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 macroalgae (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 extracellular and intracellular accumulation of Bi determined 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 4,200, 1,700 and 600 L kg^{-1}, and greatest internalisation (about 33% of total accumulated Bi) was exhibited by *C. crispus*, the only macroalga to display a toxic response in the exposures. A comparison of the 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 Bi phytotoxicity to macroalgae is low relative to other heavy metals like Ag and Tl.

Capsule

Bismuth is accumulated by three species of macroalga but exhibits only moderate toxicity to a rhodophyte

Keywords: bismuth; macroalgae; toxicity; accumulation; adsorption; internalisation

1. Introduction

Bismuth is the heaviest chemical element in Group 15 of the Periodic Table whose only naturally occurring isotope, ^{209}Bi, is radioactive ($t_{1/2} \sim 10^{19}$ 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 $\mu g\ g^{-1}$ and its minerals, including native bismuth, bismuthinite (Bi_2S_3) and bismite (Bi_2O_3), rarely occur alone (Das et al., 2006). Bismuth 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 (Lui 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 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 thermodynamic constants for Bi reported in the literature revealed such a lack of data validation and variety of inconsistencies and errors that inorganic aqueous speciation cannot be stated with confidence (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 and hydrothermal sulphides (Bertine et al., 1996) and a 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 (the latter 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 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 containing local sea water 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 the same medium in an aerated, acid-cleaned (10% HNO_3 for 24 h), 10 L polyethylene aquarium at 15±1 ^{o}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 acclimatised 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); fronds 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 fronds averaged 87.4 and 53.2 mg).

2.2. Experimental

For each macroalga, exposures were performed 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. 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^{TM} 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 3,500 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 ($F_v/F_m = [F_m - F_o]/F_m$).

2.4. Algal extraction and digestion

After measuring chlorophyll fluorescence, discs or fronds were immersed, individually, in 20 ml of 3 mM EDTA (Fisher Chemical) in 0.6 M NaCl (Sigma Aldrich) 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 plasma-mass spectrometry (ICP-MS) using a Thermo X-series II (Thermoelemental, Winsford UK) with a concentric glass nebuliser and conical spray chamber. RF power was set at 1400 W and coolant, auxiliary, nebuliser and collision cell gas flows rates were 13 L Ar min^{-1}, 0.70 L Ar min^{-1}, 0.85 L Ar min^{-1} and 3.5 mL 7% H_2 in He min^{-1}, respectively. The instrument was calibrated using 4 standards and a blank made up in either 0.1 M HNO_3 or 3 mM EDTA, and a standard was analysed after every ten samples in order to check for any drift in instrument sensitivity. Data were acquired over a dwell period of 10 ms, with 50 sweeps per reading and three replicates. Limits of detection and quantification, based on 3 σ and 10 σ arising from multiple measurements of the different blanks, ranged from about 0.05 to 0.2 $\mu g\ L^{-1}$ and 0.06 to 0.3 $\mu g\ L^{-1}$, respectively.

3. Results

3.1. Bi in controls and recovery in exposures

Concentrations of Bi in unamended sea water were close to or below the limits of detection of the ICP and have been neglected during data treatment. Bismuth concentrations digested by acid and extractable by EDTA in control algae were above detection limits (and up to 0.2 $\mu g\ g^{-1}$ on a dry weight basis) and have, therefore, been subtracted from the corresponding results arising from the exposures.

Because of the tendency of Bi to adsorb to container surfaces (Bertine et al., 1996), the recovery of Bi added to the exposures was determined by comparing the summed concentrations of the metal in each phase (sea water, algal extract and algal digest) with the corresponding concentrations added from the working stock. Recovery averaged about 70% overall, but was highly variable and displayed no clear differences among the different macroalgae or consistent trends with concentration of Bi. Acid rinses of exposure beakers after the residual contents had been discarded revealed that up to 20% of Bi had undergone progressive adsorption to the interior surfaces of the containers throughout the exposures and that about 80-90% of the metal was now accounted for. Although the nature and means of loss of remaining Bi are unknown (possibilities include strong adsorption to containers that could not be recovered by acid rinsing and loss to flasks used to prepare working stock solutions), it is important that concentrations in the current study were presented and treated as measured rather than as nominal.

3.2. Chlorophyll fluorescence quenching

The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) as a measure of the quantum efficiency of PSII photochemistry is shown for the three species of macroalga and as a function of Bi concentration in Figure 1. Note here that concentrations of Bi on the *x*-axis represent those measured at the end of the 48-h exposures and as computed from the summed concentrations of Bi in sea water and in the alga. For *U. lactuca* and *F. vesiculosus*, values of F_v/F_m are about 0.7 in the absence of added Bi, and display no significant differences ($p > 0.05$ according to one-way ANOVA) in the presence of Bi up to concentrations of about 30 μg L^{-1}. For *C. crispus*, F_v/F_m in the Bi-free control was lower than that for *U. lactuca* and *F. vesiculosus* (about 0.5). It is unclear why this is the case but we note similar values reported in the literature for a variety of red macroalgae, including *C. crispus*, maintained under

laboratory conditions (Dummermuth et al., 2003; Baumann et al., 2009). In the presence of Bi, F_v/F_m results for *C. crispus* are more variable and at the highest two concentrations measured (25 to 30 μg L^{-1}) there was a significant ($p < 0.05$) reduction in photosynthetic capacity compared to the control of about 30%.

3.3. Bi accumulation

The dry weight concentrations of Bi accumulated by *U. lactuca*, *F. vesiculosus* and *C. crispus* over the 48-h exposure period are shown as a function of Bi concentration measured in sea water, $[Bi_{aq}]$, in Figures 2, 3 and 4, respectively. Bismuth extracted by EDTA from undried alga (but expressed on a dry weight basis), Bi_{ads}, affords a measure of adsorption at the surface of the cell wall of the alga, assuming that the product of the Bi-EDTA complex and the free ligand concentration exceeds the product of the constant defining Bi complexation at the algal surface and the concentration of surface binding sites (Hassler et al., 2004). (Although very little information exists on Bi complexation at biotic surfaces, log K_{BiEDTA}- is sufficiently large (= 26.7; Stavila et al., 2006) compared with values for metals for which the approach has been validated (log *K* typically 15-18) to justify this assumption.) Bismuth digested in the dried alga by boiling HNO_3 affords a measure of the metal that has been internalised by the organism, Bi_{int}, and total Bi accumulated, Bi_T, is the sum of adsorbed and internalised Bi:

$$[Bi_T] = [Bi_{ads}] + [Bi_{int}] \quad (1)$$

For each measure of Bi accumulation and for each alga, both non-linear (Freundlich and Langmuir) and linear sorption models were applied to the data. In all cases, data were best

249 defined (and with statistical confidence; $p < 0.05$) by a linear isotherm that intersected the
250 origin:
251
252 $[Bi_{ads}] = K_{ads}[Bi_{aq}].10^{-3}$ (2a)
253
254 $[Bi_{int}] = K_{int}[Bi_{aq}] .10^{-3}$ (2b)
255
256 $[Bi_T] = AF[Bi_{aq}] .10^{-3}$ (2c)
257
258 where 10^{-3} is a unit conversion factor and AF represents a net accumulation factor, K_{ads} an
259 adsorption constant and K_{int} an internalisation constant. Constants derived from the gradients
260 of linear fits to the data are given in Table 1 along with the percentages of Bi adsorbed and
261 internalised for each macroalga and as derived from K_{ads}/AF and K_{int}/AF, respectively. These
262 constants reveal that net accumulation is in the order: *F. vesiculosus* > *C. crispus* > *U.*
263 *lactuca*; and that percentage adsorption is about 90 and greatest (or internalisation about 10
264 and lowest) for the fucoid.
265
266 **4. Discussion**
267 The ability of Bi(III) to interact with macroalgae, coupled with its affinity for container
268 surfaces, indicates that there is at least one reactive form of the aqueous metal in seawater.
269 Although Bi^{3+} has a higher affinity for chloride than Pb^{2+}, its period 6 neighbour, a larger
270 charge-radius ratio ensures much stronger hydrolysis with the result that $Bi(OH)_3^0$ is predicted
271 to be the dominant inorganic species over a broad pH range (Ure and Davidson, 2008). While
272 this form is able to undergo adsorption to biotic and abiotic surfaces (Fowler et al., 2010), a
273 review of thermodynamic constants for Bi reported in the literature suggests that a number of

oxy, hydroxyl and oxychloro complexes may also occur in sea water, including the two cationic species, BiO^+ and $Bi(OH)_2^+$ (Filella, 2010). No constants exist for Bi binding to heterogeneous ligands, but its removal in the upper layers of the ocean, intermediate status in the HSAB (Hard Soft Acid-Base) classification and ability to interact with metallothioneins and other biomolecules suggest that organic complexation is likely to be significant.

Although a few measurements of Bi in marine macrophytes (including macroalgae) have been reported previously (Bertine et al., 1996; Richir and Gobert, 2014), the present study appears to be the first to address the nature, mechanisms and effects of Bi uptake by seaweeds. Linear isotherms indicate that both extracellular accumulation (adsorption) and intracellular accumulation (internalisation) are proportional to the concentration of external, aqueous Bi over the range of concentrations tested, and suggest that the corresponding constants derived from data fitting are applicable to environmentally realistic levels of the metal. Extracellular adsorption likely involves ion exchange and complexation with surface groups of the cell wall, and in particular carboxyl and amino groups, while intracellular accumulation may be passive and diffusive or active and metabolically-dependent. Among the seaweeds studied, the order of Bi adsorption and accumulation (*F. vesiculosus* > *C. crispus* > *U. lactuca*) is consistent with more general results derived from biosorption studies employing a variety of metal ions and different macroalgae (Sanchez-Rodriguez et al., 2001; Hashim and Chu, 2004; Brinza et al., 2007; Murphy et al., 2007). Thus, greatest sorption by *F. vesiculosus* may be attributed to the abundance of cell wall polysaccharides and extracellular polymers on brown seaweeds, and in particular on fucoids (Davies et al., 2003), while greater sorption to *C. crispus* than *U. lactuca* results from the presence of additional gelifying sulphated polysaccharides in certain rhodophytes (Romero et al., 2007).

Despite qualitative consistency with the biosorption literature on other metals, the extent of Bi adsorption by living macroalgae is not a useful predictor of its propensity to internalise. For example, among the algae studied greatest adsorption by *F. vesiculosus* is accompanied by the lowest internalisation, in terms of both the percentage and absolute concentration of intracellular Bi, while *C. crispus* exhibited the greatest internalisation according to both measures; as a quantitative comparison, the highest concentration of added Bi resulted in intracellular concentrations of 2.1 μg g^{-1} and 13.7 μg g^{-1} for *F. vesiculosus* and *C. crispus*, respectively. The degree of internalisation is, presumably, related to the ability of Bi to cross the cell membrane and bind with intracellular ligands, including protein carboxyl groups and –SH residues, processes that *C. crispus* appears to facilitate more effectively than either *F. vesiculosus* or *U. lactuca*. A consequence of the relatively high degree of internalisation exhibited by *C. cripsus* is a toxic response in terms of photosynthetic activity at the two highest concentrations of added Bi. Thus, here, it is possible that there exists an excess of intracellular Bi that is able to interact with specific biomolecules. The modes and mechanisms by which Bi may interfere with processes at the cellular level in plants are unknown, but in human cells Bi^{3+} ions are believed to replace catalytic or structural metals, like Fe, Ni and Zn (Sadler et al., 1999).

The phytotoxicity of Bi relative to that of other heavy metals may be evaluated by consulting similar exposure studies that have employed different metals. Specifically, we have studied chlorophyll fluorescence quenching, but without dark adaption (= $\Delta F/F_{m'}$), of *U. lactuca* exposed to Tl(I) and Ag over a 48-h period and using a similar range in metal concentrations (Turner and Furniss, 2012; Turner et al., 2012). Thus, while Bi failed to elicit a toxic response to this macroalga up to concentrations of about 30 μg L^{-1} (~ 140 nM), or about 15 μg g^{-1} (~ 70 nmol g^{-1}) on an accumulated dry weight basis, both Tl and Ag (as HSAB "soft" acids)

exhibited significant internalisation and caused measurable reductions in fluorescence quenching. For Tl, toxicity was observed at about 10 μg L^{-1} (~ 50 nM), or about 10 μg g^{-1} (~ 50 nmol g^{-1}) on an accumulation basis, and by 25 μg L^{-1} (~ 120 nM) $\Delta F/F_{m'}$ had reduced to about 25% of the control value. For Ag, a significant, progressive reduction in $\Delta F/F_{m'}$ was observed relative to the control from 2.5 μg L^{-1} to 30 μg L^{-1} (~ 23 nM to 280 nM), or about 30 μg g^{-1} to 100 μg g^{-1} (280 nmol g^{-1} to 900 nmol g^{-1}) on an accumulation basis, with a minimum value of $\Delta F/F_{m'}$ that was about 60% of the control.

Based on Bi AF values reported here and volume concentration factors cited for phytoplankton (between about 10^5 and 10^7; Fowler et al., 2010), we infer that macroalgae are less efficient accumulators of Bi than plankton. This is, presumably, because of the significantly smaller size and greater surface area for sorption of the latter. However, we note that a comparison of the (background) concentrations of Bi in coastal macrophytes with those of Rh, a trivalent metal that is considerably less reactive than Bi, reveals little fractionation from sea water to algae, despite intense Bi-Rh fractionation from sea water to mineral phases and sediments (Bertine et al., 1996). We also note that measurements of Rh uptake by the chlorophyte, *U. lactuca*, conducted under experimental conditions similar to those presented herein in terms of timescale and metal concentration, reveal both an AF (~ 1,400 ml g^{-1}) and percentage internalisation (~ 40%) that are greater than respective values for Bi (Turner et al., 2007). In summary, it appears that Bi has an intrinsic affinity for macroalgae that is rather low compared with its affinity for other biotic and abiotic surfaces, possibly because of the abundance of "hard" (HSAB) functional groups on the macroalgal surface coupled with an "intermediate" classification of Bi according to HSAB theory.

5. Conclusions

In smmary, Bi accumulation by macroalgae is proportional to the concentration of aqueous metal and the order of accumulation by the chlorophyte, phaeophyte and rhodophyte is qualitatively consistent with the order displayed by other heavy metals and with the surface functionalities of each alga. Internalisation of Bi, as evaluated by EDTA extraction, was low compared with other metals and only resulted in a toxic response (as chlorophyll fluorescence quenching) for *C. crispus* at the highest exposure concentrations employed. While only moderately toxic, relatively high extracellular Bi on macroalga suggests that the metal is likely to be readily available to consumers and for accumulation at higher trophic levels.

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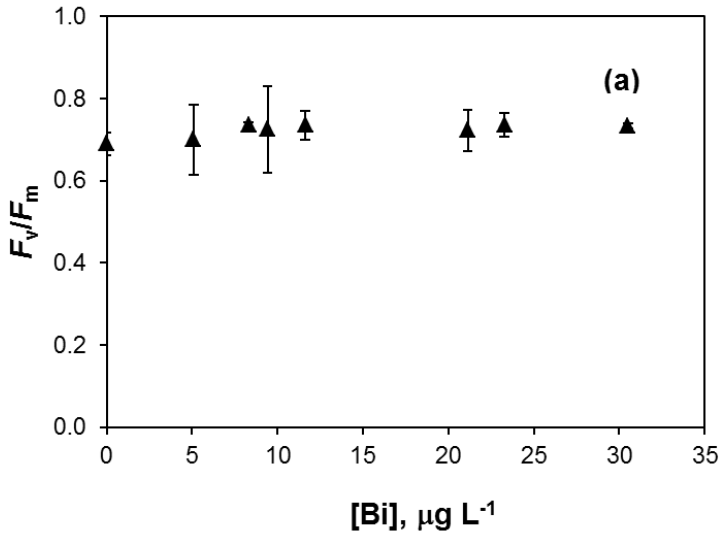
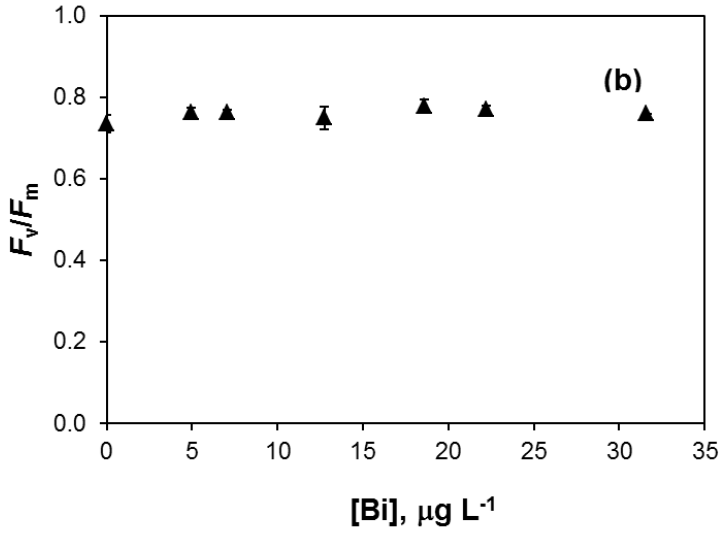
460 Figure 1: The ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) for (a) *U. lactuca*,
461 (b) *F. vesiculosus* and (c) *C. crispus* exposed to different concentrations of Bi. Errors denote
462 the one standard deviation about the mean of three independent measurements (note that *x*-
463 axis error bars are not shown for clarity) and asterisks denote a significant ($p < 0.05$)
464 difference from the corresponding control.


(a)
F_v/F_m
[Bi], μg L^{-1}


(b)
F_v/F_m
[Bi], μg L^{-1}

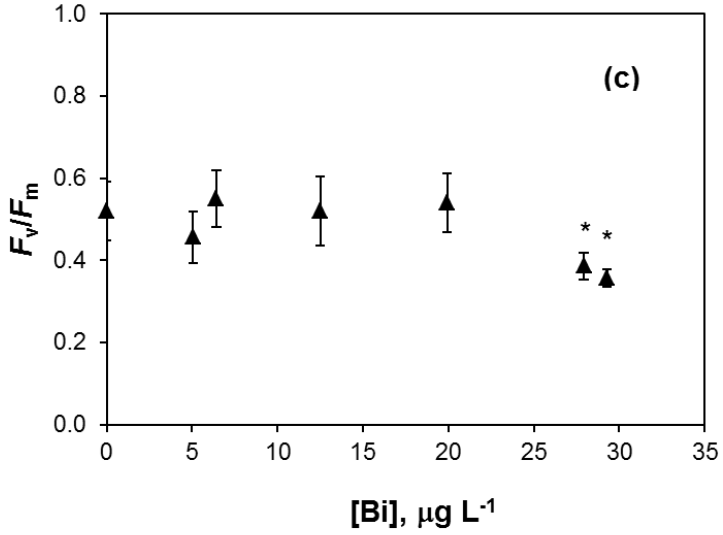

(c)
F_v/F_m
[Bi], μg L^{-1}

Figure 2: Dry weight concentrations of (a) total, (b) adsorbed and (c) internalised Bi as a function of aqueous Bi for the exposures involving *U. lactuca*. Lines denote best fits to the data according to linear regression analysis.

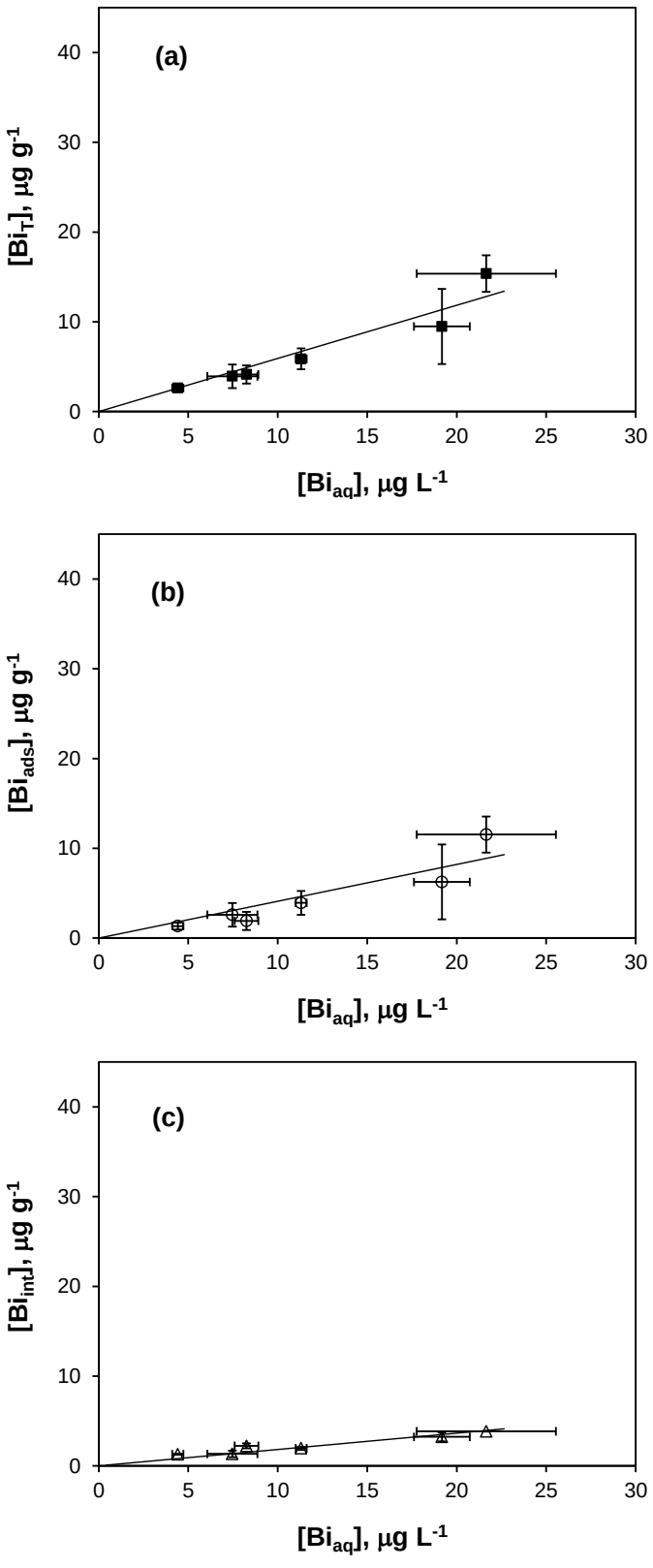


Figure 3: Dry weight concentrations of (a) total, (b) adsorbed and (c) internalised Bi as a function of aqueous Bi for the exposures involving *F. vesiculosus*. Lines denote best fits to the data according to linear regression analysis.

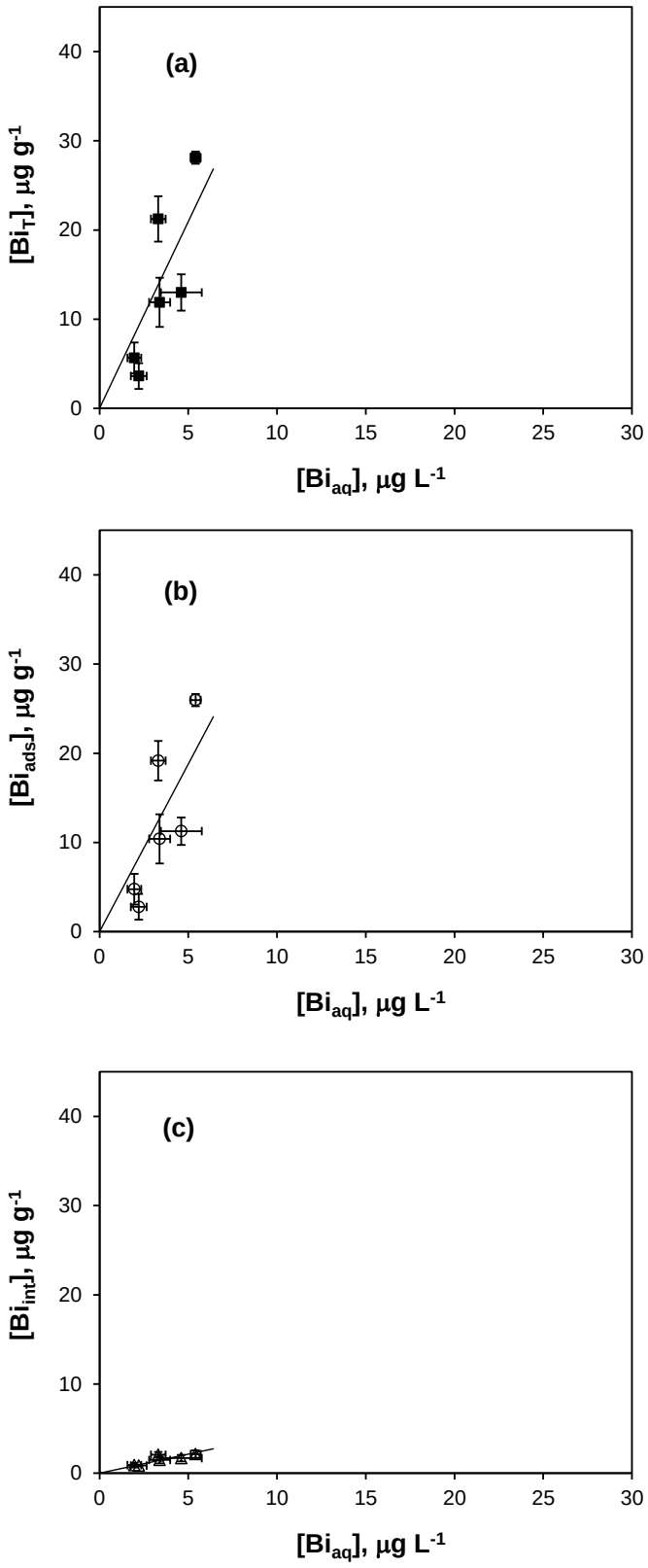


Figure 4: Dry weight concentrations of (a) total, (b) adsorbed and (c) internalised Bi as a function of aqueous Bi for the exposures involving *C. crispus*. Lines denote best fits to the data according to linear regression analysis.

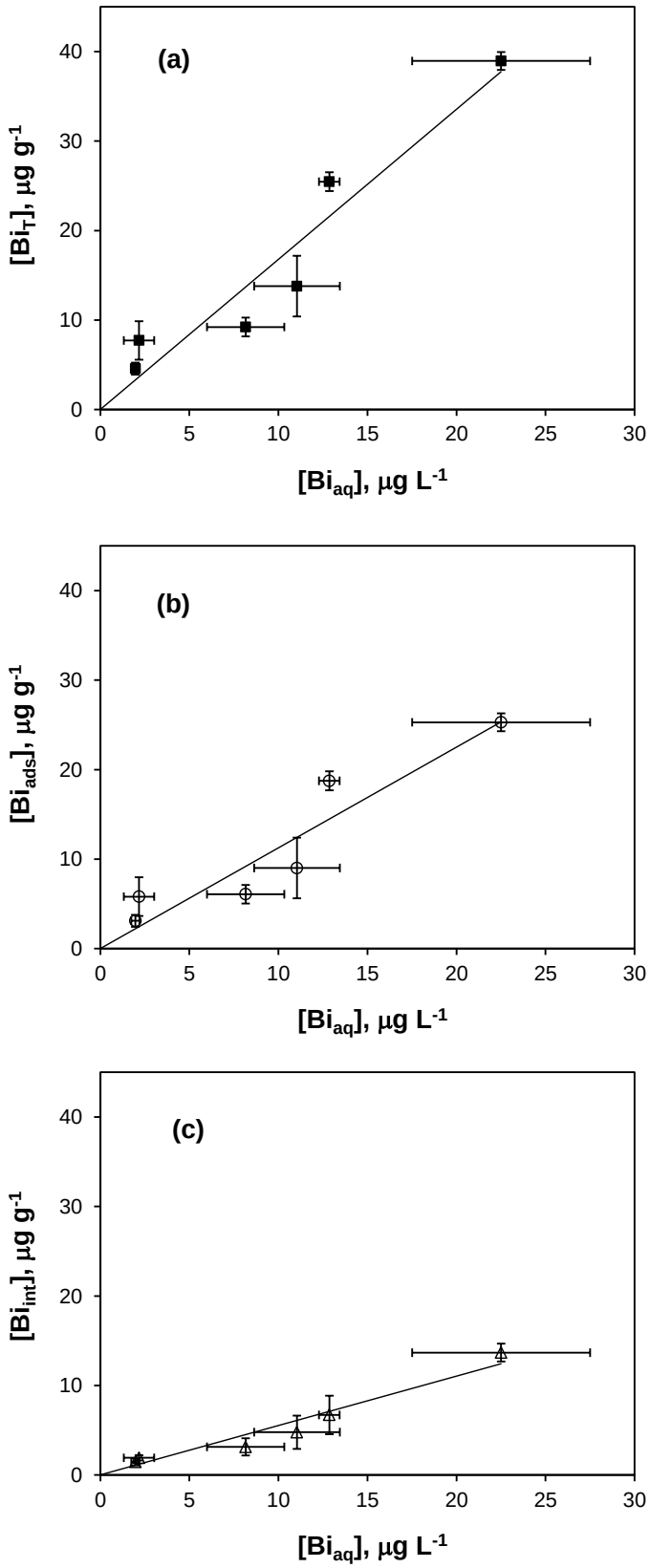


Table 1: Constants derived from regression analysis of the adsorption, internalisation and accumulation data for Bi (Figures 2-4).

	net accumulation		adsorption			internalisation		
macroalga	AF, L kg^{-1}	r^2	K_{ads}, L kg^{-1}	r^2	% adsorbed	K_{int}, L kg^{-1}	r^2	% internalised
U. lactuca	592	0.899	410	0.825	69.3	182	0.899	30.7
F. vesiculosus	4190	0.616	3760	0.596	89.7	427	0.644	10.3
C. crispus	1680	0.911	1120	0.864	67.1	551	0.943	32.9