby hospitals, the nearest one (< 3 km) renowned for cancer therapy.

The results obtained from the three contrasting sites suggest that short-term processes, such as diel cycles of photosynthesis and respiration may help understanding the marine biogeochemistry of Pt. In less urbanized sites with high primary productivity, algal Pt sorption and release appear as major factors in Pt biogeochemical cycle. In contrast, in more confined and/or urbanized coastal water bodies, such biogeochemical signals may be masked by the dynamics of Pt inputs from diverse sources, such as wastewater, run off or urban aerosols.

4.2. Platinum sorption by phytoplankton - consequences for the trophic chain

Phytoplankton cells are efficient scavengers of trace elements, accumulating high concentrations from the surrounding medium (Sanders and Riedel, 1998). Both active and passive mechanisms can be involved in trace element sorption and the relative importance of different sorption pathway depends on the target element (Sanders and Riedel, 1998). Therefore, the observed Pt sorption by phytoplankton may reveal either: (i) active uptake of Pt by phytoplankton cells with/without relevant biological functioning (e.g., mimicking other bio-elements) or (ii) adsorption of Pt on plankton material surface. For marine algae, the chemical speciation of the element and the presence of other ions or chelators able to regulate speciation are of major importance for bioavailability (Sanders and Riedel, 1998; Sunda, 1989). There is only limited thermodynamic information relevant to the speciation of Pt in aquatic environments. Available data suggest that in seawater Pt(IV) dominates such as $\text{PtCl}_5(\text{OH})^{2-}$ (Cobelo-García et al., 2014a; Gammons, 1996; Mashio et al., 2017), but no experimental data on uptake of these species by phytoplankton is available. Laboratory experiments showed linear increase of freshwater periphyton Pt uptake following exposure to dissolved Pt(II) and Pt(IV). Although no significant toxicity was determined, a significant linear decrease of the photosynthetic activity occurred in reference water which was related to bio-uptake (Rauch et al., 2004). For the marine environment, Caetano and Vale (2003) reported the relative importance of plankton and seston in the distribution of trace elements with plankton net material showing clearly higher metal (Cu) concentrations than the lithogenic fraction, i.e. detrital particles retained on 0.45 μm membranes (seston).

It remains unclear, if the observed Pt levels in plankton may induce any adverse effects on these cells, but phytoplankton being the first link in the marine trophic chain, transfer to and accumulation in higher organisms cannot be excluded. Such biotransformation may alter (enhance or reduce) compound toxicity to other organisms and associated adverse biological effects (Sanders and Riedel, 1998).

Considering that nighttime plankton material from the Gironde Estuary mouth mainly consists in zooplankton, we could assume mean Pt concentrations of $\sim 0.5 \text{ ng.g}^{-1}$ for these organisms (Figure 2D). Accordingly, Pt_{PK} in zooplankton would be lower than in phytoplankton. Moreover, Pt concentrations in wild oysters from the Gironde Estuary showed even lower values of $\sim 0.3 \text{ ng.g}^{-1}$ (Table 1), suggesting that no biomagnification of Pt concentrations occurred at this trophic level. Note that bivalves may display important seasonal variations in soft body metal concentrations due to dilution effects related with growth and/or reproduction cycles (e.g. Enríquez-Díaz et al., 2009). Since sampling campaigns aimed at determining the role of phytoplankton in Pt biogeochemical cycles, bivalves also were sampled in Spring (algal

bloom), suggesting that the observed Pt concentrations in oysters and mussels of the present study may be somewhat different (lower) from the respective winter values. However, seasonal variations in Pt concentrations in bivalves from various sites along the northwestern Mediterranean Coast were generally lower than 30 % (Abdou et al., unpublished), suggesting that the BCF determined in the present work may also be suitable for winter bivalves. In both urbanized sites, the Arcachon Bay and the Genoa Harbor, Pt concentrations in phytoplankton were $\sim 12 - 17$ times greater than in bivalves, whereas in the Gironde Estuary mouth the ratio Pt_{PK} / Pt_{BV} was ~ 4 (Table 1). The latter may be due to the fact that the Gironde Estuary phytoplankton reflects open coastal ocean conditions, whereas the oysters from the La Fosse site are exposed to inner-estuary conditions (Figure 1A). Furthermore, Pt concentrations in phytoplankton may vary as phytoplankton communities undergo species succession and changes in dominant species (Cobelo-García et al., 2012), which may induce a clear shift in the phytoplankton composition and therefore potentially on trace metal sorption. Given the short lifetime of phytoplankton, these cells could be a promising biomonitor for contaminant pressure at short time scales. More data would be necessary to evaluate the potential transfer of Pt from phytoplankton to different bivalve species, with respect to accumulation from ambient seawater as a prerequisite for reliable multi-species biomonitoring of Pt contamination as reported in other coastal environments (Conti and Cecchetti, 2003). In fact, phytoplankton, zooplankton, and bivalves may respond differently to a particular metal fraction of the water body (different speciation and partition) according to their energy harvesting behavior and detoxification mechanisms.

5. Conclusions and perspectives

This study reports on Pt partitioning at high temporal resolution in three contrasting coastal environments. In the Gironde Estuary mouth, intense primary production resulted in Pt depletion of seawater, together with increasing Pt in phytoplankton, which may imply different sorption processes such as uptake inside algal cells and/or adsorption onto algal cell surface. Phytoplankton grazing by zooplankton may lead to Pt addition to the dissolved phase due to biomass degradation. In contrast, in more confined systems influenced by various local Pt sources such as the Arcachon Bay and the Genoa Harbor, these processes were partly masked. In the Gironde Estuary, no biomagnification of Pt seems to occur neither from phytoplankton to zooplankton; nor from phytoplankton to corresponding bivalves in the three sampling sites.

To the best of our knowledge, this work constitutes the first study on Pt partitioning between seawater and phytoplankton material, providing bioconcentration factors in the marine algal compartment ($\sim 10^4$). By reflecting the general state of Pt contamination of these three contrasting sites and showing relatively high bioconcentration factors, phytoplankton may be considered as an important variable in coastal short-term biogeochemical Pt cycles.

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This study provides knowledge on factors influencing Pt biogeochemical cycles in coastal environments. Accordingly, phytoplankton may exert a non-negligible role on Pt partitioning between dissolved and particulate phase in estuarine seawater end-members and more generally in the coastal zone. The main outcomes from this study are the following:

- ✓ Platinum sorption (uptake and/or adsorption) by phytoplankton may occur with increasing primary production activity. Nocturnal cell destruction and potential sinking could cause additional Pt release into the dissolved phase.
- ✓ Higher organisms in the aquatic food chain (zooplankton and bivalves) do not show biomagnification of Pt.
- ✓ Phytoplankton Pt concentrations reflect the general contamination level of coastal environments and show relatively higher Pt bioconcentration factors than other marine organisms (bivalves, BCF $\sim 10^4$).

4.6. Conclusion

Numerous field observations of Pt concentration, distribution and behavior in various contrasting sites allowed to increase the knowledge on Pt distribution and behavior in coastal environments, including the biological compartment, at different temporal and spatial scales.

Main outcomes from field observations are summarized as follows:

Biota contamination

Successful application of bivalves as a monitoring tool for Pt contamination was performed in various sampling sites. First assumptions of Pt concentrations in phytoplankton reflect a greater transfer of Pt from the dissolved phase to algal cells ($BCF \sim 10^4$, whereas it is of 10^3 for bivalves). Platinum sorption on phytoplankton might cause significant effect on Pt biogeochemical cycles in coastal environments.

Temporal scale

Historical records of Pt levels, through the analysis of bivalve and sediment cores, proved the increasing impact of Pt inputs from anthropogenic activities. Platinum contamination is therefore reflected at the multi-decadal timescale, and recent increases in environmental levels were correlated with increasing Pt use in car catalytic converters. Additional Pt sources may occur or have occurred locally, and should not be neglected. Such sources comprise historical sources such as oil-refining and coal-combustion, and emerging sources such as Pt-based anti-cancer drugs. According to the type of Pt source and to surrounding physical/chemical parameters, Pt partitioning will be influenced. Such processes may occur at very short-term scales (within few hours), and may profoundly affect Pt distribution, speciation and the associated bioavailability.

Spatial scale

Platinum concentrations were determined in environmental samples (seawater, particles, sediments, and living organisms) from two key European coastlines: the Atlantic and the Mediterranean coasts. Generally, the Atlantic samples displayed lower Pt levels than those from the Mediterranean. Particularly, some sites along this coast reflected high anthropogenic pressure and associated Pt contamination: the Toulon Bay and the Genoa Harbor.

Overall, successful application of (bio)monitoring studies in contrasting sites allowed to report Pt contamination at various spatial and temporal timescales.

Chapter 5

General conclusions and perspectives

General conclusions

Growing human population and associated human pressure on coastal systems lead to global Pt contamination of different environmental compartments. The present study aimed at contributing to a general assessment of Pt occurrence and distribution in coastal environments, as well as producing original information on Pt behavior, including partitioning between dissolved and particulate phases and transfer to marine organisms. Given the extremely low (ultra-trace) Pt levels and the complex matrixes in environmental samples (e.g. seawater, organisms), analytical methods, comprising quality control, and experimental setups were developed/optimized. The combined outcomes from laboratory experiments and field observations have provided additional insight into Pt biogeochemical cycles in coastal environments. Major conclusions from this study are the following:

1. In the absence of suitable certified reference material for Pt concentrations in biota, successful inter-method comparison allowed to validate Pt measurements in biological tissues.

An inter-method comparison was performed using two completely independent digestion protocols and measurement techniques: Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Comparing results from both methods shows that Pt concentrations in bivalves and phytoplankton can be reliably measured by ICP-MS applying interference correction, when sufficient sample mass is available. The ICP-MS method is therefore an alternative to AdCSV for detection of Pt in biological samples and was successfully applied in the present study. The ICP-MS method allows for the detection of isotopes implying the possibility of using isotope-specific spikes and detection which has great advantages over classical methods using mono-elementary spikes and total Pt detection in biological accumulation studies.

2. The use of isotopically-labelled Pt in exposure experiments allows for (i) tracing short-term and low-level metal uptake and (ii) distinguishing the “natural” Pt content from the accumulated Pt in the same individual oyster (whole soft body or organ level).

Oysters (*Crassostrea gigas*) from an unpolluted estuary were exposed to the stable isotope ^{194}Pt in seawater at a wide range of concentrations (from environmentally-relevant to very high) during 35 days. Oysters rapidly accumulate dissolved Pt from seawater showing that Pt in seawater is bioavailable to marine organisms. In this exposure study, we performed simultaneous determination of metal concentrations accumulated from isotopically-labelled spikes and the amount of metal naturally present in the same individual organism, i.e. excluding (i) biases due to variability between individuals or (ii) information loss due to the use of organism pools. Such information is not possible to obtain with other analytical methods measuring total element concentrations.

3. Platinum exposure through seawater at a relatively high exposure concentration of 10,000 ng.L⁻¹ for 35 days did not cause mortality but significant adverse physiological effects in oyster *Crassostrea gigas*.

A battery of cell and tissue level biomarkers and their integration into the Integrative Biological Response (IBR) Index for environmental health assessment showed clear toxicological effects in oysters following Pt exposure. When exposed to high Pt levels (10,000 ng.L⁻¹) in seawater, IBR Index suggests the occurrence of a cascade of responses (oxidative stress, intralysosomal metal accumulation, and lipid depletion). Enhanced lysosome alterations together with increasing Pt contents in faeces along with Pt exposure levels suggest effective excretion of accumulated Pt. Dose-related linear accumulation in tissues and whole organisms over time suggests that excretion of metal is relatively slow compared to the uptake.

4. Oysters exposed to environmentally-relevant Pt concentration in seawater rapidly (after 21 days) reached accumulation steady state, supporting their potential to serve as environmental sentinel (biomonitors) of Pt contamination.

Experimentally exposed oysters *Crassostrea gigas* showed i) efficient Pt accumulation without suffering mortality, ii) a high Pt BCF with respect to seawater Pt levels, and iii) a linear correlation between Pt accumulation in the organism and Pt in ambient seawater. Therefore, monitoring Pt concentrations in wild oysters, integrating and amplifying the environmental signal, may provide unique information on spatial and temporal trends in local dissolved Pt levels in seawater. Oysters and other sedentary marine bivalves are promising sentinel species for Pt contamination in seawater.

5. Marine bivalves bioconcentrate Pt and the degree of accumulation reflects environmental Pt levels. Wild and farmed oysters and mussels were successfully tested as sentinel organisms of ambient Pt concentrations in contrasting sites.

By bio-concentrating seawater Pt by a factor of $\sim 10^3$ oysters *Crassostrea gigas* and mussels *Mytilus galloprovincialis* amplify the environmental signal, facilitating the evaluation of temporal and special trends in Pt seawater concentrations in natural environments. Accordingly, we have used these marine bivalves to assess Pt contamination in various coastal systems.

6. Historical records of Pt concentrations in sediment cores and bivalves reflect the evolution of anthropogenic Pt inputs in coastal environments, revealing ancient and emerging Pt sources.

Historical samples of sediments from the Gironde Estuary watershed (1950 - 2001) and the Toulon Bay (Mediterranean Sea; ~ 1900 - 2010) showed the evolution of anthropogenic Pt contamination in the past, mainly attributed to historical mining/metallurgic activities (Gironde Estuary watershed), industry/naval activities covering oil-refining and/or coal-combustion (Toulon Bay). Recently (post-1985) increase in anthropogenic Pt signals reflect increasing inputs from emerging sources, such as car catalytic converters and inputs of Pt-based anticancer drugs. The strong increase and relative dominance of Pt demand for car catalytic converters (70 % of European Pt demand) are consistent with both, signals in surface sediment and oysters/mussels from both sites. These results suggest that Pt contamination may be a common feature in coastal areas of developed countries, potentially posing a threat to living marine resources.

7. Mapping of Pt levels in various environmental samples from contrasting coastal environments allowed (i) establishing regional seawater Pt background levels for the Atlantic and the western Mediterranean coasts and (ii) assessing a relative order of Pt contamination in bivalves from these sites.

In sediment cores, regional background concentrations as deduced from grain-size normalized Pt concentrations in presumably uncontaminated sediment were somewhat different from the Upper Continental Crust (UCC) Pt content of 0.5 ng.g^{-1} . Accordingly, freshwater reservoir sediments from the Gironde fluvial-estuarine continuum appeared to be impoverished regarding UCC Pt content showing regional background concentrations of 0.2 ng.g^{-1} . On the contrary, sediments from the Mediterranean coast (Toulon Bay) dating from the early 1900's showed clearly higher Pt background concentrations ($\sim 6 \text{ ng.g}^{-1}$). Dissolved Pt concentrations of 0.08 ng.L^{-1} (as measured in the Ligurian Current and south from the Balears Islands) may be considered as a reference value for western Mediterranean surface seawater. In the Atlantic coast, the dissolved Pt background concentration was estimated at 0.05 ng.L^{-1} . In samples displaying Pt concentrations clearly greater than the respective background levels, Pt concentrations were attributed to anthropogenic contamination. Accordingly, the Gironde Estuary mouth showed relatively lower Pt concentrations in both seawater and biota (plankton material and bivalves) than the Arcachon Bay. Generally, the Mediterranean coast appears as more contaminated than the Atlantic coast especially in highly urbanized and semi-enclosed areas such as the Toulon Bay and the Genoa Harbor.

8. Platinum partitioning between dissolved and particulate phases reflects various factors including (i) overlapping local sources, (ii) biogeochemical processes modifying the nature, surface properties and abundance of suspended particles and (iii) production/degradation of biomass.

Mapping dissolved and particulate Pt along two profiles in the Genoa Harbor provides first information on Pt distribution and reactivity in an industrialized and urbanized harbor. Gradients in both dissolved and particulate Pt concentrations were combined with major biogeochemical variables, tracing input and degradation of urban sewage inside the relatively stagnant harbor waters. The results strongly suggest that biogeochemical processes induce profound changes in Pt partitioning and carrier phases. Such mobilization of anthropogenic Pt may enhance its bioavailability, bio-uptake and induce potential effects on marine organisms. However, both spatial and temporal variations at relatively small scales were partly attributed to variable Pt inputs from nearby sources (highway, hospital), and subsequent equilibration with

ambient conditions. These observations highlight the importance of adapted sampling and observation strategies when studying biogeochemical processes in dynamic coastal systems.

9. Phytoplankton production and degradation may greatly influence Pt partitioning in marine environments.

Intense primary production resulted in Pt depletion of seawater, together with increasing Pt in phytoplankton, which may imply different processes such as Pt inside the cells uptake and/or adsorption onto algal cell surface. Grazing had the opposite effect, suggesting Pt release into seawater due to biomass degradation. Although these processes are partly masked by fluctuations due to external factors such as tide or local sources, there is clear evidence that biogeochemical processes may induce diel cycles in Pt partitioning and the related Pt distribution in the coastal environment. Studies of the fate and effects of Pt in marine environments must therefore consider biological processes and timescales. This is in line with, Luoma et al. (1998) stating that sampling designs for contaminant monitoring programs are incomplete unless they also consider the effects of phytoplankton.

Overall, the present work provides valuable and original knowledge on Pt biogeochemical cycles in coastal environments under various spatial and temporal aspects. In addition to documenting records of emerging and increasing Pt contamination in contrasting sites from the Atlantic and Mediterranean coastal environment, this work covers diverse aspects of Pt transfer to living organisms, highlighting both (i) their role in coastal biogeochemical Pt cycle and (ii) their potential to serve as biomonitors. Consequently, this study provides a baseline for further investigations in Pt biogeochemistry in marine environments.

Perspectives

Based on these conclusions further investigations should focus on the following aspects:

- From the analytical point of view there is an evident need for Certified Reference Materials (CRMs) representative of environmental Pt concentrations in seawater, biota, and sediments. The few existing CRM for Pt have relatively high Pt levels, and are not representative of pristine sediments. Furthermore no CRMs exist for marine environmental samples. If performing inter-method comparisons is a means of overpassing this major quality control problem for certain sample types (here: biota), limitations in analytical methods available for other matrices (e.g. seawater) or the absence of more than one reliable method available in most laboratories highlight the priority need for adequate CRMs.
- Platinum partitioning in seawater proved to be highly dependent on suspended particle matter origin and composition. Further investigations should focus on the understanding of Pt interaction with biogenic particles, including adsorption on phytoplankton cells and their exudates. Regarding primary producers, we need better characterization of mechanisms involved in Pt sorption by phytoplankton: true uptake or adsorption on cell surface? Such research may consist in performing laboratory experiments of sorption kinetics and pathways using stable Pt isotope spikes and phytoplankton cells.
- In order to better understand the solubility of Pt derived from car catalyst exhausts in seawater at short-term timescale, laboratory experiments should also be conducted. They may consist in desorption experiment using environmentally relevant amount of ground catalyst material added in seawater. Dissolution of Pt should be observed at short-term timescale i.e. hourly timescale in order to reflect rapid changes of Pt geochemistry that may occur in coastal systems under high anthropogenic pressure.
- This work suggests the major importance of direct uptake of dissolved Pt from seawater as contamination pathway for living organisms. In fact, oyster gills showed the greatest Pt accumulation after direct exposure under laboratory conditions and in the field there was no evidence for biomagnification of Pt along the aquatic trophic chain consisting in phytoplankton, zooplankton, and bivalves. However, one cannot exclude Pt accumulation through the trophic pathway. Future work should address this point, e.g. through laboratory experiments exposing algae to stable Pt isotope spikes in seawater and subsequent exposure of higher organisms to those isotopically-labelled algae cells (zooplankton, bivalves).

- In light of recent work reporting a shift toward the dominant use of Pd as the catalyst of choice in more recent years (Wiseman et al., 2016) and given the higher environmental mobility and bioaccessibility of Pd (Jarvis et al., 2001), there is a growing need for environmental assessment of Pd contamination in coastal systems. Similarly, the biogeochemical cycles of other PGEs, which are also considered as TCE and emerging contaminants, are necessary to fill the huge knowledge gaps for marine, and more generally, all aquatic environments.
- Considerable progress in understanding the environmental fate and ecotoxicological impact of the more ‘traditional’ contaminants, including the definition of environmental guidelines, policies and laws, has been achieved over the past decades. However, no clear quality guidelines exist for TCEs, because even the most fundamental information on their cycles, transfer mechanisms and effects are lacking. For terrestrial environments no safety guidelines have been established, since very low levels were recorded in food crops and their intake has not increased since 1994 (Rose et al., 2010). Accordingly, studies conclude that current dietary exposures to Pt are unlikely to be of toxicological concern (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, COT, 2008). However such guidelines are not established for marine resources. The present work clearly suggests recently increasing Pt levels by factors of up to 8 - fold since ~1985 in seafood (mussels, oysters) which indicates the need for more research focusing on potential consequences and seafood safety guidelines.
- Although there is clear evidence of increasing Pt contamination of all environmental compartments, mostly in a small number of developed countries, many third countries introduce PGE-based technologies such as automobile catalysts without establishing infrastructures and policy measures supporting their efficient use (Rauch et al., 2006) or surveillance. Extremely high environmental PGE concentrations as observed in Ghana (Esumang, 2008; Kylander et al., 2003) may become a worldwide feature because anthropogenic PGE contamination from automobile, two and three wheelers can be substantial in countries with a growing automobile industry such as developing Asiatic countries (India and China with more than two billion inhabitants; Sen (2013). Comprehensive assessment of Pt levels in aquatic systems in such countries as well as in PGE producing/processing countries exposed to extreme PGE emissions (Almécija et al., 2017; Rauch and Fatoki, 2013) requires extended research in the future.

Résumé étendu

Résumé étendu

Les facteurs déterminant la distribution des populations humaines sont fondamentaux pour comprendre les relations entre les populations et l'environnement (Small et Cohen, 2004). Environ 60 % de la population mondiale est concentrée dans un rayon de moins de 100 km de la côte (Vitousek et al., 1997). Comme l'indiquent Small et Cohen (2004), cette distribution reflète un développement urbain intense dans les zones côtières et le long des rivières navigables qui offrent des avantages économiques et stratégiques dans le cadre de la globalisation.

Par conséquent, l'environnement côtier est fortement affecté par l'activité humaine (Vitousek et al., 1997). L'anthropisation progressive des zones côtières à travers le monde entraîne des changements dans les cycles biogéochimiques élémentaires en affectant durablement le fonctionnement des écosystèmes (Oursel et al., 2013). Sous la pression anthropique, les milieux côtiers deviennent des systèmes vulnérables, influencés par une grande diversité de pollution. Parmi la variété de composés chimiques libérés dans l'environnement côtier, cette étude portera sur les métaux traces.

Les métaux traces sont des constituants naturels des matériaux de la croûte terrestre qui atteignent les milieux aquatiques par divers processus naturels et liés à l'activité humaine. Parmi la variété des métaux, le platine, de symbole chimique Pt, fait partie des métaux du groupe du platine (MGP). Tandis que la recherche sur les MGP est effectuée depuis plus de deux décennies, leur distribution et leurs gammes de concentration dans les écosystèmes naturels et en particulier dans les environnements marins demeurent sous-documentées et peu précises. Jusqu'à présent, une évaluation claire des risques environnementaux liés à l'utilisation exponentielle des MGP ne peut pas être réalisée compte tenu de la rareté des études de terrain (Ruchter et al., 2015). Ce manque de données environnementales pertinentes pourrait être en partie lié au challenge analytique que représente la détermination des MGP dans les gammes de concentrations trouvées dans la nature. Pourtant, il est maintenant certain que la perturbation anthropique des cycles géochimiques naturels du platine, surtout lié aux émissions des pots catalytiques des véhicules, se produit dans tous les compartiments terrestres (Sen et Peucker-Ehrenbrink, 2012). Des niveaux de concentrations en platine croissants sont enregistrés, même dans des zones très reculées comme la couverture neigeuse en Antarctique (Soyol-Erdene et al., 2011). Les émissions de platine devraient augmenter dans les prochaines années en raison de l'augmentation de la charge en MGP suivant une réglementation de plus en plus stricte des émissions dans les pays développés et de l'introduction de pots catalytiques dans les pays en voie de développement (Sen, 2013).

Les objectifs principaux de la présente thèse sont les suivants:

- Déterminer, dans des conditions de laboratoire contrôlées, **le taux et la cinétique de l'accumulation de Pt chez les bivalves marins et les effets physiologiques** associés à différents niveaux d'organisation biologique.
- **Évaluer la possibilité d'utiliser des bivalves marins comme sentinelles** de la contamination de Pt dans les environnements côtiers.
- **Identifier et documenter la contamination et les sources passées et présentes de Pt** en combinant l'étude **d'enregistrements historiques et de campagnes de (bio)surveillance** de sites côtiers contrastés (relativement propres à fortement pollués) le long des côtes atlantiques et méditerranéennes.
- **Caractériser et comprendre les facteurs contrôlant la dynamique à court terme de Pt et sa répartition entre l'eau de mer et les particules en suspension**, y compris les organismes phytoplanctoniques.

Ces objectifs ont été étudiés principalement selon quatre approches:

- *Validation des procédures analytiques.* Afin d'obtenir des données environnementales pertinentes et précises sur le platine, la première étape consiste à appliquer des méthodes d'échantillonnage, ainsi que des procédures de traitement et d'analyse des échantillons appropriées. Pour cela, un soin particulier a été apporté à la collecte des échantillons et les procédures analytiques ont été validées, si possible en utilisant des matériaux de référence certifiés et / ou en effectuant une inter-comparaison entre des techniques indépendantes.
- *Etablissement d'un jeu d'échantillons original.* Une expérimentation de laboratoire utilisant des huîtres exposées à des isotopes stables de Pt exposés a été réalisée. De plus, au moyen de différents réseaux de surveillance environnementale, un ensemble original d'échantillons a été constitué et analysé. En appliquant des méthodologies pertinentes, les analyses de Pt ont été effectuées sur des enregistrements historiques, y compris des échantillons de programmes de biosurveillance. Plusieurs campagnes de terrain ont été réalisées, permettant des échantillonnages ponctuels et des études à haute résolutions

temporelle et spatiale. Ces échantillons ont permis de définir les contaminations anciennes et actuelles de Pt dans divers environnements côtiers.

- *Etude de l'accumulation du platine et des effets biologiques sur les organismes marins.* L'expérimentation en laboratoire a permis d'observer la biodisponibilité de Pt et de tracer son accumulation dans un organisme marin modèle. De plus, les niveaux de Pt ont été mesurés dans diverses espèces marines clés, en appliquant une sélection appropriée à l'écosystème étudié.
- *Détermination de la teneur, du comportement et de la dynamique du platine dans diverses matrices environnementales.* Les cycles biogéochimiques de Pt dans les environnements côtiers ont été étudiés en mesurant les niveaux de Pt dans diverses matrices environnementales. Cela comprend des échantillons dissous et particuliers, permettant notamment l'observation du comportement et de la dynamique de Pt dans l'eau de mer. Afin de renforcer la pertinence des observations, de telles mesures ont été effectuées dans plusieurs zones côtières clés et à différentes échelles de temps.

Après une description du contexte scientifique (*Chapitre 1*) et du Matériel et des méthodes appliquées (*Chapitre 2*), les résultats du présent travail sont présentés dans deux chapitres. Ils comprennent des articles révisés par des pairs et des manuscrits prêts à être soumis, fournissant de nouvelles connaissances sur les cycles biogéochimiques de Pt. Ils sont présentés comme suit:

Chapitre 3: Accumulation du platine et réponse physiologique de l'huître Crassostrea gigas

Ce chapitre présente les résultats d'une expérience de laboratoire exposant des huîtres (*Crassostrea gigas*) à différentes concentrations de Pt à l'aide d'ajout d'isotope stable dans de l'eau de mer pendant 35 jours. Différents critères ont été étudiés à travers cette étude de laboratoire.

La première partie consiste en l'étude de la cinétique d'accumulation du platine dans l'huître, révélant l'évolution des concentrations de Pt dans les tissus mous d'organismes entiers au cours du temps. Seulement trois jours après le début de l'expérimentation, les huîtres ont accumulé des quantités significatives de Pt, y compris lorsqu'elles sont exposées à la plus basse teneur (50 ng.L^{-1}). Ces résultats confirment la biodisponibilité de Pt en phase dissoute comme il était déjà observé pour les bivalves d'eau douce (Ruchter, 2012; Sures and Zimmermann, 2007).

Lorsqu'ils sont exposés à des concentrations en Pt pertinentes d'un point de vue environnemental, les tissus mous des huîtres montre une augmentation linéaire au cours du temps et ce jusqu'à 21 jours d'exposition. Par la suite, un plateau d'accumulation est atteint (état d'équilibre) suggérant le fait que l'assimilation est compensée par l'excrétion et des mécanismes de régulation efficaces. De plus, une relation linéaire existe entre les concentrations en Pt dissous et les concentrations dans les tissus mous des huîtres. Ce résultat confirme l'utilisation des huîtres comme espèces sentinelles des niveaux de Pt dans l'eau de mer avec des facteurs de bioconcentration expérimentaux relativement élevés (~ 500). A des concentrations d'exposition plus fortes de 10 000 ng.L⁻¹, les concentrations en Pt n'atteignent pas de plateau d'accumulation à la fin de la durée d'exposition. Les résultats de cette étude sont publiés dans la revue scientifique *Science of the Total Environment*, sous le titre « **Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species in coastal marine environments** ».

La seconde partie du chapitre se concentre sur les réponses biologiques de l'huître à la suite de l'exposition au Pt et ceci à différents niveaux d'organisation biologique. Premièrement une étude de l'organotropisme de Pt a été effectuée par dissection des organismes exposés à 10 000 ng.L⁻¹. Cette étude montre que la distribution de Pt dans les différents organes diffère de la distribution originelle après seulement 3 jours d'exposition. La plus forte accumulation est observée dans les branchies (~ 70 %), et dans une moindre mesure dans le manteau (~ 25 %). Les branchies sont donc l'organe cible de la contamination de Pt par voie directe (phase dissoute). Une accumulation linéaire de Pt, sans atteindre de plateau d'accumulation, est observée dans tous les organes étudiés. De plus la teneur en Pt mesurée dans les fèces est dépendante du temps et de la concentration d'exposition suggérant l'intensification des mécanismes de défense (excrétion). Au niveau tissulaire, malgré l'observation de l'altération de l'intégrité des tissus, particulièrement dans l'épithélium digestif, aucune conclusion claire n'a pu être obtenue à ce niveau d'organisation biologique. Cependant, au niveau cellulaire les huîtres exposées à des fortes concentrations en Pt de 10 000 ng.L⁻¹ montrent une augmentation de l'accumulation en lipofuscine suggérant l'initiation de réactions d'oxydation dans la glande digestive par Pt. De plus une diminution de la teneur en lipides neutres est observée, montrant une utilisation accrue des ressources énergétiques pour faire face à l'exposition au Pt. L'index de réponse biologique (Index of Biological Response, IBR) reflète une dégradation de l'état de santé général des organismes. Par conséquent, cette étude donne une base de connaissances sur l'accumulation et les effets biologiques causés par le Pt dans les organismes marins tels que les huîtres. Les résultats de cette étude sont prêts à être soumis à la revue scientifique

Environmental Science and Pollution Research sous le titre « **Organotropism and biomarker response in oyster *Crassostrea gigas* exposed to platinum in seawater** ».

Chapitre 4: Aspects temporel et spatial des cycles biogéochimiques du platine dans les environnements côtiers

Ce chapitre est divisé en quatre parties présentant les résultats d'études sur le terrain réalisées dans des sites d'étude contrastés et comprenant des analyses dans diverses matrices environnementales.

La première partie présente l'évolution des concentrations en Pt dans des sédiments et des huîtres du continuum fluvio-estuarien de la Gironde (Sud-Ouest de la France) comprenant la rivière du Lot. Des enregistrements sédimentaires du Lot datant de 1952 à 2001 et des échantillons d'huîtres sauvages de l'embouchure de l'estuaire de la Gironde récoltés de 1981 à 2013 ont permis de retracer la contamination passée et présente de Pt dans ce système aquatique européen majeur. Dans les sédiments, le bruit de fond géochimique local de Pt a été déterminé à $\sim 0,2 \text{ ng.g}^{-1}$ en amont de la source de pollution en Pt. En aval, les sédiments révèlent la présence d'une ancienne source de pollution de Pt (concentrations de $\sim 1.6 \text{ ng.g}^{-1}$ pour les sédiments les plus profonds) probablement associée aux anciennes activités industrielles (métallurgie) du bassin versant du Lot. De plus, les huîtres de l'estuaire montrent l'évolution des sources de Pt dans le continuum fluvio-estuarien, et suggèrent l'importance d'une source plus récente de Pt. L'augmentation des concentrations mesurées dans les huîtres (de 0.36 ng.g^{-1} en 1980 atteignant 0.60 ng.g^{-1} en 2006) reflète en effet l'augmentation de l'utilisation de Pt pour les pots catalytiques des voitures. Cette étude de terrain prouve que les huîtres sont de bons organismes sentinelles de la contamination de Pt en milieu marin, bioconcentrant le Pt de la phase dissoute par un facteur de $\sim 10^3$. Les résultats de cette étude sont publiés dans la revue scientifique à comité de lecture *Marine Chemistry*, sous le titre « **Past and present platinum contamination of a major European fluvial-estuarine system: Insights from river sediments and estuarine oysters** ».

La deuxième partie du chapitre présente une étude réalisée dans un autre milieu côtier, la zone côtière du nord-ouest de la Méditerranée. Les concentrations en platine ont été déterminées dans (i) des carottes sédimentaires et des moules d'élevage de la baie de Toulon afin d'évaluer la contamination de Pt dans un port fortement anthropisé et (ii) des moules sauvages (*Mytilus galloprovincialis*) échantillonnées le long du littoral Méditerranéen afin de déterminer la biodisponibilité de Pt chez cette espèce et l'état de contamination de divers sites d'échantillonnage. Les couches profondes des sédiments de la baie de Toulon montrent des

concentrations en Pt de référence relativement fortes ($\sim 6 - 10 \text{ ng.g}^{-1}$). Ce résultat reflète potentiellement l'origine marine des particules ou une remobilisation chimique de Pt conduisant à la présence d'une contamination récente dans des couches sédimentaires plus profondes. Les émissions en Pt issus des pots catalytiques sont probablement à l'origine de l'augmentation récente des concentrations en Pt (atteignant $\sim 15 \text{ ng.g}^{-1}$) alors qu'une source plus ancienne possiblement liée à l'industrie du charbon a été identifiée dans une des carottes. L'évolution temporelle des teneurs en Pt des moules d'élevage montre une augmentation générale d'un facteur 8 sur la période 1984 à 2014 (de 0,1 à 0,8 ng.g^{-1}). En particulier, l'intense augmentation dans le milieu des années 1990 reflète l'intensification de l'utilisation des pots catalytiques. L'étude de biosurveillance des moules sauvages de la côte méditerranéenne montre une disparité spatiale des concentrations qui semble être liée au degré d'anthropisation des différents sites d'étude. Particulièrement, les sites de la baie de Toulon et du port de Gênes montrent des niveaux de Pt dans les moules relativement plus élevés. La moule *Mytilus galloprovincialis* est une espèce sentinelle pertinente pour la détermination de la contamination en Pt dans les systèmes côtiers. Les résultats de cette étude de terrain sont prêts à être soumis dans la revue scientifique à comité de lecture *Chemosphere*, sous le titre « **Platinum in sediments and mussels from the northwestern Mediterranean coast: temporal and spatial aspects** ».

La troisième partie présente une étude de cas rapportant la distribution des concentrations en Pt dans les phases dissoutes et particulaires le long d'une cartographie effectuée dans un port très pollué, le port de Gênes (Nord-Ouest de l'Italie). Les résultats de cette étude permettent d'établir une valeur de référence pour les eaux de surface méditerranéenne de $\sim 0,08 \text{ ng.L}^{-1}$. La mesure des concentrations en Pt dans différents sites du port de Gênes révèle la contamination anthropique en Pt de ce site industriel. Les apports en Pt et la dégradation des rejets d'eaux usées dans les eaux stagnantes du port induisent des gradients de concentrations dissoutes et particulaires de Pt. Ainsi, de l'intérieur du port vers la mer, les concentrations en Pt dissous augmentent de 0,10 à 0,18 ng.L^{-1} tandis qu'elles diminuent de 2,5 à 0,5 ng.g^{-1} dans la phase particulaire. Ces gradients impliquent d'importants changements dans la distribution de Pt dans le port et la par nature de sa phase porteuse. Différentes sources potentielles de Pt sont ainsi identifiées: les pots catalytiques mais aussi les rejets d'hôpitaux, fournissant des connaissances sur les gradients de concentration de Pt présents dans ce système et suggérant des variations temporelles de l'intensité de la contamination à court terme (échelle horaire).

Les résultats de cette étude sont prêts à être soumis dans la revue scientifique à comité de lecture *Estuarine, Coastal and Shelf Science*, sous le titre « **Spatial variability and sources of platinum in the Genoa Harbor – from geochemical background to urban levels** ».

La dernière partie du chapitre rend compte des niveaux de Pt dans un compartiment original du biote : la phase planctonique. La détermination des niveaux de Pt dans ces échantillons est d'une importance majeure afin d'acquérir des connaissances sur l'influence des producteurs primaires sur les cycles biogéochimiques de Pt dans les eaux côtières. Pour cette étude à haute résolution temporelle, des échantillons ont été collectés dans trois zones côtières clés : l'estuaire de la Gironde, la baie d'Arcachon et le port de Gênes. Cette étude prouve la sorption (assimilation et / ou adsorption) de Pt par le phytoplancton durant la phase de productivité primaire (la journée) avec des concentrations en Pt variant de 0,2 à 0,3 ng.g⁻¹ en seulement quelques heures. L'importante influence de la marée rend complexe l'observation d'une réciprocité au niveau des concentrations de Pt dans la phase dissoute. Toutefois, la nuit, la dégradation des cellules phytoplanctoniques au travers du broutage par le zooplancton semble conduire à une augmentation des niveaux de Pt dissous. Les organismes plus hauts dans la chaîne trophique (zooplancton et bivalves) ne montrent pas de biomagnification de Pt. Enfin, les concentrations en Pt du phytoplancton reflètent la contamination globale en Pt des environnements côtiers, présentant un facteur de bioconcentration plus élevé que celui des bivalves (d'un facteur 10 environ). Les résultats de cette étude sont prêts à être soumis dans la revue scientifique à comité de lecture *Marine Pollution Bulletin*, sous le titre « **Short-term variations in platinum partitioning in contrasting European coastal environments: the role of primary production and local point sources** ».

En combinant les résultats des études de terrain et des résultats obtenus en laboratoire, la présente thèse fournit donc une meilleure caractérisation et compréhension de la biogéochimie de Pt dans les systèmes côtiers incluant une évaluation de sa distribution entre l'eau de mer, les particules et les organismes vivants. Les conclusions générales de ce travail sont résumées comme suit:

1. En l'absence de matériel de référence certifié approprié pour les concentrations de Pt dans le biote, une comparaison de deux méthodes indépendante a permis de valider les mesures de Pt dans ces matrices.
2. L'utilisation d'isotopes stables permet de suivre l'accumulation de Pt et fournit des informations sur la partie "naturelle" de la teneur en Pt et sur la contribution isotopique de Pt accumulée à partir des ajouts d'isotope, et ce dans un même organisme.
3. L'exposition au Pt dans l'eau de mer à une concentration relativement élevée de 10 000 ng.L⁻¹ pendant 35 jours dans l'huître *Crassostrea gigas* n'a pas causé de mortalité mais des effets nocifs importants au niveau cellulaire incluant une augmentation des réactions d'oxydation et une diminution des réserves lipidiques.
4. Les huîtres ont atteint un état d'équilibre d'accumulation après une exposition au Pt dans l'eau de mer à une concentration pertinente d'un point de vue environnemental de 50 ng.L⁻¹ pendant 35 jours. Une relation linéaire positive existe entre la concentration en Pt dans l'eau de mer et celle des tissus mous, ce qui légitime expérimentalement l'utilisation des huîtres comme espèce sentinelle de la contamination en Pt.
5. Les bivalves marins bioconcentrent Pt et reflètent la contamination environnementale. De telles observations permettent une application réussie sur le terrain des bivalves sauvages et d'élevage comme organismes sentinelles des concentrations naturelles de Pt en milieu côtier.
6. L'étude des enregistrements historiques des concentrations de Pt dans les organismes vivants et dans les carottes sédimentaires reflète l'évolution des apports anthropiques de Pt dans les environnements côtiers révélant des sources anciennes et actuelles de Pt.
7. La détermination des niveaux de Pt dans divers échantillons environnementaux des côtes atlantiques et méditerranéennes a permis d'effectuer une cartographie des niveaux de base régionaux de Pt et de la contamination générale d'environnements côtiers contrastés.
8. La distribution du platine entre la phase dissoute et la phase particulaire pourrait être conditionnée par divers facteurs, comprenant des sources locales concomitantes, des processus biogéochimiques modifiant la nature, les propriétés de surface et l'abondance des particules en suspension, la sorption sur des particules biogènes.

9. Les organismes phytoplanctoniques peuvent grandement influencer la distribution de Pt dans les milieux marins.

Cette étude fournit une base de référence pour d'autres recherches concernant la biogéochimie de Pt dans les environnements marins. Ces futurs travaux devraient considérer les aspects suivants:

- Sur le plan analytique, il manque des matériaux certifiés de référence à des concentrations pertinentes d'un point de vue environnemental et pour tous les types de matrices. Un travail de constitution de ces matériaux de référence devrait être effectué par des laboratoires scientifiques.
- La distribution du platine dans l'eau de mer s'est révélée fortement dépendante de l'origine et de la composition des particules en suspension. D'autres études devraient être menées sur la compréhension de la complexation de Pt avec la matière organique, y compris l'adsorption sur les cellules phytoplanctoniques et leurs exsudats. En ce qui concerne les producteurs primaires, nous devrions mieux caractériser si la sorption de Pt par le phytoplancton se produit par absorption ou adsorption sur la surface cellulaire.
- Afin de mieux comprendre la solubilité à court terme de Pt provenant des pots catalytiques dans l'eau de mer, des simulations expérimentales devraient également être réalisées en laboratoire.
- Notre étude suggère l'importance majeure de Pt dissous comme voie de contamination pour les organismes vivants. L'échantillonnage dans d'autres sites d'étude, y compris différents organismes clés de la chaîne alimentaire aquatique, aidera à confirmer nos hypothèses. De plus, une expérience en laboratoire pourrait être menée en utilisant des algues exposées à un isotope stable de Pt dopé dans l'eau de mer et des organismes supérieurs exposés à ces algues (zooplancton, bivalves).
- De plus, les futures recherches devraient mieux caractériser les cycles biogéochimiques des autres MGP qui sont aussi émis par les véhicules, et ce dans divers régions du globe incluant les pays en voie de développement. Ceci devrait aider à établir des normes et réglementations appropriées à ces contaminants émergents dans l'environnement.

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Scientific production

Scientific production

Peer Reviewed Publications

- 1. Abdou M.**, Dutruch L., Schäfer J., Zaldibar B., Medrano R., Izagirre U., Gil-Díaz T., Bossy C., Catrouillet C., Hu R., Coynel A., Lerat A., Cobelo-García A., Blanc G., Soto M. (2018). Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species for Pt concentrations in coastal marine environments. *Science of the Total Environment*, 615, 652–663.
- 2. Abdou M.**, Schäfer J., Cobelo-García A., Neira P., Petit J.C.J., Auger D., Chiffolleau J.-F., Blanc G. (2016). Past and present platinum contamination of a major European fluvial-estuarine system: Insights from river sediments and estuarine oysters. *Marine Chemistry*, 185, 104–110.
- 3.** Gil-Díaz T., Schäfer J., Pougnet F., **Abdou M.**, Dutruch L., Eyrolle-Boyer F., Coynel A., Blanc G. (2016). Distribution and geochemical behaviour of antimony in the Gironde Estuary: A first qualitative approach to regional nuclear accident scenarios. *Marine Chemistry*, 185, 65–73.
- 4.** Cuartero M., Crespo G., Cherubini T., Pankratova N., Confalonieri F., Massa F., Tercier-Waeber M.-L., **Abdou M.**, Schäfer J., Bakker E (2018). In Situ Detection of Macronutrients and Chloride in Seawater by Submersible Electrochemical Sensors. *Analytical Chemistry*, 90, 4702–4710.

Abstract

A new submersible probe for the in situ detection of nitrate, nitrite and chloride in seawater is presented. Inline coupling of a desalination unit, an acidification unit and a sensing flow cell containing all-solid-state membrane electrodes allows for the potentiometric detection of nitrate and nitrite after removal of the key interfering ions in seawater, chloride and hydroxide. Thus, the electrodes exhibited attractive analytical performances for the potentiometric detection of nitrate and nitrite in desalinated and acidified seawater: fast response time ($t_{95} < 12$ s), excellent stability (long-term drifts of < 0.5 mV.h⁻¹), good reproducibility (calibration parameter deviation of < 3 %) and satisfactory accuracy (uncertainties < 8 %Diff compared to reference technique). The desalination cell, which can be repetitively used for about 30 times, may additionally be used as an exhaustive, and therefore calibration-free, electrochemical sensor for chloride and indirect salinity detection. The detection of these two parameters together with nitrate and nitrite may be useful for the correlation of relative changes in macronutrients levels with salinity cycles, which is of special interest in recessed coastal water bodies. The system is

capable of autonomous operation during deployment, with routines for repetitive measurements (every two hours), data storage and management, and computer visualization of the data in real time. In situ temporal profiles observed in the Arcachon Bay (France) showed valuable environmental information concerning tide-dependent cycles of nitrate and chloride levels in the lagoon, which are here observed for the first time using direct in situ measurements. The submersible probe based on membrane electrodes presented herein may facilitate the study of biogeochemical processes occurring in marine ecosystems by the direct monitoring of nitrate and nitrite levels, which are key chemical targets in coastal waters.

Manuscript ready for submission

1. Abdou M., Zaldibar B., Medrano R., Schäfer J., Izagirre U., Dutruch L., Coynel A., Blanc G., Soto M. Organotropism and biomarker response in oyster *Crassostrea gigas* exposed to platinum in seawater. Ready for submission to *Environmental Science and Pollution Research*.

2. Abdou M., Schäfer J., Hu R., Gil-Díaz T., Garnier C., Brach-Papa C., Chiffolleau J.-F., Charmasson S., Dutruch L. Platinum in sediments and mussels from the northwestern Mediterranean coast: temporal and spatial aspects. Ready for submission to *Chemosphere*.

3. Abdou M., Schäfer J., Gil-Díaz T., Tercier-Waeber M.-L., Catrouillet C., Massa F., Castellano M., Magi E., Povero P. Spatial variability and sources of platinum in the Genoa Harbor – from geochemical background to urban levels. Ready for submission to *Estuarine, Coastal and Shelf Science*.

4. Abdou M., Gil-Díaz T., Schäfer J., Catrouillet C., Bossy C., Dutruch L., Blanc G., Cobelo-García A., Massa F., Castellano M., Magi E., Povero P., Tercier-Waeber M.-L. Short-term variations in platinum partitioning in contrasting European coastal environments: the role of primary production and local point sources. Ready for submission to *Marine Pollution Bulletin*.

Conference and other reports

1. Akter, M., Auguste, M., Bir, J., Blanco, E., Briaudeau, T., Daputo, G., de Cerio, D., Coccoli, C., Creemers, M., Espino, M., Gain, D., Gil-Uriarte, E., Grimaldi, C., Khalil, S.M.I., Lopez, A., Rementeria, A., Roch, M., Rodriguez, A., Roman, O., Ruoyu, H., **Abdou, M.**, Crespi, M.C., Botia, M.C., Figuera, M., Gil-Diaz, T., Kowal, J.L., Luxenburger, F., Mirasole, C., Pankratova, N., Penezic, A., Zieger, S., Tercier-Waeber, M.-L., Nardin, C. (2017). SCHeMA EU Project Summer School Report (Bilbao June 16 – 17, 2016). *Chimia*, 71, 607–610.

Abstract

This conference report describes the training activities that took place in the frame of the Integrated in Situ Chemical MAPPING probe (SCHeMA) summer school organized from the 14th to the 16th of June 2016 in Bilbao (Spain).

2. Tercier Waeber M.-L., Bakker E., Nardin C., Mongin S., Prado E., Cuartero Botia M., Mazaikoff B., Luxenburger F., Klimant I., Mistlberger G., Mueller B., Van Der Val P., Figuera M., Fernandez Sanchez J., Medina Castillo A. L., Valero Navarro A., Schäfer J., **Abdou M.**, Novellino A., D'Angelo P., Confalonieri F., Castellano M., Magi E., Massa F., Povero P. (2015). FP7-OCEAN-2013 - SCHeMA: integrated in Situ Chemical MAPPING Probes. 978-1-4799-8736-8/15/\$31.00 ©2015 **European Union**.

Abstract

Coastal areas are vulnerable ecosystems comprising unique biological niches that require protection. Identification of relevant types of hazards at the appropriate temporal and spatial scale is crucial to detect their sources and origin, to understand the processes governing their magnitude and distribution, and to ultimately evaluate and manage their risks and consequences preventing economic losses. SCHeMA aims to provide an open and modular sensing solution for autonomous in situ high resolution mapping of a range of anthropogenic and natural chemical compounds coupled to master bio-physicochemical parameters. User-friendly data discovery, access and download as well as interoperability with other projects will be achieved via dedicated interface compatible with INSPIRE and GEO/GEOSS standards and principles.

Oral Communications

- 1. Abdou M.**, Schäfer J., Dutruch L., Hu R., Zaldibar B., Medrano R., Izagirre U., Gil-Díaz T., Coynel A., Blanc G., Soto M. (2018). Platinum accumulation in marine organisms – outcomes from an exposure experiment and field observations; COST Workshop on Technology Critical Elements 19th – 20th April 2018, Tallinn, Estonia.
- 2. Abdou M.**, Schäfer J., Dutruch L., Bossy C., Zaldibar B., Medrano R., Izagirre U., Catrouillet C., Hu R., Coynel A., Gil-Díaz T., Lerat A., Blanc G., Soto M. (2017). Platinum bioaccumulation kinetics in marine bivalve (oyster *Crassostrea gigas*) – a potential sentinel species for TCEs; Goldschmidt Conference 13-18th August 2017, Paris (France).
- 3. Abdou M.**, Schäfer J., Cobelo-García A., Neira P., Petit J.C.J., Auger D., Chiffolleau J.-F., Blanc G. (2017). Recent platinum contamination of a major European estuarine system: a bivalve biomonitoring study; COST Workshop on Technology Critical Elements 19th – 20th January 2017, Rehovot (Israël).
- 4. Abdou M.**, Schäfer J., Tercier-Waeber M.-L., Gil-Díaz T., Coll-Crespi M., Bossy C., Derriennic H., Blanc, G. (2016). Diel cycles of arsenic (As) speciation and partitioning: High resolution monitoring in a coastal system; Oral presentation, 32nd International SEGHI Conference 4-8th July 2016, Brussels (Belgium).

- 1.** Gil-Díaz T., Schäfer J., Dutruch L., Keller V., Eiche E., Mößner C., Lenz M., **Abdou M.**, Gardes T., Rementeria A., Zaldibar B., Eyrolle-Boyer F. Tellurium and selenium reactivity in estuarine gradients and bioaccumulation in wild oysters; COST Workshop on Technology Critical Elements 19th – 20th April 2018, Tallinn, Estonia.

- 2.** **Abdou M.**, Catrouillet C., Schäfer J., Gil-Díaz T., Tercier-Waeber, M.-L., Cobelo-García, A., Derriennic, H., Bossy, C., Coynel, A., Blanc, G. (2017). Diel variations of dissolved platinum in seawater: preliminary results of a high resolution monitoring study; Poster presentation, 14th IEBS (International Estuarine Biogeochemistry Symposium) 4-7th June 2017, Rimouski, Canada.

- 3.** **Abdou M.**, Schäfer J., Cobelo-García A., Neira P., Petit J.C.J., Auger D., Blanc G., Chiffoleau J.-F. (2016). Past and present platinum contamination of a major European fluvial-estuarine system: insights from river sediments and estuarine oysters; Poster presentation, SETAC Europe 26th Annual Meeting, 22-26th May 2016, Nantes France.

- 4.** Gil-Díaz T., Schäfer J., Pougnet F., **Abdou M.**, Dutruch L., Eyrolle-Boye F., Coynel A. Antimony fate modelling approach for regional nuclear risk assessment; Poster presentation, SETAC Europe 26th Annual Meeting, 22-26th May 2016, Nantes (France).

- 5.** **Abdou M.**, Schäfer J., Cobelo-García A., Neira P., Petit J.C.J., Auger D., Blanc G., Chiffoleau J.-F. (2015). Long-term records of platinum contamination and sources in sediments and oysters from a fluvial-estuarine system (Gironde Estuary, SW France); Poster presentation, 13th IEBS (International Estuarine Biogeochemistry Symposium) 7-10th June 2015, Bordeaux, France.

- 6.** Gil Díaz T., Makni L., Schäfer J., Pougnet F., **Abdou M.**, Mikolaczyk M., Coynel A., Dutruch L., Bossy C., Husson A., Derriennic H., Blanc G. (2015). Spatiotemporal distribution of dissolved and particulate inorganic antimony along the Gironde fluvial-estuarine continuum: a preliminary approach to regional nuclear accident scenarios; Poster presentation, 13th IEBS (International Estuarine Biogeochemistry Symposium 7-10th June 2015, Bordeaux, France.

SCHeMA Project Deliverables

In the scope of the SCHeMA Project, I have been actively involved in the organization of field campaigns, validation of sensor measurements and writing of scientific deliverables to the European Commission:

D8.1. Preparation and organization of field campaigns – *April 2015 – Confidential Report*

Schäfer J., **Abdou M.**, Povero P., Magi E., Tercier-Waeber M.-L.

D8.2. Adaptation/improvement of laboratory based techniques– *October 2015 – Public Report*

Schäfer J., **Abdou M.**, Magi E., Castellano M., Povero P.

Available on the SCHeMA website: <http://www.schema-ocean.eu/Dissemination/Public-deliverables>

D8.3. Field application of the SCHeMA system in European coastal key areas– *October 2016 – Confidential Report*

Schäfer J., **Abdou M.**, Magi E., Castellano M., Povero P., Massa F., Mirasole C., Tercier-Waeber M.-L., Cuartero M., Zieger S., Luxenburger F., Confalonieri F.

D8.4. SCHeMA chemical probes and complementary data bank and visualization– *September 2017 – Confidential Report*

Schäfer J., **Abdou M.**, Catrouillet C., Magi E., Castellano M., Povero P., Massa F., Mirasole C., Tercier-Waeber M.-L., Cuartero M., Zieger S., Dettenrieder C., Confalonieri F.