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

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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 ^{194}Pt 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 more than 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 5.10^3 (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 $ng.L^{-1}$ 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 are described for Pt determination in previous publications: Adsorptive Cathodic Stripping Voltammetry (AdCSV) and Isotope Dilution Inductively Coupled Mass Spectrometer (ID-ICP-MS) with respective detection limits (expressed as three times the standard deviation of blank measurements) for seawater of $3.9\ 10^{-3}\ ng.L^{-1}$

and 2.9 10^{-3} ng.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-Żyłkiewicz, 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…). 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 $PtCl_4^{2-}$ and $PtCl_5(OH)^{2-}$, 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 species include fish such as the eel *Anguilla anguilla* (Zimmermann et al., 2004), or the 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 biomonitoring 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) 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 ± 1 mg.L^{-1} (n = 5), corresponding to a nominal total Pt concentration of 650 mg.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 (~ 90 ± 5 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 µm (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 twelve 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 twelve 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 ng.L^{-1} Pt (B condition); 100 ng.L^{-1} Pt (C condition); 10 000 ng.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 ng.L^{-1} Pt and 100 ng.L^{-1} Pt respectively. These concentrations represent about 500 and 1 000 times the Pt concentrations encountered in clean coastal environments (mean Pt estuarine concentrations ~ 0.1 ng.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 ~ 7 ng.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 ng.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 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. 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 ± 0.07 L seawater 27± 4 ng total Pt in tanks B, 53 ± 7 ng total Pt in tanks C, 5311 ± 737 ng total Pt in tanks D, and $7*10^7$ 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 20h 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 250mL Teflon bottles. Water samples were filtered immediately through 0.2 µm polycarbonate filters (Nucleopore®) with a filter-syringe (Sartorius®). Filtrates were collected in acid-cleaned 60mL Teflon tubes previously rinsed with an aliquot of the filtrate, acidified to 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% HNO_3; 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:

(1) CI = Visceral Content (wet weight; g) / Shell (wet weight; g) * 100, (Strady et al., 2011a).

Soft tissues were deep-frozen (- 80 °C), freeze-dried, weighed (dry-weight), and crushed in an agate mortar to obtain a homogenous powder.

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 HNO_3 (30% HCl and 65% HNO_3 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 3h. 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 1 000 $\mu g.mL^{-1}$ 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 $^{178}Hf^{16}O$, $^{179}Hf^{16}O$, $^{180}Hf^{16}O$ or $^{181}Hf^{16}O$ 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),

239 which was applicable to the samples of the exposure experiment ($^{178}Hf/^{194}Pt$ and $^{179}Hf/^{195}Pt < 50$).
240 Correction of Hf oxide (HfO^+) interference was performed using the ^{193}Ir signal that is highly interfered
241 by HfO^+ (Djingova et al., 2003) and given that Ir concentration in our samples was assumed negligible.

242 The following equations were applied:

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

244 where S_{corr} is the corrected signal of the analyte (^{194}Pt or ^{195}Pt), S_{meas} the measured signal of the analyte
245 (^{194}Pt or ^{195}Pt), S_{inter} the signal of the interference (estimated from mass 193 signals representing HfO^+
246 interference), A is the % of formation of HfO^+ on the masses 194 and 195, and B the % of formation of
247 HfO^+ on mass 193 (modified from Djingova et al., 2003).

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

250 (3a) $^{194}Pt_{corr} = L*A^{194}Pt_L + N*A^{194}Pt_N$

251 (3b) $^{195}Pt_{corr} = L*A^{195}Pt_L + N*A^{195}Pt_N$

252 Where $^{194}Pt_{corr}$ and $^{195}Pt_{corr}$ are the interference-corrected count numbers of ^{194}Pt and ^{195}Pt corresponding
253 to the sum of natural and isotopically-labelled Pt signals, E corresponds to the number of counts for
254 isotopically-labelled Pt, N corresponds to the number of counts for natural Pt, $A^{194}Pt_L$ corresponds to
255 the abundance of isotope ^{194}Pt in the isotopically-labelled solution, $A^{195}Pt_L$ corresponds to the abundance
256 of isotope ^{195}Pt in the isotopically-labelled solution, $A^{194}Pt_N$ and $A^{195}Pt_N$ correspond to the respective
257 natural abundances of the isotopes ^{194}Pt and ^{195}Pt.

258 Then L and N were determined as follows:

259 (4a) $L = (^{194}Pt_{corr} * A^{195}Pt_N - {}^{195}Pt_{corr} * A^{194}Pt_N) / (A^{194}Pt_L * A^{195}Pt_N - A^{195}Pt_L * A^{194}Pt_N)$

260 (4b) $N = (^{194}Pt_{corr} * A^{195}Pt_L - {}^{195}Pt_{corr} * A^{194}Pt_L) / (A^{195}Pt_L * A^{194}Pt_N - A^{194}Pt_L * A^{195}Pt_N)$

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

264 Finally, the total Pt concentrations in oyster samples were obtained from the sum CL + CN. This enables
265 an estimate of the total Pt uptake over exposure time at the individual scale, as described elsewhere
266 (Mikolaczyk et al., 2016). However, these calculations were only possible for the B and C exposure
267 conditions i.e. 50 $ng.L^{-1}$ and 100 $ng.L^{-1}$ spiked concentrations. In the D tanks Pt was spiked at 10 000
268 $ng.L^{-1}$ and the oysters accumulated high amounts of Pt very rapidly making these calculations impossible

because $^{194}Pt/^{195}Pt$ ratios in the oysters were similar to the $^{194}Pt/^{195}Pt$ 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 $^{194}Pt/^{195}Pt$ ratios in the oysters (i.e. values between natural and spike $^{194}Pt/^{195}Pt$ 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 ^{195}Pt signal ($^{195}Pt_{corr}$) 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 150µm, 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 of concentrated HNO_3 (30% HCl and 65% HNO_3 Suprapur®, Merck) was added to cooled crucibles. After 1h, 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 H_2SO_4 (93-98% Trace metal™ grade, Fisher Chemical), and evaporated again until no more fumes were observed (i.e. only H_2SO_4 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 1 000 $\mu g.mL^{-1}$

PLASMACAL, 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 H_2O_2 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 4 000-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 equation (5):

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

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

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

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

With $[Pt]_{oyster}$ the total Pt concentration in the oyster soft tissue ($ng.g^{-1}$) and $[Pt]_{seawater}$ 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

335 from GSJ). Analyses of these CRMs by ICP-MS gave satisfactory recovery values of 87% and 101%
336 respectively (n = 3). Platinum concentrations in these CRMs were also analyzed by AdCSV and gave
337 satisfactory recovery of 89% for BCR®-723 and 98% for Jsd-2 (n = 3). Furthermore inter-method
338 comparison of ICP-MS and AdCSV methods were realized on the same biological samples in order to
339 assess accuracy and precision of Pt determination in this matrix. Limit of detection of Pt in biological
340 matrix for AdCSV and ICP-MS methods (calculated as 3 * blank standard deviation) were of
341 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.

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

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

352

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 O_2 concentration were measured daily, 4h 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 O_2 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 equation (1). Despite some minor fluctuations over time, CI showed overall similar values (CI = 20 ± 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.

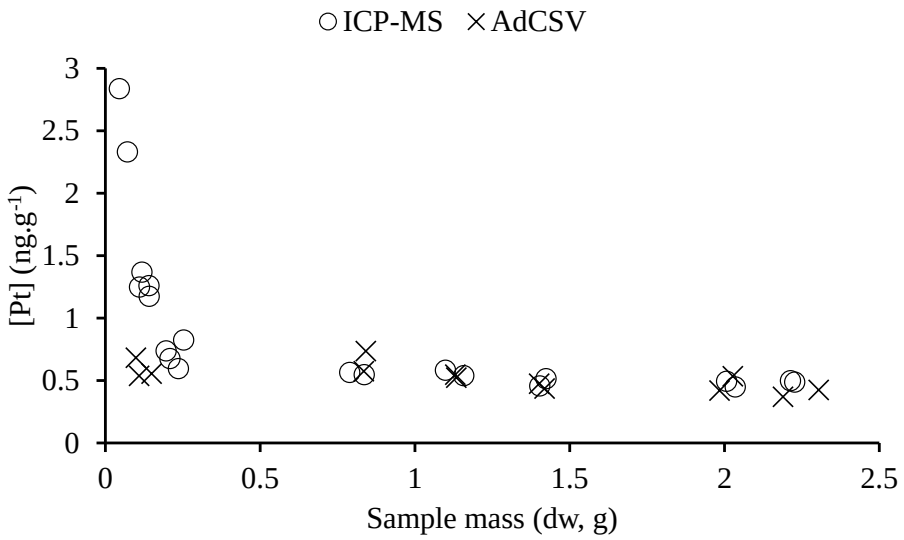


Figure 1: Comparison of Pt concentrations (ng.g^{-1}, dry weight) measured in an oyster pool sample by ICP-MS (round symbols) and AdCSV (cross symbols) as a function of sample mass (dw: dry weight).

Aliquots of the same dry, homogenized oyster soft tissue pool have been digested, covering a wide range of sample masses, i.e. 0.05 g to 2 g. Voltammetrical analyses gave similar Pt concentrations of 0.52 ± 0.10 ng.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 greater than 0.25 g with average Pt concentrations of 0.51 ± 0.04 ng.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 twice (20/21 and 30/31 days) 1h and 20 h after spiking the water in order to estimate Pt concentrations before and after the daily exposure period. Three other samplings were performed 20h after the spike (33, 34, and 35 days after exposure) in order to monitor the exposure level (Table 1).

*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 * 2) 1 h after the spike, and at five sampling times (n = 2 * 5; n = 2 * 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 ($ng.L^{-1}$)	0.25	52	102	9910
	SD ($ng.L^{-1}$)	0.06	2.6	3.0	270
	n	4	4	4	4
20 h after the spike	Mean Pt concentrations ($ng.L^{-1}$)	0.22	36	70	7170
	SD ($ng.L^{-1}$)	0.07	3.4	5.1	610
	n	4	10	10	10

Platinum concentrations determined in tanks A control are relatively low with mean concentrations of 0.25 ± 0.06 $ng.L^{-1}$ 1h after the spike performed in the exposure tanks and remained low 20h after the spike performed in the exposure tanks (0.22 ± 0.07 $ng.L^{-1}$). 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 ± 270 $ng.L^{-1}$, 1h after the spikes were performed (n = 4 for each exposure condition; Table 1). A decrease of ~ 30% in dissolved Pt concentration occurred 20h 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 $^{194}Pt/^{195}Pt$ in oyster tissues

The use of stable isotope spiking allows for tracing metal uptake and to determine Pt concentrations initially present in oyster tissues as previously described. Oysters from tanks B and C showed progressive changes in $^{194}Pt/^{195}Pt$ ratio during the experiment, starting from T = 3. Measurements of the ^{194}Pt and ^{195}Pt isotopes in those organisms provide information on the "natural" Pt content part and the contribution of isotopically-labelled Pt accumulated from the spikes in the same individual oyster.

Table 2: Comparison of natural Pt concentrations (ng.g^{-1}, dry weight) obtained from ^{195}Pt measurements in oysters from tanks B and C with Pt concentrations in T0 individuals and control oysters (tanks A replicates).

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

The "natural" part of Pt concentrations (determined from ^{195}Pt) 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}Pt/^{195}Pt ratio is 0.973. The isotope ratio in the isotopically-labelled solution was ^{194}Pt/^{195}Pt = 5.51. Isotopic ratios in oyster tissues 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}Pt/^{195}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}Pt/^{195}Pt ratio = 4.9 at T = 3 and ^{194}Pt/^{195}Pt 5.5 at T = 35. In contrast, oysters from tanks D displayed ^{194}Pt/^{195}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.

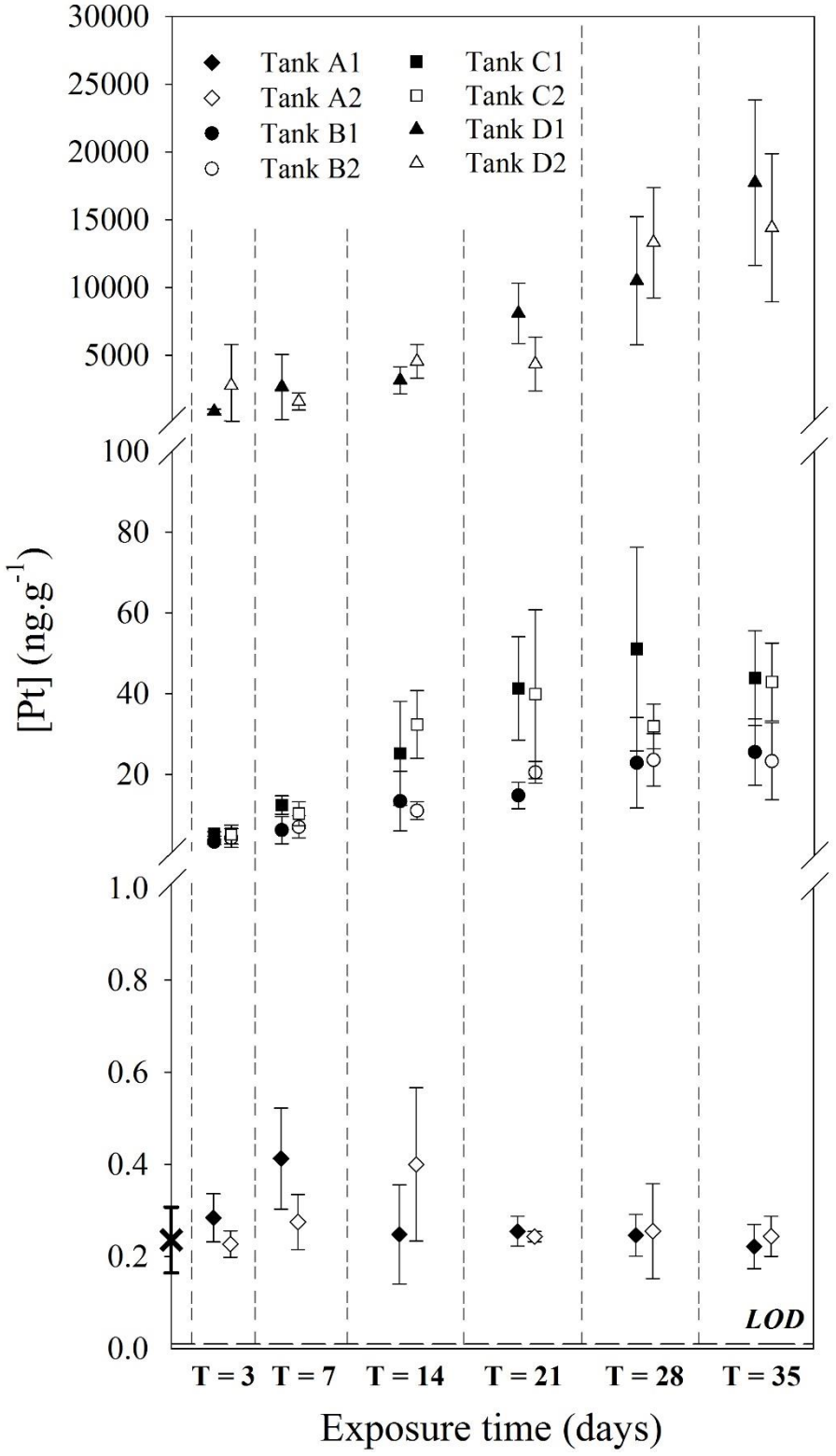


Figure 2: Accumulation kinetics of Pt in total oyster soft tissues. Oyster mean Pt concentrations (n = 5, dry weight) for each replicate (replicate 1: full symbols, replicate 2: empty symbols) of tanks B (round symbols), C (square symbols), and D (triangular symbols) as well as in control tanks (A, diamond 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 pg.g^{-1}). Note the discontinued scale of the concentration axis. Error bars represent standard deviation (SD).

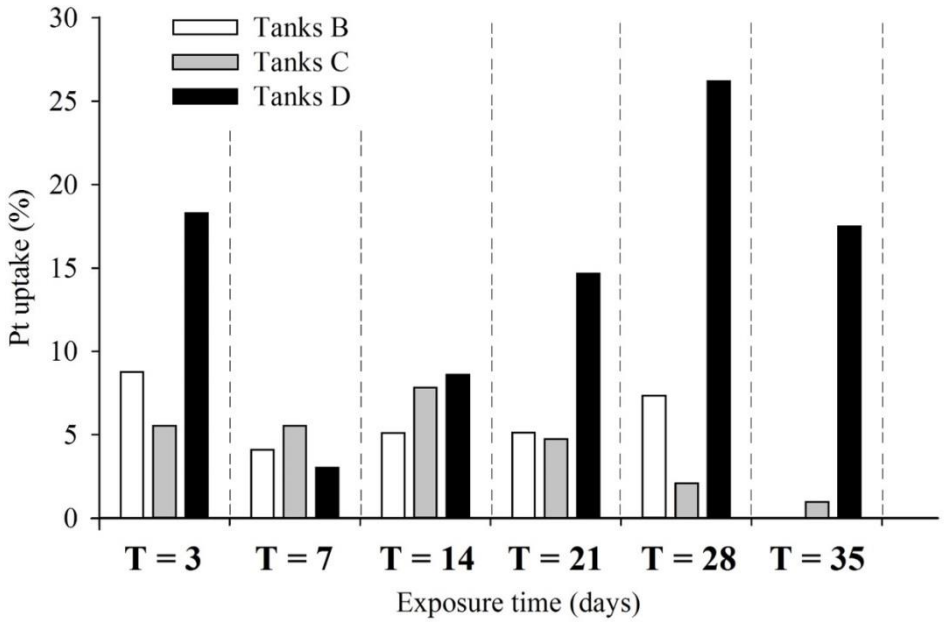
Oysters from the control tanks (A tanks) showed average values between 0.2 and 0.4 $ng.g^{-1}$, i.e. remained similar to initial values throughout the experiment (about 0.25 $ng.g^{-1}$; Fig. 2). Slightly different values were independent from the replicate series and most likely due to inter-individual 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 in soft tissues by ICP-MS 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 $ng.L^{-1}$ Pt; B tanks), Pt concentrations in total oyster soft tissues increased with time and were ten times higher than those in control individuals after only three days of exposure (~ 4 $ng.g^{-1}$ Pt, significant difference). Concentrations increased continuously thereafter to values of ~ 25 $ng.g^{-1}$ 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 $ng.L^{-1}$ (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 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 $ng.g^{-1}$, 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 $ng.L^{-1}$ 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 ~ 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 $ng.g^{-1}$ 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.

476 *3.7. Platinum uptake kinetics*

477 The overall Pt uptake kinetics was determined with the equation (5) in oysters from each exposure
478 conditions.



479

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

483 The relative Pt uptake by oysters in the tanks B and C compared to the total amount of Pt spiked was
484 ~ 5 - 10% until T = 28 days when the plateau was reached. At T = 35, Pt uptake for oysters from tanks
485 B is near 0% and reaches only 1% for oysters from tanks C. In oysters from tanks D, % Pt uptake was
486 more variable and clearly higher in most cases (Fig. 3).

487

488

489

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 $ng.g^{-1}$ ± 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 (less than 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 $ng.g^{-1}$ 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 Resolution)-ICP-MS and quadrupole ICP-MS. However, those samples presented much higher Pt concentrations (~ 10 $ng.g^{-1}$). 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 present 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). 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.

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 ng.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 ng.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 – 0.25 ng.L^{-1}).

556 *4.3. Platinum accumulation kinetics*

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

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

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

586 In the tanks B and C, Pt concentrations in oysters steadily increased with time, following a linear pattern
587 until T = 21. However, oysters from the same tanks sampled at the following exposure times (i.e. T =
588 28 and T = 35) showed similar Pt concentrations, suggesting that Pt concentrations in oysters might have
589 reached a plateau of accumulation, at which metal uptake is compensated by excretion (Singer et al.,
590 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 suggest 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 $ng.L^{-1}$ 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; Zimmerman et al., 2002; Zimmermann et al., 2004…). Ruchter (2012) exposed the freshwater clam *Corbicula sp.* to the same environmentally relevant levels Pt concentrations i.e. 50 and 100 $ng.L^{-1}$ 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 $\mu g.L^{-1}$ 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 equation (5) for each exposure condition through time (Fig. 3). Since seawater in the thanks 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 $ng.L^{-1}$) 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 at T = 21 and T = 28, 1h 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 $\mu g.L^{-1}$ to ~ 70 $\mu g.L^{-1}$ 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 twelve 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 uptake (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 $ng.L^{-1}$), Pt accumulation is controlled by oyster elimination mechanisms, whereas at exposure up to 10 000 $ng.L^{-1}$ 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 report on environmental Pt concentrations in estuarine areas and the open ocean suggesting 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 $ng.L^{-1}$ 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 $ng.L^{-1}$ 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 $ng.L^{-1}$ 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).

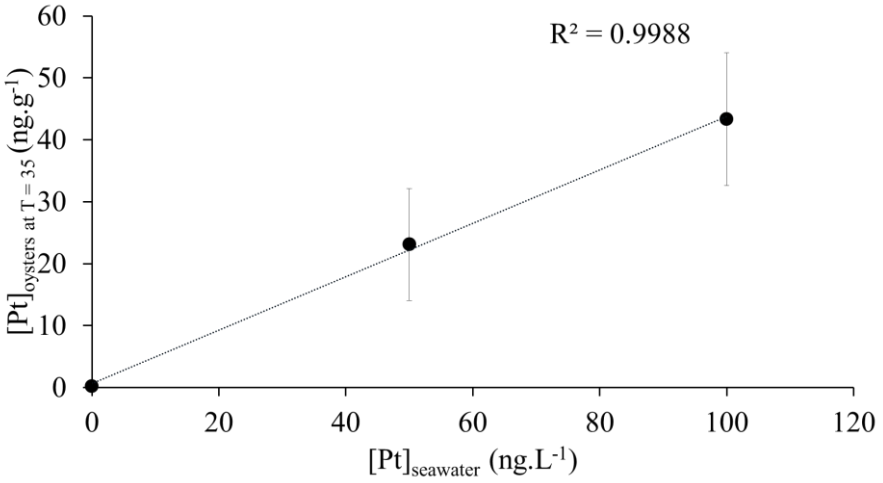


Figure 4: Correlation between Pt concentration in seawater (ng.L^{-1}) and mean Pt concentration (n = 10) in oyster tissues from tanks A (control), and from exposure tanks B and C after 35 days of experiment (ng.g^{-1}, dry weight). Error bars represent standard deviation.

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 equation (6). Following this calculation, both oyster groups exposed to environmentally relevant Pt concentrations of 50 and 100 ng.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 ($[Pt]_{seawater}$ = 10 000 ng.L^{-1}; $[Pt]_{oyster}$ = 16 060 ng.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 ~ $3*10^3$; Abdou et al., 2016) and in control oysters of the present experiment (BCF ~ $1.3*10^3$). 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 macroalga *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).

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. more than 0.25 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 $ng.L^{-1}$) revealed that at environmentally relevant levels (50 and 100 $ng.L^{-1}$) Pt accumulation may be controlled by excretion mechanisms, whereas at 10 000 $ng.L^{-1}$ 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 (~ 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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