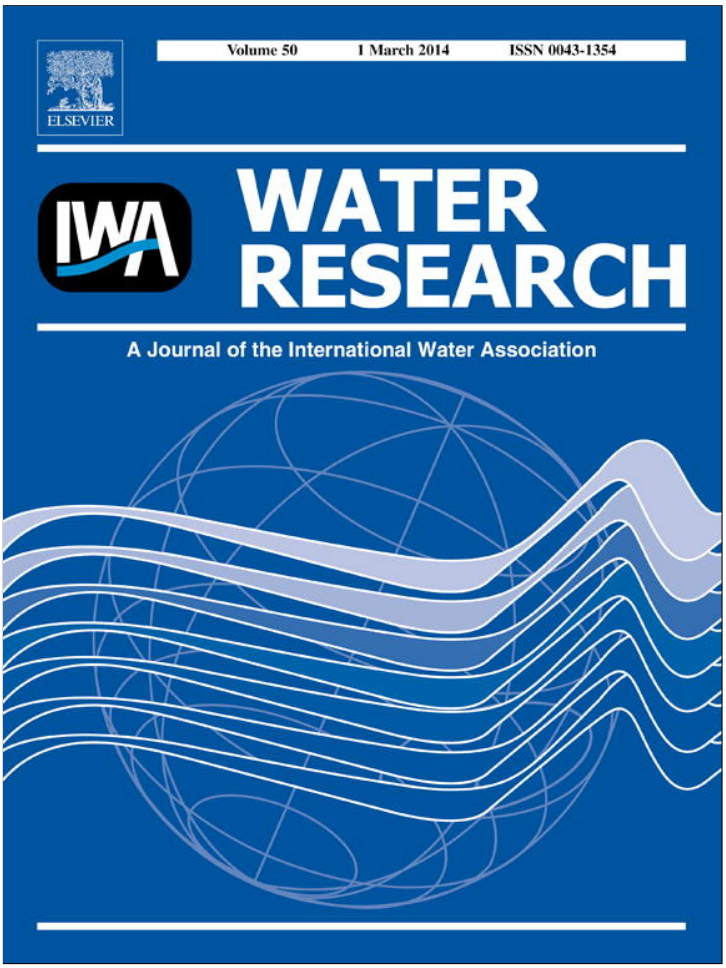

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Extra- and intra-cellular accumulation of platinum group elements by the marine microalga, *Chlorella stigmatophora*

Leyla Shams [a], *Andrew Turner* [a,*], *Geoffrey E. Millward* [b], *Murray T. Brown* [c]

[a] *School of Geography, Earth and Ocean Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK*
[b] *Marine Institute, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK*
[c] *School of Marine Science and Engineering, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK*

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ABSTRACT

To better understand the marine biogeochemistry of the platinum group elements (PGE), Rh(III), Pd(II) and Pt(IV) were added in combination and at ppb concentrations to cultures of the marine microalga, *Chlorella stigmatophora*, maintained in sea water at 15 °C and under 60 μmol m^{-2} s^{-1} PAR. The accumulation of PGE was established in short-term (24-h) exposures, and under varying conditions of algal biomass and PGE concentration, and in a longer-term exposure (156-h) by ICP-MS analysis of sea water and nitric acid digests and EDTA washes of the alga. In short-term exposures, and under all conditions, the extent of accumulation by *C. stigmatophora* was in the order: Rh > Pd >> Pt; and Pd was internalised (or resistant to EDTA extraction) to a considerably greater extent than Rh and Pt. Accumulation isotherms were quasi-linear up to added PGE concentrations of 30 μg L^{-1} and all metals displayed a significant reduction in accumulation on a weight-normalised basis with increasing density (biomass) of *C. stigmatophora*, an effect attributed to the production of exudates able to stabilise metals in sea water through complexation. In the longer-term exposure, kinetic constraints on the reactivities of Rh and, in particular, Pt, resulted in final degrees of accumulation and internalisation by *C. stigmatophora* that were greatest for Rh and similar between Pd and Pt. Among the PGE, therefore, Rh is predicted to participate in biological removal and transport processes in the marine environment to the greatest extent while decoupling in the biogeochemistries of Pd and Pt is predicted in shorter-term or more transient processes.

1. Introduction

Rhodium, palladium and platinum (and hereafter collectively referred to PGE) are used, predominantly, in automobile catalytic converters to modify the composition of exhaust gases. However, as the washcoat of the catalytic converter abrades and deteriorates, fine particles of PGE are emitted with the exhaust (Ravindra et al., 2004). A consequence of emissions from vehicles is that concentrations of PGE are both elevated and increasing in roadside dusts and soils (Mihajevic et al.,

* *Corresponding author*. Tel.: +44 1752 584570; fax: +44 1752 584710.
E-mail address: aturner@plymouth.ac.uk (A. Turner).

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2013). Moreover, the fine, particulate association of PGE has facilitated their long range transport to regions remote from any urbanisation (Soyol-Erdene et al., 2011).

Through urban runoff and atmospheric deposition, PGE are transported to the aquatic environment and, ultimately, to coastal and oceanic waters where they are gradually solubilised into aqueous forms (Colombo et al., 2008). Dissolved Rh(III) is predicted to form relatively strong chloride complexes in sea water ($[RhCl_{6-x}(H_2O)_x]^{x-3}$, where $x = 0–6$) that are characterised by variable ligand replacement times ranging from a few minutes to a few months (Bertine et al., 1996; Gerber et al., 2010), but no thermodynamic information exists concerning its complexation with organic ligands. The inorganic speciation of Pd(II), Pt(II) and Pt(IV) in sea water is dominated by chlorides and mixed hydroxychlorides (Gammons, 1996) and the relative abundance of the respective free ions is vanishingly small (e.g. $[Pd^{2+}/[PdCl_4^{2-}] < 10^{-10.5}$ and $[Pt^{2+}/PtCl_4^{2-}] \sim 10^{-13}$ at equilibrium; Cosden and Byrne, 2003). Because of their strong interactions with soft ligands, cations of both metals are predicted to complex readily with natural organic ligands and surface functional groups (Wood, 1990; Wood and Middlesworth, 2004). However, the slow rearrangements in the coordination spheres of Pt(II) and Pt(IV) mean that reactions involving this metal are kinetically hindered compared with those involving Pd (Cosden et al., 2003).

Despite increasing anthropogenic emissions of PGE, coupled with the known toxicities of many complexes of these metals (Schmid et al., 2007; Wiseman and Zereini, 2009), little is known about their biogeochemical behaviour in the aquatic environment, and in particular in the marine environment. Empirically derived constants defining the adsorption of PGE by estuarine sediment suspended in sea water and their accumulation by the marine macroalga, *Ulva lactuca* have been reported (Cosden et al., 2003; Turner, 2007; Turner et al., 2007; Turner and Xu, 2008). What is lacking, however, is mechanistic and kinetic information on the interactions of PGE with marine microalgae. These organisms are excellent model systems for investigating the processes controlling metal accumulation at the cellular level (Vasconcelos and Leal, 2001a; Quigg et al., 2006) and, as decaying and settling particles, they also represent an important vehicle for the vertical transport of contaminants in the marine environment (Gonzalez-Davila, 1995; Twining et al., 2011). The unicellular marine microalga, *Chlorella stigmatophora*, has been previously utilised as a model planktonic organism for trace metal studies (Christensen et al., 1979; Rebhun and Ben-Amotz, 1984) because it is relatively fast growing, with a cell size ranging between about 2 and 5 μm, and can be manipulated under controlled conditions in the laboratory. As with other marine green algae, *C. stigmatophora* produces polysaccharides on its cell walls which can affect the specificity of its metal complexing capacity (Kaplan et al., 1987). The production of these compounds may also regulate the fraction of metal bound to the external cell walls relative to that which is internalised.

The present study examines the interactions of PGE with *C. stigmatophora* under carefully controlled laboratory conditions. Specifically, we investigate the net accumulation and surface bound-internalised distributions of PGE in a series of short-term (24-h) exposures and, because of the kinetic constraints on the reactivity of Rh and Pt, we also examine the rates of these interactions in a longer-term (156-h) exposure.

2. Materials and methods

2.1. Materials and reagents

Except when purchased new or sterile, all plastic- and glassware used in the experiments and for sample and analyte storage were soaked in 1 M HCl for 24–48 h and subsequently rinsed three times with double distilled water (DDW). Unless otherwise stated, chemical reagents were purchased from Fisher Scientific, VWR or Sigma and were of analytical grade or better. The stock culture of *Chlorella stigmatophora* was provided by the Marine Biological Association of the UK and English Channel sea water (pH ~ 7.8; salinity 34.1–34.3) was collected in bulk and supplied to the laboratory from fibreglass storage tanks via polymer piping and was double filtered online through 5 μm and 0.6 μm extruded carbon filters.

2.2. Algal culturing

Algal culturing was performed according to established protocols (Andersen, 2005). Sea water used for culturing and experimental work was enriched by addition of nutrients (except silicate), trace metals (Fe, Mn, Zn, Co, Cu, Mo), EDTA and vitamins in accordance with Guillard's f/2 formulation (Guillard, 1975). As required, 5 mL of stock cells were transferred to 400 mL of sea water in a series of sterile 500 mL borosilicate bottles and the contents incubated at 15 °C and under 60 μmol m^{-2} s^{-1} PAR supplied by fluorescent lighting on a 14 h:10 h light:dark photoperiod in a Snijders Scientific controlled environment cabinet. The bottle was continuously aerated using a Pasteur pipette containing non-absorbent cotton connected to an air pump via polyethylene airline tubing. For the experiments, cells of *C. stigmatophora* in their mid-exponential growth phase were used. (An example of a

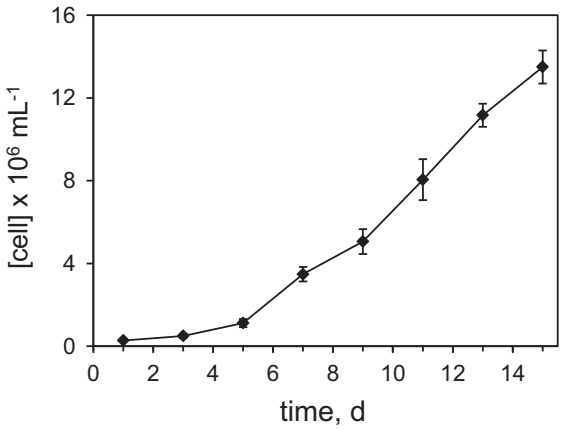

Fig. 1 – Typical growth curve of *C. stigmatophora* cultured for a fifteen day period. The specific growth rate between 5 and 15 days was 0.25 ± 0.02 d^{-1}. Errors represent the standard deviation about the mean of three independent measurements.

typical growth curve, derived from measurements made using a Neubauer improved ruling double chamber haemocytometer, is shown in Fig. 1.) Cells were centrifuged at 4000 rpm for 10 min and the residual pellet was resuspended in an appropriate volume of nutrient-amended sea water and allowed to acclimatise for 24 h under the conditions described above. Subsequent microscopic observations of samples from a variety of exposure conditions indicated no damage to cells from centrifugation.

2.3. *Exposure experiments*

"Short-term" exposures were undertaken in triplicate or quadruplicate as follows. Cell suspensions of 120 mL and about 10^6 cells mL^{-1} (or 10–20 mg dry weight L^{-1}) were added to 150 mL sterile Styrolux (crystal polystyrene) beakers and spiked with a mixed, 200 mg L^{-1} solution of Rh(III), Pd(II) and Pt(IV), prepared by serial dilution of Spectrosol plasma emission standards in 0.4 M HCl, to obtain a concentration of 20 μg L^{-1} for each element. Beakers were loosely covered and then agitated at 85 rpm on a Denley orbital mixer or Heidolph UNIMAX2010 shaker (depending on the number of containers) for 24 h and at 15 °C under the light conditions described above. The pH was measured at regular intervals and with a precision of 0.01 units using a Denver Instruments pH Meter. Alterations of pH of more than 0.5 units were compensated by dropwise addition of either 1 M HCl or 1 M NaOH. Control treatments were performed under identical conditions in the absence of PGE.

At the end of the exposure period, 15 mL aliquots from each beaker were vacuum-filtered through pre-weighed 0.2 μm polycarbonate filter membranes (Cyclopore, 47 mm diameter, Whatman) using a polysulphone filtration unit. Ten mL filtrates were transferred to clean 60 mL polystyrene containers and acidified with 40 mL of 1 M HNO_3 to reduce effects arising from the saline matrix during subsequent analysis (see below). Filters were rinsed with 10 mL of DDW before being immersed in 15 mL of 5 mM EDTA in order to extract extracellular or adsorbed PGE (Turner et al., 2007; Levy et al., 2008). After cells had been re-filtered through the original filter, 10 mL of the EDTA extracts were stored in 30 mL polypropylene tubes and filters were allowed to dry at room temperature for 24 h before being reweighed. Filters were then digested in 2 mL of concentrated HNO_3 for 40 min in new 30 mL polypropylene tubes while being agitated on a roller mixer before being diluted to 20 mL in 1 M HNO_3.

In similar experiments, the density (biomass) of *C. stigmatophora* was varied between about 10^6 and 10^7 mL^{-1} by inoculation of 120 mL of sea water with different quantities of the stock culture. Accumulation isotherms were conducted as above but the concentrations of PGE were varied (up to 30 μg L^{-1}) by addition of different quantities of the PGE stock solution.

A "longer-term" exposure was undertaken in triplicate over a period of 156 h in 1.5 L cell suspensions contained in sterile 2 L narrow necked Nalgene polycarbonate bottles. Reactors were continuously aerated using a Pasteur pipette connected to an air supply, rather than being agitated, under conditions that were otherwise identical to those described earlier. Subsamples were abstracted and filtered after 30 min and thereafter every 12 h, and filtrates and filters were processed as above. The pH was recorded initially and before subsampling throughout the experiment and was adjusted, when necessary, by microlitre additions of either 1 M HCl or 1 M NaOH. This exposure was also undertaken in triplicate in sea water that had not been amended with nutrient trace metals, vitamins and EDTA in order to ascertain any potential confounding effects (e.g. competition, complexation) arising from the presence of these chemicals.

2.4. *PGE analysis*

Inductively coupled plasma-mass spectrometry (ICP-MS) was employed for the determination of Rh (as ^{103}Rh), Pd (as ^{108}Pd) and Pt (as ^{196}Pt) in acidified filtrates, EDTA washes and acid digests using a Thermo Elemental PlasmaQuad PQ2+ with a Meinhard nebuliser. The instrument was calibrated over the range 0–7 μg L^{-1} using multi-element standards prepared by dilution of individual plasma emission standards in either 1 M HNO_3 (for digested cells), a 1:4 mixture of sea water:1 M HNO_3 (for acidified filtrates) or 5 mM EDTA (for EDTA washes). Internal standardisation was achieved by the addition of 10 μg L^{-1} of ^{115}In and ^{191}Ir to all samples and standards. A PGE standard was analysed after every seven samples as a check and the instrument was flushed between samples and standards with 0.1 M HNO_3. Samples and standards were read three times during the analysis, while blanks of each matrix were read nine times. Limits of detection, based on three standard deviations of replicate readings of blanks, varied between the different matrices and the different experiments but were generally greatest in acidified sea water (up to about 0.2 μg L^{-1}, 0.8 μg L^{-1} and 0.08 μg L^{-1}, after dilution correction, for Rh, Pd and Pt, respectively).

2.5. *Data presentation and analysis*

Molecular ion interferences were detected in the filtrates, EDTA washes and digests of the control treatments, and signals were subtracted from the corresponding concentrations in samples that had undergone PGE exposure. Corrected concentrations of PGE in the alga are presented on a dry weight basis, although cell density is also given for each set of results. Errors shown in each case represent one standard deviation arising from replicate experimental measurements ($n = 3$ or 4). Errors were generally greatest for Rh because of analytical interferences in the acid digests and aqueous concentrations that were often close to the detection limit of the metal.

3. Results

3.1. *Recovery of PGE*

In all experiments, the recovery of PGE was examined by comparing the total w/v concentration analysed (that is, the summed concentrations in sea water, the EDTA extract and algal digest) with that added at the beginning of the exposure period. Thus, in the short-term exposures, recovery of Pt was close to 100%, while between about 20 and 40% of Rh and

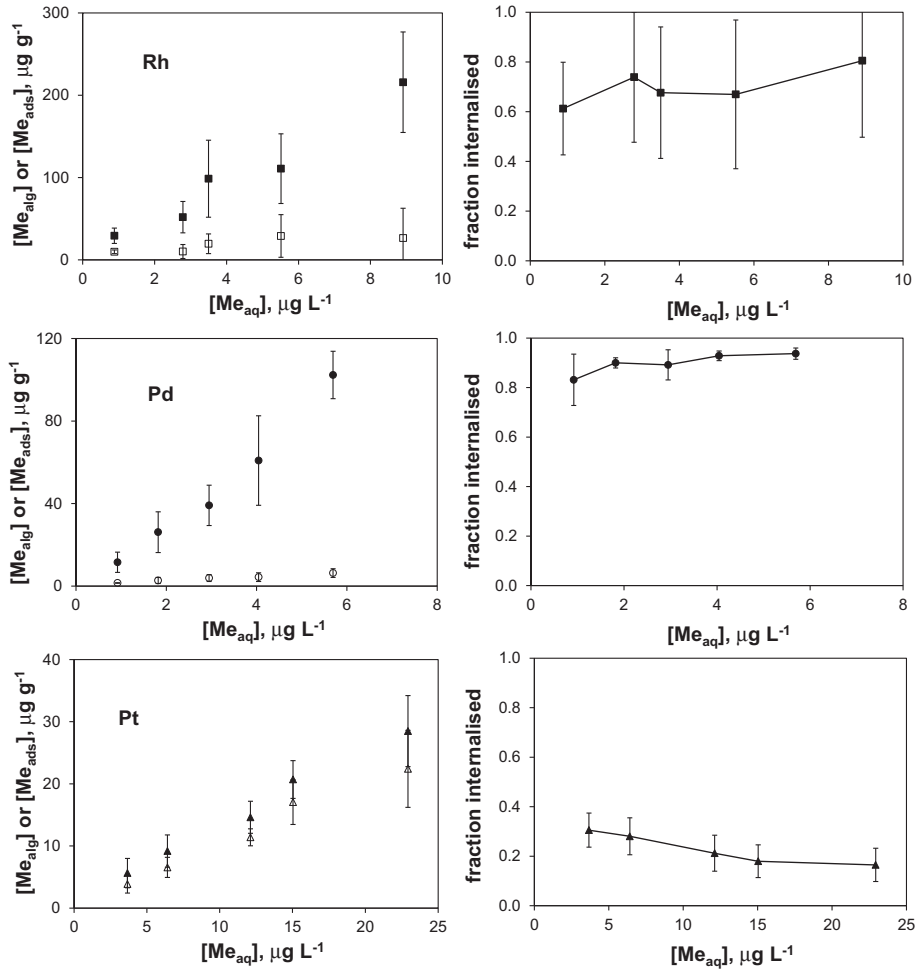

Fig. 2 – Concentrations of PGE accumulated (filled symbols) and adsorbed (open symbols) and the fraction of PGE internalised (right-hand panels) by ~10^6 cells mL^{-1} of *C. stigmatophora* over a 24-h period as a function of PGE concentration remaining in sea water. Errors represent the standard deviation about the mean of four independent measurements. Constants quantifying the accumulation and adsorption isotherms are given in Table 1.

about 50 and 70% of Pd was unaccounted for; loss of these metals was largely independent of density of algae and the concentration of metal added. In the longer-term experiment, loss of Pd and Pt increased over the exposure period such that about 80% was unaccounted for after 156 h; in contrast, Rh loss was about 20% and exhibited no clear dependence on exposure time. Presumably, loss of metals arises from their rapid (Rh) or progressive (Pd and Pt) adsorption to the container surfaces, an effect that has been described in detail for different polymeric materials by Cobelo-Garcia et al. (2007). It is reasonable to assume that metal lost from the system to the container surfaces has no impact on its partitioning between *C. stigmatophora* and sea water. Clearly, however, for an accurate assessment of the algal-sea water distribution of PGE it is critical that concentrations in both phases are measured.

3.2. *Accumulation/adsorption isotherms*

In Fig. 2, the dry w/w concentrations of PGE adsorbed by *C. stigmatophora*, [Me_{ads}] (extracted by EDTA), and that accumulated by the alga, [Me_{alg}] (derived from the sum of metal concentrations in the EDTA extract and HNO_3 digest), are shown as a function of the corresponding PGE concentrations remaining in sea water, [Me_{aq}]. Note that, for clarity, error bars are shown for [Me_{alg}] and [Me_{ads}] but not for [Me_{aq}]. In each case, data could be defined with statistical confidence ($p < 0.05$) by a linear isotherm:

$$[Me_{alg}] = AF[Me_{aq}] \quad (1a)$$

$$[Me_{ads}] = K_{ads}[Me_{aq}] \quad (1b)$$

where AF (v/w) represents an accumulation factor and K_{ads} (v/w) an adsorption constant. However, marginally better fits were obtained with a Freundlich-type equation:

$$[Me_{alg}] = AF_F[Me_{aq}]^n \quad (2a)$$

$$[Me_{ads}] = K_F[Me_{aq}]^n \quad (2b)$$

where AF_F and K_F are the corresponding Freundlich constants and n is a measure of the non-linearity of the relationship. Constants derived from both linear and non-linear fitting of the data are given in Table 1. Also shown in Fig. 2 is the fraction of accumulated PGE that is internalised, and as operationally

Table 1 – Constants defining the accumulation and adsorption of PGE by *C. stigmatophora* according to Equations (1) and (2).

PGE	Equation (1a)		Equation (1b)		Equation (2a)			Equation (2b)		
	AF, mL g^{-1}	r^2	K_{ads}, mL g^{-1}	r^2	AF_F	n	r^2	K_F	n	r^2
Rh	23,400	0.956	3830	0.374	41,400	0.847	0.933	28,500	0.507	0.725
Pd	16,300	0.956	1160	0.918	8730	1.160	0.991	2910	0.753	0.989
Pt	1280	0.984	1010	0.979	2190	0.886	0.992	1110	0.980	0.990

defined by the proportion of total metal accumulated by *C. stigmatophora* that is resistant to EDTA extraction:

$$\text{fraction internalised} = \frac{[Me_{alg}] - [Me_{ads}]}{[Me_{alg}]} \quad (3)$$

Measures of internalisation are variable amongst replicates but, with increasing $[Me_{aq}]$, mean values exhibit a decrease (Pt), an increase (Pd), or are relatively invariant (Rh).

Results of experiments in which the density of *C. stigmatophora* was varied are shown in Fig. 3. Here, metal accumulation is expressed as an accumulation factor, AF (v/w), calculated from the ratio of w/w concentration of metal associated with the algae to concentration of metal in the aqueous phase (Equation (1a)). For all metals, a reduction in AF with increasing cell density is observed, an effect defined by an equation of the form:

$$AF = a[\text{cell}]^{-b} \quad (4)$$

where a and b are empirical constants whose values are annotated in each case. The magnitude of a, or the AF normalised to a cell density of 10^6 mL^{-1}, is an order of magnitude greater for Rh than Pd and Pt; the magnitude of b, or the gradient of the effect, is greatest for Rh and Pt.

3.3. Accumulation kinetics

Results of the longer-term experiment, in which *C. stigmatophora* was exposed to 20 μg L^{-1} of PGE over a 156-h period, are shown in Fig. 4. Here, PGE are expressed in terms of the percentage of total analytical metal that is accumulated by *C. stigmatophora*:

$$\%\ \text{accumulated} = \frac{[Me_{alg}][alg]\cdot 100\%}{[Me_{aq}] + [Me_{alg}][alg]} \quad (5)$$

where [alg] is the concentration of alga in the treatment on a dry weight basis. In this experiment, pH exhibited no significant change over the exposure period but algal density increased from about 50 mg L^{-1} at the beginning of the experiment to about 90 mg L^{-1} at its termination. Continuous, relatively rapid accumulation of Rh occurs during the first 50 h, followed by a period of slower accumulation in which equilibrium appears to be attained. Palladium exhibits rapid accumulation within the first 24 h and apparent equilibrium thereafter, while Pt exhibits continuous accumulation over the timeframe of the exposure.

The fraction of PGE internalised by the alga and calculated according to Equation (3) is also shown in Fig. 4 as a function of time over the 156-h exposure period. For Rh, internalisation increases within the first 50 h and thereafter is almost completely internalised. Platinum exhibits a continuous increase in internalisation during the experiment whereas the internalisation of Pd increases rapidly during the first 12 h and subsequently declines throughout the remainder of the exposure. By the end of the experiment, the fraction of both Pd and Pt internalised is about 0.4.

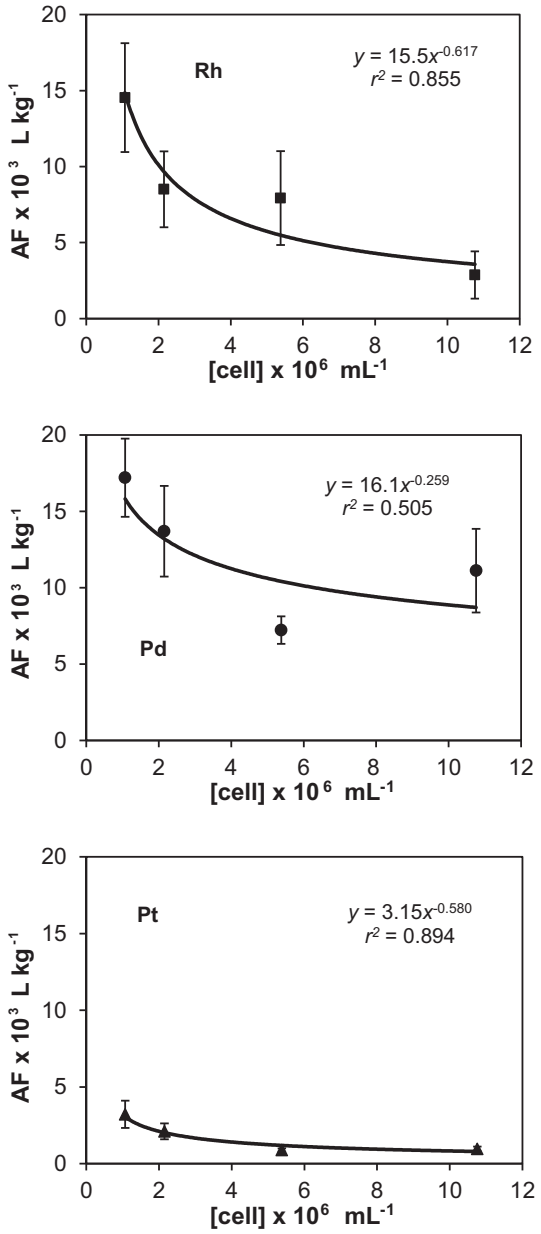

Fig. 3 – The accumulation of PGE by *C. stigmatophora* (shown in terms of accumulation factors) as a function of cell density over a 24-h period. Errors represent the standard deviation about the mean of four independent measurements. Also shown are the best fit equations to the accumulation data.

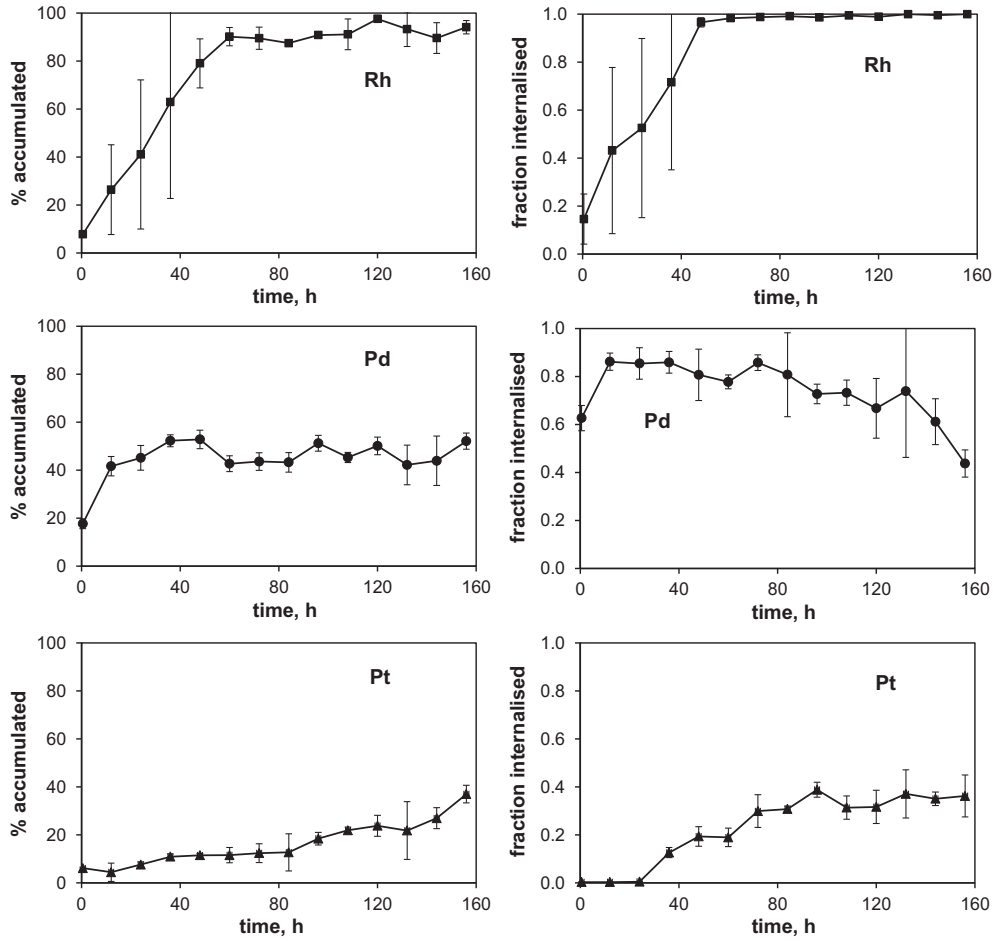

Fig. 4 – The percentage of PGE accumulated and the fraction internalised (right-hand panels) by *C. stigmatophora* (original cell concentration ~10^6 mL^{-1}) as a function of time. Errors represent the standard deviation about the mean of three independent measurements.

Two measures of reaction rate were calculated for the experiment. Firstly, the instantaneous rate, λ (%), was determined from the slope of the line between the origin and accumulation after 30 min; this measure was also derived after PGE results had been recalculated on a w/w basis; i.e. λ (μg g^{-1}). Secondly, the time for 50% accumulation, $t_{1/2}$, was defined as the time required for the accumulation of 50% of that taken up by the microalga by the end of the exposure period; note that the end-point appears to represent equilibrium conditions for Rh and Pd, but not for Pt. Results of these calculations, shown in Table 2, reveal that the instantaneous reactivity of Pd is approximately double that of Rh and Pt, and that the 50% accumulation period of both Rh and Pt is an order of magnitude greater than that for Pd.

The kinetic experiment was also repeated in unamended sea water (in the absence of added nutrient trace metals, vitamins and the complexant, EDTA). Results, as both percentage of PGE accumulated and fraction of PGE internalised by the microalga, were statistically indistinguishable from the corresponding results derived in the amended medium ($p > 0.05$ according a series of independent samples t-tests). For clarity, the data are not shown in Fig. 4; however, the reaction rate constants, derived from the kinetic profiles and shown in Table 2, are very similar to those defining the reactions in which the additional chemicals were present.

4. Discussion

This study is the first to examine the interactions of an important group of emerging contaminants (PGE) with marine microalgae, and is one of a very limited number of studies to investigate the accumulation of these contaminants by aquatic algae (Cosden et al., 2003; Godlewska-Żyłkiewicz, 2003; Turner et al., 2007). As such, the results make an

Table 2 – Instantaneous reaction rates and half-lives defining the accumulation of PGE by *C. stigmatophora*. Values in parentheses represent constants derived in unamended sea water (in the absence of nutrient trace metals, vitamins and EDTA).

PGE	λ, %	λ, μg g^{-1}	$t_{1/2}$, h
Rh	7.8 (7.1)	0.85 (0.75)	36 (39)
Pd	17.7 (26.2)	1.85 (2.64)	4 (2)
Pt	6.1 (5.8)	0.91 (0.84)	91 (91)

important contribution to our understanding of the biogeochemistry of PGE in coastal and oceanic waters. Many of the observations also have more general implications for both our understanding of trace metal behaviour in the marine environment and with regard to the practical implications and limitations inherent in kinetic studies of metal-algal interactions under laboratory conditions (e.g. Vasconcelos and Leal, 2001b; Levy et al., 2008; Varma et al., 2013).

Because significant and variable fractions of added PGE are lost to the container walls in this type of experiment, it is critical that accumulation and kinetic constants defining metal-algal interactions are derived from an analysis of both the algae and the aqueous phase. Clearly, any study in which PGE concentrations accumulated by algae that are computed from the difference between the added concentration and the quantity remaining in solution may be overestimated unless such losses are quantitatively accounted for (Cosden et al., 2003; Turner et al., 2007). Loss of PGE to the containers also means that significant proportions of added metal would not be available to the algal biomass in any toxicity studies.

An experimental and environmental variable that had a significant impact on the accumulation of PGE by *C. stigmatophora* was the weight to volume concentration (or density) of the algae. Thus, with decreasing algal density, an increase in the weight-normalised AF was observed for Rh, Pd and Pt, an effect that could not be quantitatively accounted for by the relatively small degree of non-linearity in the accumulation isotherms. Equivalent observations have been made in adsorption studies of trace metals, including PGE, in sediment and soil suspensions (Turner and Millward, 2002; Zhou et al., 2003; Turner and Wu, 2007) but, to our knowledge, the relationship has not been previously documented for marine microalgae.

With respect to sediment- or soil-water interactions, the "particle concentration effect" has been attributed to a reduction in particulate surface area (through aggregation) with increasing particle concentration, or to the presence (pre-existence or collisional generation) of a third, filterable (colloidal) phase, acting as a sorbent or complexant and whose abundance co-varies with that of filter-retained particles. While aggregation of living algae is unlikely and was not supported by light microscope imaging of selected algal samples in the present study, it is possible that a third, filterable phase consists of complexing algal exudates (Vasconcelos and Leal, 2001a; Levy et al., 2008; Strmecki et al., 2010). Requirements for our observations, therefore, are that these exudates are able to bind PGE and their abundance is related to the abundance of *C. stigmatophora*. If this is indeed the reason for the inverse dependence of AF on algal density, the effect is likely to be neither specific to *C. stigmatophora* nor to PGE. Accordingly, accumulation factors used for modelling microalgal-metal interactions in sea water may not only be specific to the metal and algal species but also to the precise density of algae present (hence the stage of the plankton bloom, time of day, season etc). Significantly, an increase in the biomass is not necessarily associated with a proportional reduction in the amount of metal remaining in sea water because algae appear to be able to buffer the concentration of metal that can be removed from the aqueous phase. Biogeochemical scavenging or uptake models that rely on accumulation factors may require revision or reconsideration, and future studies involving metal-algal interactions should incorporate biomass as a key variable in their protocols.

The accumulation isotherms are consistent with results of other short-term experiments using a variety of biotic and abiotic solids (e.g. Turner, 2007; Turner et al., 2007) in that the net order of PGE accumulation by *C. stigmatophora* (or the magnitude of AF or AF_F) is Rh > Pd > Pt. That isotherms are quasi-linear suggests available binding sites on the algal surface do not approach saturation over the PGE concentration range studied and constants defining accumulation (Table 1) are likely to be applicable under environmentally realistic conditions and PGE concentrations (on the order of pM in sea water; Yang, 1989; Bertine et al., 1996). However, the differential aqueous reaction kinetics displayed by Rh, Pd and Pt means that information on the rates of these interactions are critical for a more complete biogeochemical understanding of PGE in the marine environment. Thus, although the equilibrium chemistries and ionic radii of Pt(IV) and Pd(II) are very similar, rearrangements in the coordination spheres of both Pt(II) and Pt(IV) are extremely slow and Pt complexation is, therefore, kinetically hindered (Cosden et al., 2003). Rhodium forms relatively strong chloride complexes of the form: $[RhCl_{6-x}(H_2O)_x]^{x-3}$, where $x = 0–6$; but first-order rate constants for ligand replacement are highly variable and range from a few minutes to a few months (Bertine et al., 1996).

Neglecting the relatively small confounding effects associated with the increasing biomass in the containers, the results of the longer-term experiment are largely, and at least qualitatively, consistent with the relative kinetics of formation and breakdown of complexes among the PGE. Moreover, the kinetics of PGE-algal interactions appear to be unaffected by the presence of competing (nutrient) metals and complexants (EDTA) in the experimental medium. Thus, for Pt, both the adsorption and internalisation by *C. stigmatophora* proceed relatively slowly because complexation reactions involving this metal, including those with surface functional groups and intracellular molecules, are considerably constrained. In contrast, accumulation of Pd by *C. stigmatophora* proceeds much more rapidly and attains equilibrium within a few hours of exposure. However, a protracted period in which the extent of internalisation diminishes suggests some rearrangement in the location of Pd with respect to this microalga. Specifically, it appears that an efflux of intracellular Pd is compensated by adsorption to the cell surface of an equivalent quantity of Pd. Given the kinetic constraints on Pt reactivity, it is likely that this rearrangement is the result of the progressive competition with Pt for mutually accessible intracellular sites. Because of this competition and, more generally, their common equilibrium chemistries, Pd and Pt exhibit similar degrees of adsorption and internalisation by the end of the exposure (despite possible kinetic constraints on the extraction of internally bound Pt by EDTA and on its complexation with algal exudates). Results for Rh indicate that interactions with *C. stigmatophora* are kinetically constrained, but at equilibrium (or after about 50 h), extents of both accumulation and internalisation considerably exceed those of either Pd or Pt.

The broader implications of our observations for the marine biogeochemical behaviour of PGE may be summarised as

follows. Of the metals studied, and despite evidence that its accumulation by *C. stigmatophora* is kinetically hindered, Rh is predicted to be removed to the greatest extent by microalgae and other biological surfaces in the marine environment, presumably through interactions involving Rh^{3+} and cationic chloride complexes. Thus, Rh is likely to exhibit greatest involvement in biological transport mechanisms from surface to deeper waters, an assertion supported by observations of surface water depletion of aqueous Rh in the Pacific Ocean (Bertine et al., 1996). Interactions with algae involving Pd and Pt likely involve the free ion and, possibly, anionic chloride complexes (Godlewska-Zyłkiewicz, 2003). Palladium is predicted to be involved in biological removal and transport processes to a greater extent than Pt because of considerable kinetic constraints on the biological reactivity of the latter, and differential removal of Pd and Pt is likely to be greatest by motile organisms and in transient algal blooms. Because of the more rapid biological reactivity of Pd, it is predicted to display greater nutrient-like behaviour in the coastal and oceanic water column and ratios of dissolved Pt:Pd are expected to be higher in surface waters than in deeper waters.

5. Conclusions

This study represents the first investigation into the accumulation of platinum group elements by marine microalgae. Results of short-term (24-h) exposure experiments suggest that the accumulation of Rh, Pd and Pt by *Chlorella stigmatophora* is determined by the reactivity of the algal surface, the abundance of reactive forms of PGE and the nature and concentration of algal exudates that are able to bind metals in solution. Given the similar equilibrium chemistries of Pd and Pt, differences in their adsorption and internalisation are attributed to differences in the kinetics of their interactions with surface functional groups and intracellular molecules. Results of a longer-term (156-h) exposure confirm that this is the case and reveal evidence for the progressive competition for external and internal sites between these metals. Overall, both the adsorption and internalisation by *C. stigmatophora* are greatest for Rh. Among the PGE, therefore, Rh is predicted to participate in biological removal and transport processes in the oceans to the greatest extent.

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