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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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25 **Abstract**

26 Bismuth is a heavy metal whose biogeochemical behaviour in the marine environment is
27 poorly defined. In this study, we exposed three different species of macroalgae (the
28 chlorophyte, *Ulva lactuca*, the phaeophyte, *Fucus vesiculosus*, and the rhodophyte, *Chondrus*
29 *crispus*) to different concentrations of Bi (up to 50 µg L⁻¹) under controlled, laboratory
30 conditions. After a period of 48-h, the phytotoxicity of Bi was measured in terms of
31 chlorophyll fluorescence quenching, and extracellular and intracellular accumulation of Bi
32 determined after EDTA extraction and acid digestion, respectively. For all algae, both the
33 internalisation and total accumulation of Bi were proportional to the concentration of aqueous
34 metal. Total accumulation followed the order: *F. vesiculosus* > *C. crispus* > *U. lactuca*; with
35 respective accumulation factors of about 4,200, 1,700 and 600 L kg⁻¹, and greatest
36 internalisation (about 33% of total accumulated Bi) was exhibited by *C. crispus*, the only
37 macroalga to display a toxic response in the exposures. A comparison of the results with those
38 reported in the literature suggests that Bi accumulation by macroalgae is significantly lower
39 than its accumulation by marine plankton (volume concentration factors of 10⁵ to 10⁷), and
40 that Bi phytotoxicity to macroalgae is low relative to other heavy metals like Ag and Tl.

41

42 **Capsule**

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

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

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51 **1. Introduction**

52

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

67

68 The increasing usage of Bi in industry and as a “safe” replacement for Pb in many consumer
69 products has been accompanied by the realisation that very little is known about its behaviour
70 and impacts in the environment. For example, a recent review of thermodynamic constants for
71 Bi reported in the literature revealed such a lack of data validation and variety of
72 inconsistencies and errors that inorganic aqueous speciation cannot be stated with confidence
73 (Filella, 2010). With regard to toxicity, published studies appear to be limited to those that
74 define the acute and chronic effects of Bi shotshell on waterfowl and game birds (the results
75 of which ultimately led to the approval of the product; Fahey and Tsuji, 2006) and the

76 nanotoxicity of Bi-asparagine coordination polymer spheres on zebrafish embryos (He et al.,
77 2013).

78

79 With respect to the marine environment, the principal source of Bi is the atmosphere via
80 volcanic emissions and fossil fuel combustion (Lee et al., 1985/1986). The limited oceanic
81 profiles available indicate surface enrichment from the atmosphere, removal in the mixed
82 layer, regeneration at intermediate depths, and intense scavenging in deeper waters. The
83 strong particle reactivity of Bi in the deep ocean results in enrichment in ferromanganese
84 phases and hydrothermal sulphides (Bertine et al., 1996) and a residence time of only about
85 20 years (Lee et al., 1985/1986). Radiotracer experiments conducted by Fowler et al. (2010)
86 using ^{207}Bi indicate significant accumulation by phytoplankton, with volume concentration
87 factors, VCF, between about 10^5 and 10^7 ; copepods consuming plankton were able to
88 assimilate 4% of ^{207}Bi with the remainder voided in fecal pellets (the latter also acted as
89 strong scavengers of aqueous ^{207}Bi).

90

91 In the present study, and to improve our understanding of the behaviour of Bi in the coastal
92 marine environment, we study its accumulation by and toxicity to macroalgae that are
93 exposed to variable concentrations of the metal under controlled laboratory conditions.

94 As well as playing an important role in the nutrient dynamics of near-shore systems,
95 macroalgae readily reflect changes in water quality, a trait that is widely employed to monitor
96 and characterise coastal contamination and in particular that arising from metals (Baumann et
97 al., 2009; Malea et al., 2015). Providing habitat and sustenance to a variety of organisms,
98 macroalgae can also influence the accumulation of contaminants at higher trophic levels.

99 We selected three species of seaweed that are commonly encountered on rocky shores and the
100 sublittoral zones of north western Europe; namely: *Ulva lactuca* (Chlorophyta), *Chondrus*

101 *crispus* (Rhodophyta), and *Fucus vesiculosus* (Phaeophyta). Since green, red and brown
102 seaweeds contain different surface functional groups and different pigments for capturing
103 different wavelengths of light, we would expect to see differences in both the accumulation
104 and phytotoxicity of Bi among the species selected. We employ chlorophyll fluorescence
105 quenching as a rapid, non-invasive measure of toxicity, and discriminate Bi that is adsorbed to
106 the cell walls from Bi that is internalised by means of an EDTA extract.

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108

109 **2. Materials and Methods**

110

111 *2.1. Sampling and sample preparation*

112 Coastal sea water of salinity 32, pH 8.0 and dissolved organic carbon concentration of about
113 100 μM was used for culturing and experimental work. Sea water was collected in bulk from
114 Plymouth Sound (UK) at high water and was piped to the laboratory under gravity and after
115 filtration through a 0.6 μm extruded carbon filter.

116

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

126

127 Prior to the exposures, macroalgae were cut into smaller, working samples that were
128 acclimatised for a period of 24 h in new aquaria but under the conditions described above. For
129 *U. lactuca*, the sharpened end of a 30 mm diameter polyethylene cylinder was used to cut
130 discs from the central portions of the thalli (dry weights of discs averaged 23.1 mg); fronds of
131 *F. vesiculosus* (without air bladders) and *C. crispus* were cut to lengths of about 35 mm and
132 30 mm, respectively, using a stainless steel scalpel (respective dry weights of fronds averaged
133 87.4 and 53.2 mg).

134

135 2.2. *Experimental*

136 For each macroalga, exposures were performed in triplicate and in 100 ml aliquots of sea
137 water in a series of sterilised 150 ml polyethylene terephthalate beakers that had been rinsed
138 twice with the exposure medium. In separate beakers, Bi was added to concentrations of 0, 5,
139 10, 20, 40 and 50 $\mu\text{g L}^{-1}$ from a stock solution of 1 mg L^{-1} Bi in distilled water that had been
140 prepared immediately before use by serial dilution of a 10 g L^{-1} BDH “Aristar” solution of
141 Bi(III) in 1.6 M HNO_3 . (Note that serial dilution was not performed in acid in order to
142 minimise any pH changes of the exposure medium.) A single algal disc or frond tip was then
143 added to each beaker using a pair of plastic tweezers before beakers were loosely covered
144 with their lids and agitated on a Heidolph Unimax 2010 orbital shaker at 100 rpm for 48 h.

145

146 At the end of the exposures, 1 ml water samples for Bi analysis were pipetted from each
147 beaker into individual 30 ml screw-capped polypropylene tubes containing 9 ml of 0.1 M
148 HNO_3 (Fisher Chemical TraceMetalTM Grade). Discs or frond tips were retrieved using
149 tweezers and shaken gently to remove excess sea water before being measured for
150 fluorescence quenching and extracted-digested for accumulated Bi (see below). Meanwhile,

151 and in order to evaluate loss of Bi to the container surfaces, selected beakers whose remaining
152 contents had been discarded were rinsed with 10 ml of 0.1 M HNO₃ for about 5 min before
153 rinsates were transferred to 30 ml polypropylene tubes pending analysis.

154

155 *2.3. Chlorophyll fluorescence measurements*

156 Exposed algal samples were placed in a series of Hansatech Handy PEA leaf clips with closed
157 shutter plates for 20 min in order to ensure algal reaction centres were fully oxidised and any
158 chlorophyll fluorescence yield fully quenched. Leaf clips were then placed individually on a
159 Hansatech Pocket PEA chlorophyll fluorimeter and algae were exposed to a single high
160 intensity beam of excitation light (up to 3,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a peak wavelength of 627
161 nm). Fluorescence origin and maximum fluorescence yield, F_0 and F_m , respectively, were
162 measured, and results expressed as the effective quantum yield of PS II and in terms of the
163 ratio of variable to maximum chlorophyll fluorescence ($F_v/F_m = [F_m - F_0]/F_m$).

164

165 *2.4. Algal extraction and digestion*

166 After measuring chlorophyll fluorescence, discs or fronds were immersed, individually, in 20
167 ml of 3 mM EDTA (Fisher Chemical) in 0.6 M NaCl (Sigma Aldrich) in a series of acid-
168 cleaned Pyrex beakers in order to extract Bi adsorbed to the algal surface. After 15 min,
169 solutions were transferred to individual 30 ml polypropylene tubes pending analysis while the
170 discs or fronds were placed in individual specimen bags before being frozen and dried for 24
171 h in an Edwards Super Modulyo freeze dryer. Dried algae were weighed using an Oxford A
172 Series A2204 balance and then digested for 50 min in 5 ml of concentrated, boiling HNO₃
173 (Fisher Chemical TraceMetal™ Grade) in a series of 25 ml, acid-cleaned Pyrex beakers
174 covered with watch glasses and on a hot plate. Digests were made up to 25 ml in a volumetric

175 flask with distilled water before being transferred to a series of polypropylene tubes pending
176 analysis.

177

178 *2.5. Bi analysis*

179 Diluted-acidified sea water samples and algal digests and extracts were analysed for ²⁰⁹Bi by
180 collision cell-inductively coupled plasma-mass spectrometry (ICP-MS) using a Thermo X-
181 series II (ThermoElemental, Winsford UK) with a concentric glass nebuliser and conical spray
182 chamber. RF power was set at 1400 W and coolant, auxiliary, nebuliser and collision cell gas
183 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% H₂ in He
184 min⁻¹, respectively. The instrument was calibrated using 4 standards and a blank made up in
185 either 0.1 M HNO₃ or 3 mM EDTA, and a standard was analysed after every ten samples in
186 order to check for any drift in instrument sensitivity. Data were acquired over a dwell period
187 of 10 ms, with 50 sweeps per reading and three replicates. Limits of detection and
188 quantification, based on 3 σ and 10 σ arising from multiple measurements of the different
189 blanks, ranged from about 0.05 to 0.2 $\mu\text{g L}^{-1}$ and 0.06 to 0.3 $\mu\text{g L}^{-1}$, respectively.

190

191

192 **3. Results**

193 *3.1. Bi in controls and recovery in exposures*

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

199

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

213

214 3.2. Chlorophyll fluorescence quenching

215 The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) as a measure of the
216 quantum efficiency of PSII photochemistry is shown for the three species of macroalga and as
217 a function of Bi concentration in Figure 1. Note here that concentrations of Bi on the x -axis
218 represent those measured at the end of the 48-h exposures and as computed from the summed
219 concentrations of Bi in sea water and in the alga. For *U. lactuca* and *F. vesiculosus*, values of
220 F_v/F_m are about 0.7 in the absence of added Bi, and display no significant differences ($p >$
221 0.05 according to one-way ANOVA) in the presence of Bi up to concentrations of about 30
222 $\mu\text{g L}^{-1}$. For *C. crispus*, F_v/F_m in the Bi-free control was lower than that for *U. lactuca* and *F.*
223 *vesiculosus* (about 0.5). It is unclear why this is the case but we note similar values reported
224 in the literature for a variety of red macroalgae, including *C. crispus*, maintained under

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

229

230 3.3. Bi accumulation

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

244

$$245 \quad [\text{Bi}_{\text{T}}] = [\text{Bi}_{\text{ads}}] + [\text{Bi}_{\text{int}}] \quad (1)$$

246

247 For each measure of Bi accumulation and for each alga, both non-linear (Freundlich and
248 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

$$252 \quad [\text{Bi}_{\text{ads}}] = K_{\text{ads}}[\text{Bi}_{\text{aq}}] \cdot 10^{-3} \quad (2a)$$

253

$$254 \quad [\text{Bi}_{\text{int}}] = K_{\text{int}}[\text{Bi}_{\text{aq}}] \cdot 10^{-3} \quad (2b)$$

255

$$256 \quad [\text{Bi}_{\text{T}}] = \text{AF}[\text{Bi}_{\text{aq}}] \cdot 10^{-3} \quad (2c)$$

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 $\text{Bi}(\text{OH})_3^0$ 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

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

279

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

298

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

316

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

324 exhibited significant internalisation and caused measurable reductions in fluorescence
325 quenching. For Tl, toxicity was observed at about $10 \mu\text{g L}^{-1}$ ($\sim 50 \text{ nM}$), or about $10 \mu\text{g g}^{-1}$ (\sim
326 50 nmol g^{-1}) on an accumulation basis, and by $25 \mu\text{g L}^{-1}$ ($\sim 120 \text{ nM}$) $\Delta F/F_m$ had reduced to
327 about 25% of the control value. For Ag, a significant, progressive reduction in $\Delta F/F_m$ was
328 observed relative to the control from $2.5 \mu\text{g L}^{-1}$ to $30 \mu\text{g L}^{-1}$ ($\sim 23 \text{ nM}$ to 280 nM), or about 30
329 $\mu\text{g g}^{-1}$ to $100 \mu\text{g g}^{-1}$ (280 nmol g^{-1} to 900 nmol g^{-1}) on an accumulation basis, with a minimum
330 value of $\Delta F/F_m$ that was about 60% of the control.

331

332 Based on Bi AF values reported here and volume concentration factors cited for
333 phytoplankton (between about 10^5 and 10^7 ; Fowler et al., 2010), we infer that macroalgae are
334 less efficient accumulators of Bi than plankton. This is, presumably, because of the
335 significantly smaller size and greater surface area for sorption of the latter. However, we note
336 that a comparison of the (background) concentrations of Bi in coastal macrophytes with those
337 of Rh, a trivalent metal that is considerably less reactive than Bi, reveals little fractionation
338 from sea water to algae, despite intense Bi-Rh fractionation from sea water to mineral phases
339 and sediments (Bertine et al., 1996). We also note that measurements of Rh uptake by the
340 chlorophyte, *U. lactuca*, conducted under experimental conditions similar to those presented
341 herein in terms of timescale and metal concentration, reveal both an AF ($\sim 1,400 \text{ ml g}^{-1}$) and
342 percentage internalisation ($\sim 40\%$) that are greater than respective values for Bi (Turner et al.,
343 2007). In summary, it appears that Bi has an intrinsic affinity for macroalgae that is rather low
344 compared with its affinity for other biotic and abiotic surfaces, possibly because of the
345 abundance of “hard” (HSAB) functional groups on the macroalgal surface coupled with an
346 “intermediate” classification of Bi according to HSAB theory.

347

348

349 **5. Conclusions**

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

358

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362

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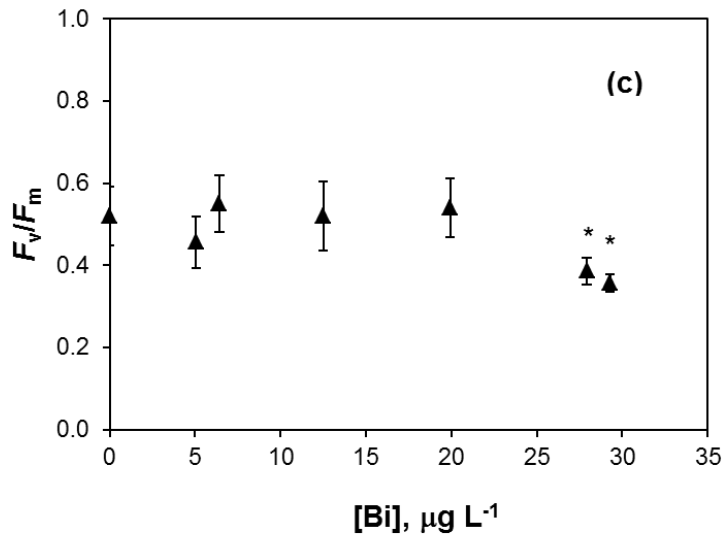
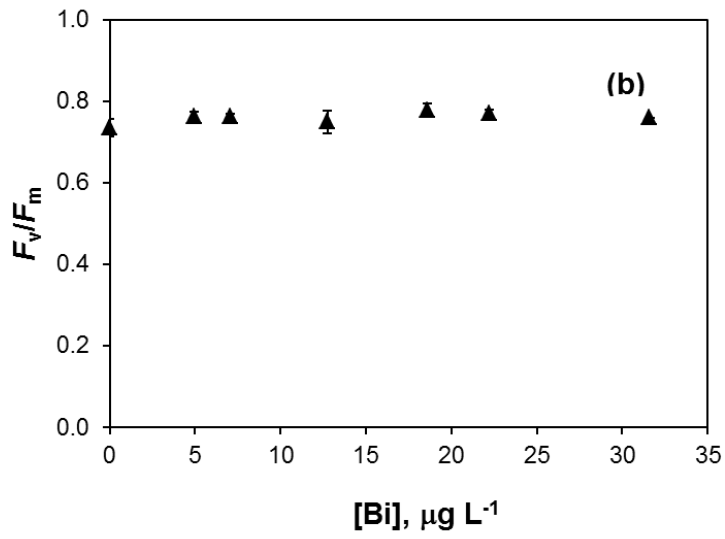
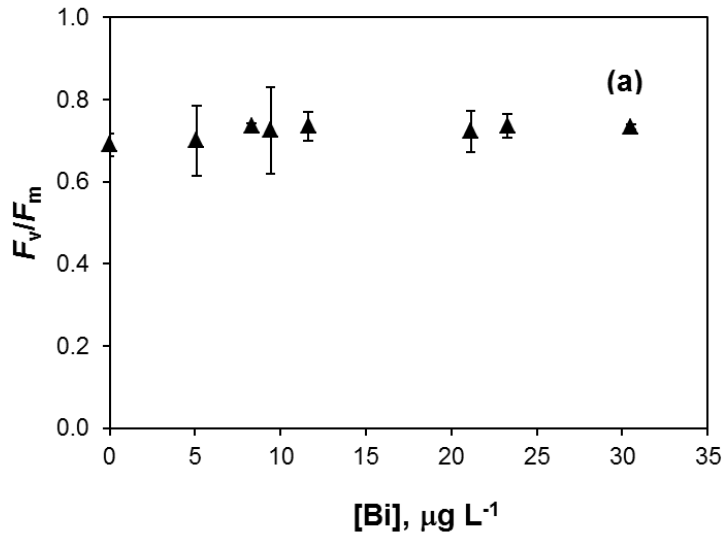
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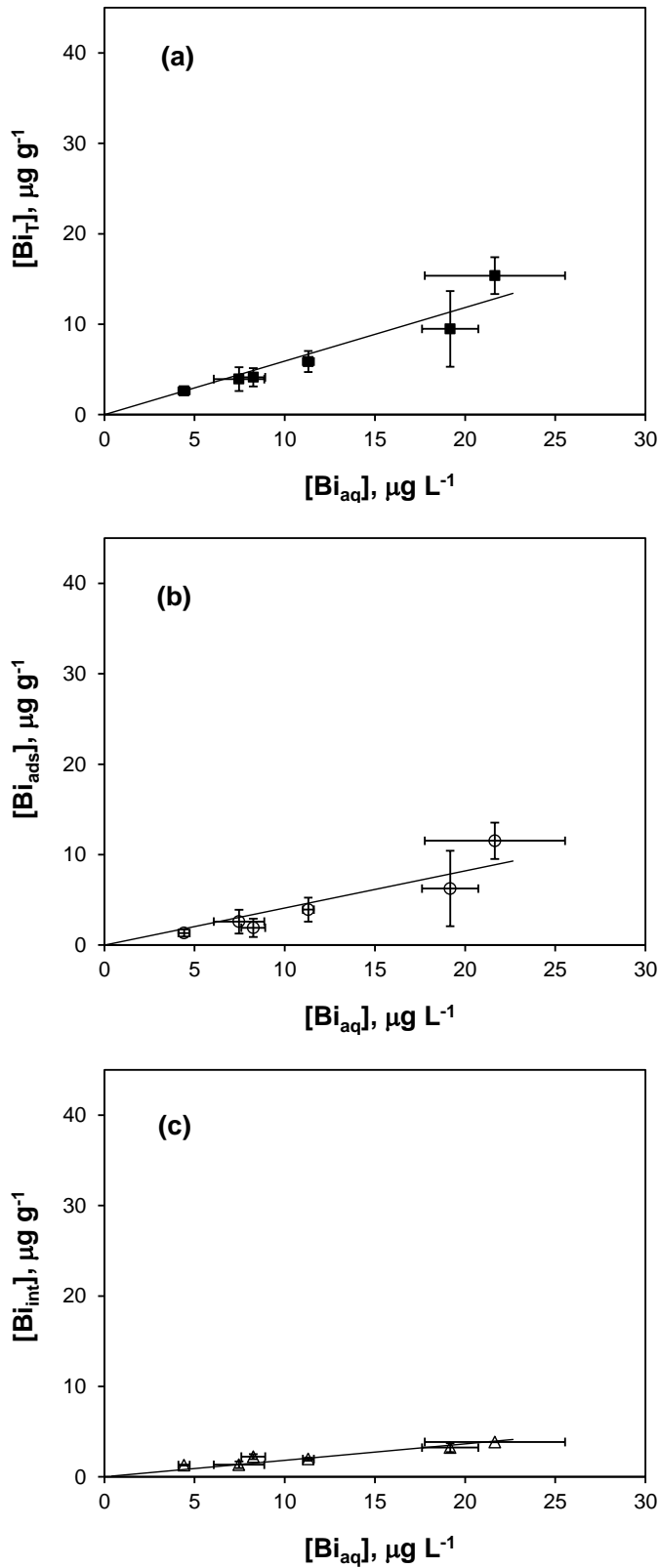
460 Figure 1: The ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) for (a) *U. lactuca*,
461 (b) *F. vesiculosus* and (c) *C. crispus* exposed to different concentrations of Bi. Errors denote
462 the one standard deviation about the mean of three independent measurements (note that x -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



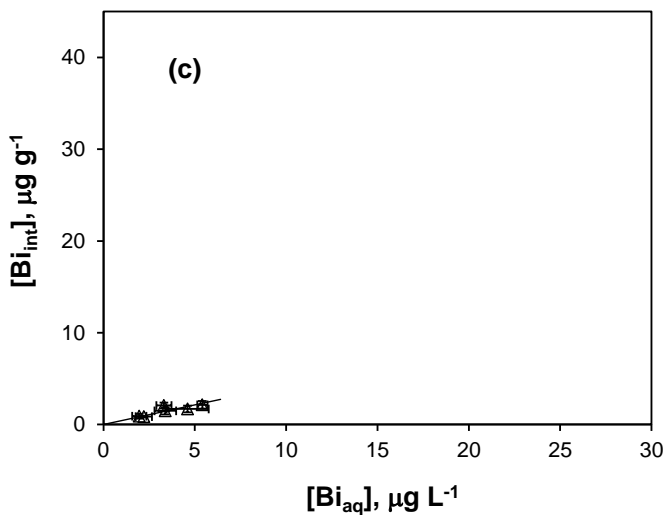
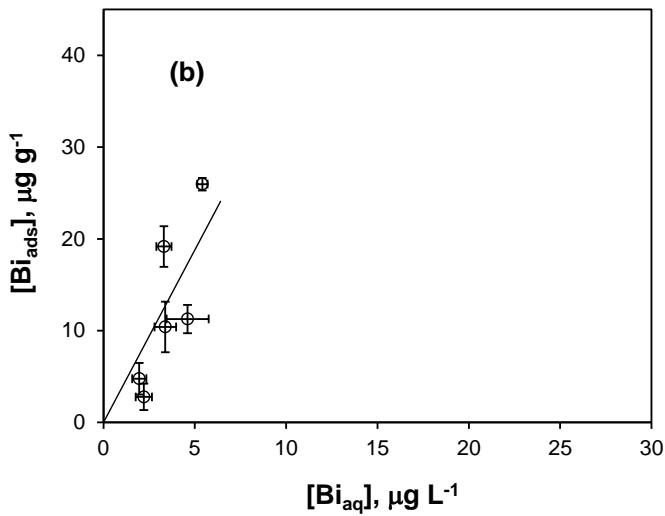
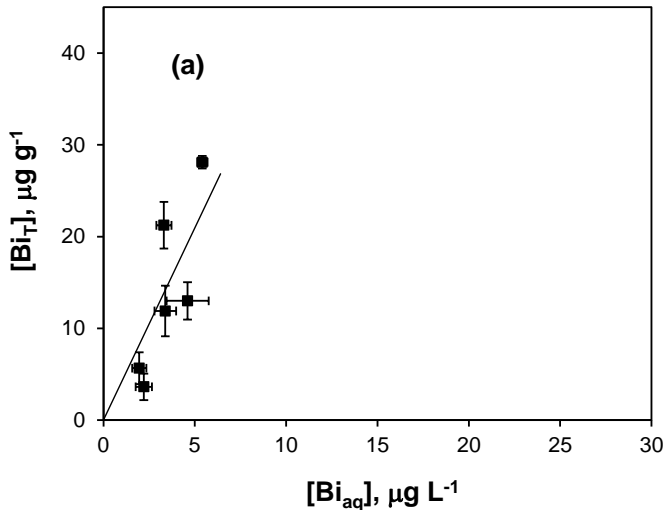
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470 Figure 2: Dry weight concentrations of (a) total, (b) adsorbed and (c) internalised Bi as a
471 function of aqueous Bi for the exposures involving *U. lactuca*. Lines denote best fits to the
472 data according to linear regression analysis.

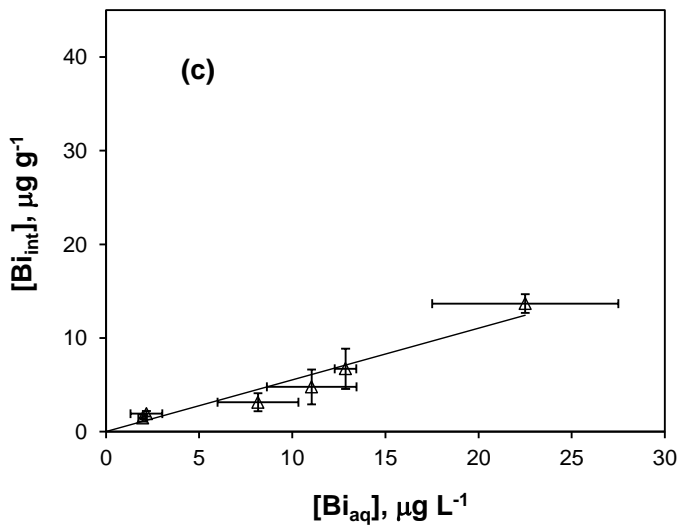
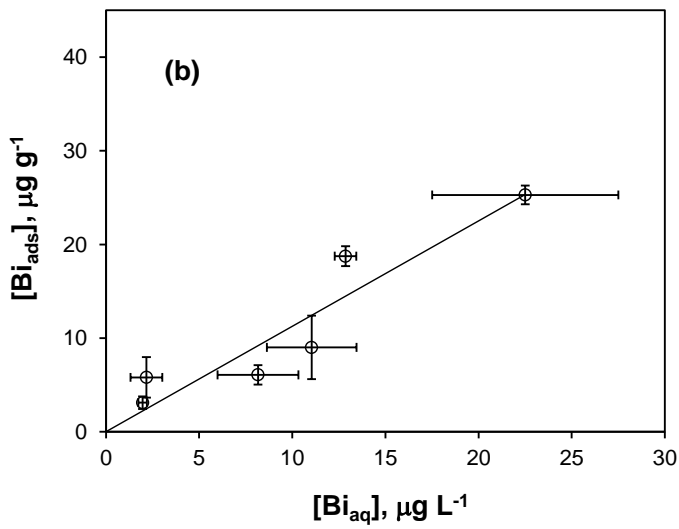
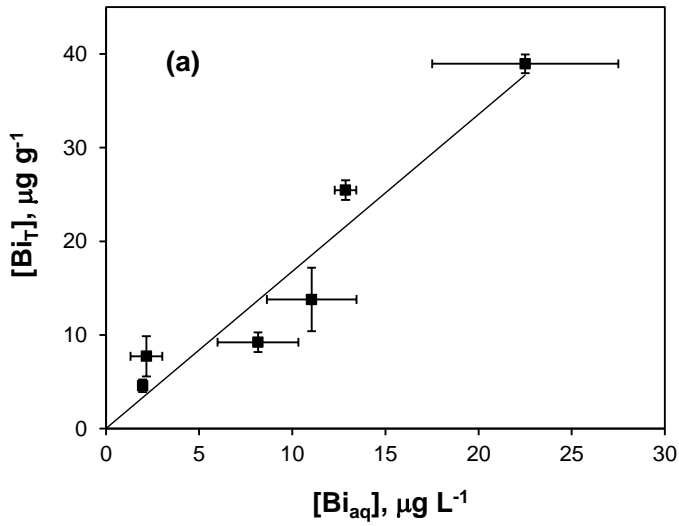
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517 Figure 3: Dry weight concentrations of (a) total, (b) adsorbed and (c) internalised Bi as a
518 function of aqueous Bi for the exposures involving *F. vesiculosus*. Lines denote best fits to
519 the data according to linear regression analysis.



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



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

611

612

613

614

net accumulation

adsorption

internalisation

615

616

617

macroalga

AF, L kg⁻¹

*r*²

*K*_{ads}, L kg⁻¹

*r*²

% adsorbed

*K*_{int}, L kg⁻¹

*r*²

% internalised

618

619

620

U. lactuca

592

0.899

410

0.825

69.3

182

0.899

30.7

621

F. vesiculosus

4190

0.616

3760

0.596

89.7

427

0.644

10.3

622

C. crispus

1680

0.911

1120

0.864

67.1

551

0.943

32.9

623

624

625