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An evaluation of the toxicity and bioaccumulation of bismuth in the coastal environment using three species of macroalga

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ABSTRACT

Bismuth is a heavy metal whose biogeochemical behaviour in the marine environment is poorly defined. In this study, we exposed three different species of macroalga (the chlorophyte, *Ulva lactuca*, the phaeophyte, *Fucus vesiculosus*, and the rhodophyte, *Chondrus crispus*) to different concentrations of Bi (up to 50 μg L^{-1}) under controlled, laboratory conditions. After a period of 48-h, the phytotoxicity of Bi was measured in terms of chlorophyll fluorescence quenching, and adsorption and internalisation of Bi determined by ICP after EDTA extraction and acid digestion, respectively. For all algae, both the internalisation and total accumulation of Bi were proportional to the concentration of aqueous metal. Total accumulation followed the order: *F. vesiculosus* > *C. crispus* > *U. lactuca*; with respective accumulation factors of about 4200, 1700 and 600 L kg^{-1}. Greatest internalisation (about 33% of total accumulated Bi) was exhibited by *C. crispus*, the only macroalga to display a phytotoxic response in the exposures. A comparison of the present results with those reported in the literature suggests that Bi accumulation by macroalgae is significantly lower than its accumulation by marine plankton (volume concentration factors of 10^5 to 10^7), and that the phytotoxicity of Bi is low relative to other heavy metals like Ag and Tl.

1. Introduction

Bismuth is the heaviest chemical element in Group 15 of the Periodic Table whose only naturally occurring isotope, ^{209}Bi, is radioactive ($t_{1/2}$ ~ 10^{19} years). It can exist in a number of oxidation states but the trivalent form is the most stable and abundant in the geosphere. The crustal content of Bi is only about 0.02 μg g^{-1} and its minerals, including native bismuth, bismuthinite (Bi_2S_3) and bismite (Bi_2O_3), rarely occur alone (Das et al., 2006). Consequently, Bi is usually obtained as a by-product from Cu and Pb ores and recovered by the reduction of the oxide by iron or charcoal (Ayres and Hellier, 1998). The metal and its compounds have a wide range of applications in the electronics, cosmetics, chemical, medical, metallurgical and nuclear industries, and increasing usage has been accompanied by an increase in anthropogenic release to the environment (Liu et al., 2011). Bismuth exhibits low toxicity to humans compared to its periodic neighbours (Pb and Po) and other group 15 elements (e.g. As and Sb) and is believed to be a non-essential element with no known biological function. It is, however, toxic to some prokaryotes and has, therefore, been used to treat various bacterial infections (including syphilis and peptic ulcers; Das et al., 2006).

The increasing usage of Bi in both industry and as a 'safe' replacement for Pb in many consumer products has been accompanied by the realisation that very little is known about its behaviour and impacts in the environment. For example, a recent review of published thermodynamic constants for Bi revealed such a variety of inconsistencies and errors and lack of data validation that precluded confidence in many aqueous speciation calculations (Filella, 2010). With regard to toxicity, published studies appear to be limited to those that define the acute and chronic effects of Bi shotshell on waterfowl and game birds (the results of which ultimately led to the approval of the product; Fahey and Tsuji, 2006) and the nanotoxicity of Bi-asparagine coordination polymer spheres on zebrafish embryos (He et al., 2013).

With respect to the marine environment, the principal source of Bi is the atmosphere via volcanic emissions and fossil fuel combustion (Lee et al., 1985/1986). The limited oceanic profiles available indicate surface enrichment from the atmosphere, removal in the mixed layer, regeneration at intermediate depths and intense scavenging in deeper waters. The strong particle reactivity of Bi in the deep ocean results in enrichment in ferromanganese phases

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and hydrothermal sulphides (Bertine et al., 1996) and an oceanic residence time of only about 20 years (Lee et al., 1985/1986). Radiotracer experiments conducted by Fowler et al. (2010) using ^{207}Bi indicate significant accumulation by phytoplankton, with volume concentration factors, VCF, between about 10^5 and 10^7; copepods consuming plankton were able to assimilate 4% of ^{207}Bi with the remainder voided in fecal pellets (that also acted as strong scavengers of aqueous ^{207}Bi).

In the present study, and to improve our understanding of the behaviour of Bi in the coastal marine environment, we study its accumulation by and toxicity to macroalgae that are exposed to variable concentrations of the metal under controlled laboratory conditions. As well as playing an important role in the nutrient dynamics of near-shore systems, macroalgae readily reflect changes in water quality, a trait that is widely employed to monitor and characterise coastal contamination and in particular that arising from metals (Baumann et al., 2009; Malea et al., 2015). Providing habitat and sustenance to a variety of organisms, macroalgae can also influence the accumulation of contaminants at higher trophic levels. We selected three species of seaweed that are commonly encountered on rocky shores and the sublittoral zones of north western Europe; namely: *Ulva lactuca* (Chlorophyta), *Chondrus crispus* (Rhodophyta), and *Fucus vesiculosus* (Phaeophyta). Since green, red and brown seaweeds contain different surface functional groups and different pigments for capturing different wavelengths of light, we would expect to see differences in both the accumulation and phytotoxicity of Bi among the species selected. We employ chlorophyll fluorescence quenching as a rapid, non-invasive measure of toxicity, and discriminate Bi that is adsorbed to the cell walls from Bi that is internalised by means of an EDTA extract.

2. Materials and methods

2.1. Sampling and sample preparation

Coastal sea water of salinity 32, pH 8.0 and dissolved organic carbon concentration of about 100 μM (and as determined using a Shimadzu TOC-5000 Analyzer) was used for culturing and experimental work. Sea water was collected in bulk from Plymouth Sound, UK, at high water and was piped to the laboratory under gravity and after filtration through a 0.6 μm extruded carbon filter.

The three different species of macroalga were collected on separate occasions and at low tide during January and February 2015 from the rock pools and rocky shores of Wembury, a protected area of coastline about 7 km to the south east of Plymouth. Samples were transported in clear, zip-lock polyethylene bags to the laboratory where they were subsequently cleaned of particulate matter and epibionts under running (laboratory) sea water with the aid of a fine nylon brush and plastic sieve. Macroalgae were then acclimatised for five days in sea water in an aerated, acid-cleaned (10% HNO_3 for 24 h), 10 L polyethylene aquarium at 15 ± 1 °C and under fluorescent lighting (250 μmol photons m^{-2} s^{-1} photosynthetic active radiation) on a 16 h:8 h light:dark cycle.

Prior to the exposures, macroalgae were cut into smaller, working samples that were allowed to recover from any trauma for a period of 24 h in new aquaria but under the conditions described above. For *U. lactuca*, the sharpened end of a 30 mm diameter polyethylene cylinder was used to cut discs from the central portions of the thalli (dry weights of discs averaged 23.1 mg); frond tips of *F. vesiculosus* (without air bladders) and *C. crispus* were cut to lengths of about 35 mm and 30 mm, respectively, using a stainless steel scalpel (respective dry weights of frond tips averaged 87.4 and 53.2 mg).

2.2. Experimental

For each macroalga, exposures were performed under the conditions above in triplicate and in 100 ml aliquots of sea water in a series of sterilised 150 ml polyethylene terephthalate beakers that had been rinsed twice with the exposure medium. Thus, in separate beakers, Bi was added to concentrations of 0, 5, 10, 20, 40 and 50 μg L^{-1} from a stock solution of 1 mg L^{-1} Bi in distilled water that had been prepared immediately before use by serial dilution of a 10 g L^{-1} BDH "Aristar" solution of Bi(III) in 1.6 M HNO_3. (Note that serial dilution was not performed in acid in order to minimise any pH changes of the exposure medium.) A single algal disc or frond tip was then added to each beaker using a pair of plastic tweezers before beakers were loosely covered with their lids and agitated on a Heidolph Unimax 2010 orbital shaker at 100 rpm for 48 h.

At the end of the exposures, 1 ml water samples for Bi analysis were pipetted from each beaker into individual 30 ml screw-capped polypropylene tubes containing 9 ml of 0.1 M HNO_3 (Fisher Chemical TraceMetal™ Grade). Discs or frond tips were retrieved using tweezers and shaken gently to remove excess sea water before being measured for fluorescence quenching and extracted-digested for accumulated Bi (see below). Meanwhile, and in order to evaluate loss of Bi to the container surfaces, selected beakers whose remaining contents had been discarded were rinsed with 10 ml of 0.1 M HNO_3 for about 5 min before rinsates were transferred to 30 ml polypropylene tubes pending analysis.

2.3. Chlorophyll fluorescence measurements

Exposed algal samples were placed in a series of Hansatech Handy PEA leaf clips with closed shutter plates for 20 min in order to ensure algal reaction centres were fully oxidised and any chlorophyll fluorescence yield fully quenched. Leaf clips were then placed individually on a Hansatech Pocket PEA chlorophyll fluorimeter and algae were exposed to a single high intensity beam of excitation light (up to 3500 μmol m^{-2} s^{-1} with a peak wavelength of 627 nm). Fluorescence origin and maximum fluorescence yield, F_0 and F_m, respectively, were measured, and results expressed as the effective quantum yield of PS II and in terms of the ratio of variable to maximum chlorophyll fluorescence ($F_v/F_m = [F_m - F_0]/F_m$).

2.4. Algal extraction and digestion

After measuring chlorophyll fluorescence, discs or frond tips were immersed, individually, in 20 ml of 3 mM EDTA (Fisher Chemical) in a series of acid-cleaned Pyrex beakers in order to extract Bi adsorbed to the algal surface. After 15 min, solutions were transferred to individual 30 ml polypropylene tubes pending analysis while the discs or fronds were placed in individual specimen bags before being frozen and dried for 24 h in an Edwards Super Modulyo freeze dryer. Dried algae were weighed using an Oxford A Series A2204 balance and then digested for 50 min in 5 ml of concentrated, boiling HNO_3 (Fisher Chemical TraceMetal™ Grade) in a series of 25 ml, acid-cleaned Pyrex beakers covered with watch glasses and on a hot plate. Digests were made up to 25 ml in a volumetric flask with distilled water before being transferred to a series of polypropylene tubes pending analysis.

2.5. Bi analysis

Diluted-acidified sea water samples and algal digests and extracts were analysed for ^{209}Bi by collision cell-inductively coupled