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

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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.,

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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

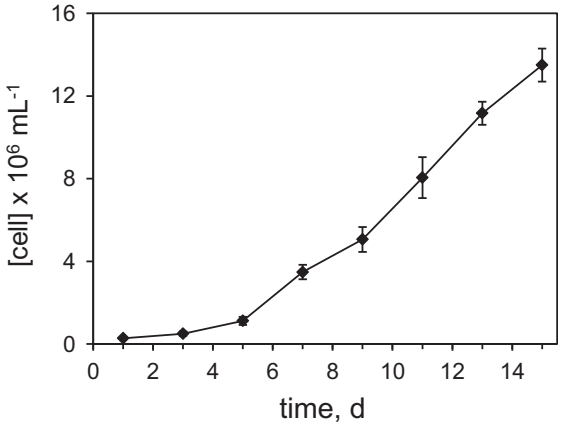

Fig. 1 – Typical growth curve of *C. stigmatophora* cultured for a fifteen day period. The specific growth rate between 5 and 15 days was 0.25 ± 0.02 d^{-1}. Errors represent the standard deviation about the mean of three independent measurements.