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**Novel use of field-portable-XRF
for the direct analysis of trace elements in marine
macroalgae**

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20 **Abstract**

21 Samples of dried marine macroalgae (*Fucus serratus*, *Palmaria palmata* and *Ulva lactuca*)
22 have been analysed for trace elements by a novel, non-destructive approach involving a
23 Niton field-portable-x-ray fluorescence (FP-XRF) spectrometer configured in a low density
24 plastics mode with thickness correction. Detection limits for a 200-second counting time
25 ranged from $< 5 \mu\text{g g}^{-1}$ for As and Pb in *F. serratus* and As in *P. palmata* to several tens of
26 $\mu\text{g g}^{-1}$ for Cd, Sb and Sn in all species tested. Arsenic, Cu, Pb and Zn were detected by the
27 XRF in samples collected from a protected beach ($n = 18$) and in samples therefrom that had
28 been exposed to additional aqueous elements in combination ($n = 72$) with concentrations
29 returned (in $\mu\text{g g}^{-1}$) ranging from 3.9 to 39.7 for As, 13.0 to 307 for Cu, 6.1 to 14.7 for Pb
30 and 12.5 to 522 for Zn. Independent measurements of trace elements in the macroalgae by
31 ICP-MS following nitric acid digestion revealed a direct and significant proportionality with
32 concentrations returned by the XRF, with slopes of the XRF-ICP relationships (As = 1.0; Cu
33 = 2.3; Pb = 2.4; Zn = 1.7) that can be used to calibrate the instrument for direct
34 measurements. The approach shows potential for the in situ monitoring of macroalgae in
35 coastal regions that is currently being investigated.

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38 **Keywords:** marine macroalgae; FP-XRF; arsenic; copper; zinc; lead

39 **1. Introduction**

40 With the miniaturisation of x-ray sources, reduction in battery power requirements, and
41 improvements in detector resolution, detection limits and fundamental parameter
42 calibrations, field-portable-x-ray fluorescence (FP-XRF) spectrometry has gained increasing
43 use for the rapid, cost-effective and non-destructive analysis of trace elements in
44 environmental solids over the past two decades (Bosco, 2013). Most publications have
45 described the analysis of dried and sieved or pulverised soils, tailings, dusts and sediments
46 (Radu and Diamond, 2009; Parsons et al., 2013; McComb et al., 2014), with many studies
47 extending the application for screening in situ (Higuera et al., 2012; Weindorf et al., 2012).
48 Recently, means of measuring trace elements by FP-XRF in low density environmental
49 particulates, like paints and plastics, have also been described and tested (Nakashima et al.,
50 2012; Turner et al., 2014; Ytreberg et al., 2015). Because polymers are composed of light
51 elements that are weak absorbers of x-rays, the intensity of characteristic fluorescent x-rays
52 is dependent, in part, on sample thickness (Piorek, 2004). To compensate for low density
53 samples that are thinner than a few mm, therefore, application of a thickness correction
54 algorithm based on measured sample thickness is an important, additional consideration in
55 the fundamental parameter XRF computations (Turner and Solman, 2016).

56

57 In the present study, we hypothesize that the XRF approach developed for use on plastics
58 and paints could be applied to the determination of trace elements in marine macroalgae,
59 whose compositional and thickness characteristics bear similarities to those of synthetic
60 polymeric films. Many species of marine macroalgae accumulate trace metals and metalloids
61 from sea water to concentrations several orders of magnitude greater than their environment
62 and serve as potentially useful sentinel organisms of local environmental contamination
63 (Varma et al., 2011; Reis et al., 2014; Malea et al., 2015). While conventional analysis of
64 macroalgae entails digestion of dried material in concentrated mineral acid and subsequent

65 analysis by, for example, anodic stripping voltammetry, atomic absorption spectrometry or
66 inductively coupled plasma (ICP) spectrometry, the throughput of multiple samples can be
67 time-consuming and labour-intensive. Here, therefore, we investigate the feasibility of a FP-
68 XRF spectrometer (Niton XL3t) calibrated for plastics and with thickness correction
69 capability for the analysis of a variety of trace metals and metalloids in dried samples of a
70 brown (*Fucus serratus*), red (*Palmaria palmata*) and green (*Ulva lactuca*) seaweed. As an
71 independent and comparative measure of the elemental content of the algae, we analyse
72 subsequently digested samples by ICP-mass spectrometry. Although the XRF study is
73 conducted in a bench-top accessory stand, we also discuss the potential for the approach to
74 be employed for in situ monitoring and screening of coastal and estuarine macroalgae.

75

76 **2. Materials and methods**

77 *2.1. Sampling and sample preparation*

78 Individuals of *Fucus serratus*, *Palmaria palmata* and *Ulva lactuca* were collected at low tide
79 during November 2015 from the intertidal rock pools at Wembury, a protected beach in
80 south Devon, SW England (50°19'03.8"N, 4°05'04.5"W). Samples were transported to the
81 Plymouth University laboratory in zip-locked polyethylene bags where they were washed in
82 a 1:9 solution of ethanol:sea water before surfaces were gently scraped with a polyethylene
83 spatula to remove particulate matter and epiphytes (Gledhill et al., 1998). Different species
84 were then grouped and transferred to ten-litre polyethylene aquaria containing aerated,
85 coastal sea water (salinity ~ 32; pH ~ 8.0) that had been collected in bulk from Plymouth
86 Sound and filtered through 0.6 µm extruded carbon. Samples were acclimated for three to
87 six days under an irradiance of 125 µmol m⁻² s⁻¹ on a 16:8 hour light:dark cycle at 14 ± 2 °C.
88
89 In a first experiment, three samples of each species were removed from the aquaria and cut
90 into two halves longitudinally. To compare drying method on XRF analysis (through

91 potential differences in sample integrity, flatness, smoothness and thickness), one half of
92 each sample was oven-dried at 80 °C for 24 h while the other half was frozen and freeze-
93 dried for 48 h using an Edwards Super Modulyo. These samples are hereafter referred to as
94 ‘baseline’ and contain ambient concentrations of metals and metalloids.

95

96 In a second experiment, 36 one-litre clear polyethylene tanks were filled with filtered sea
97 water. To 27 tanks, one of three concentrations of a combined solution of As, Cu and Zn was
98 added (the rationale for using these elements was based on results from the ‘baseline’
99 experiment and as discussed below). Specifically, a stock solution containing
100 $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (ReagentPlus®, Sigma-Aldrich) was used
101 to obtain respective concentrations of As, Cu and Zn of 5, 50 and 500 $\mu\text{g L}^{-1}$, 7.5, 75 and
102 750 $\mu\text{g L}^{-1}$ and 10, 100 and 1000 $\mu\text{g L}^{-1}$. Twelve individuals of each species were then
103 allocated to aquaria, with three replicates per treatment that included controls without
104 element addition. After seven days’ exposure under the acclimation conditions described
105 above, individuals were removed and cut in half longitudinally, with one half being oven-
106 dried and the other half freeze-dried. These samples, hereafter referred to as ‘exposed’, were
107 designed to contain a range of elevated concentrations of As, Cu and Zn representative of
108 more contaminated coastal environments.

109

110 2.2. *FP-XRF analysis*

111 In order to minimise confounding effects arising from differential accumulation of elements
112 by different parts of the macroalgae, sections from the mid-thallus were dissected from each
113 dried sample. Sections were analysed for trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se,
114 Sn and Zn) by energy dispersive FP-XRF using a battery-powered, field portable (1.3 kg)
115 Niton XRF analyser (model XL3t 950 He GOLDD+). The instrument employs an x-ray tube
116 with a silver anode operating at up to 50 kV and 200 μA as the source of sample excitation,

117 and is fitted with a geometrically optimised large area silicon drift detector to detect and
118 register characteristic fluorescent x-rays from the sample.

119

120 Elemental concentrations in macroalgal sections were determined using a low density
121 plastics mode by way of a fundamental parameters-based alpha coefficient correction model.

122 Because the intensity of fluorescent x-rays arising from low density materials is affected by

123 the depth of the sample, a thickness correction algorithm, employing a compensation for

124 mass absorption coefficient based on Compton scatter and calibrated down to 0.05 mm, was

125 also applied after sample thickness had been measured with digital callipers.

126

127 The XRF was used in the laboratory in a bench-top accessory stand (with the nose pointing
128 upwards) and was connected to a Fujitsu laptop computer via USB and a remote trigger.

129 Samples were placed on to a SpectraCertified Mylar polyester 3.6 μm film which was then

130 positioned such that the smoothest and flattest part of the sample lay directly and centrally

131 above the 8 mm XRF measurement window, a process aided by referring to real-time video

132 footage generated by an integrated CCD camera adjacent to the detector. To increase the

133 effective depth of the thinnest samples (mainly *Ulva*), sections were folded or cut and

134 stacked before being placed above the window. On closing the steel shield of the stand,

135 measurements with appropriate thickness correction were activated through the laptop for a

136 total period of 200 seconds; specifically, counting was performed for 100 seconds each in a

137 low energy range (20 kV and 100 μA) and main energy range (50 kV and 40 μA).

138 Decreasing counting time was found to reduce the number of cases in which elements were

139 detected while increasing counting time (up to 600 seconds) did not significantly increase

140 detectable cases but resulted in a reduction in counting error.

141

142 *2.3. Macroalgae digestion and analysis by ICP*

143 As an independent and more sensitive measure of the elemental composition of the
144 macroalgae, all baseline ($n = 18$) and exposed ($n = 72$) sample sections were subsequently
145 acid-digested and analysed by inductively coupled plasma-mass spectrometry (ICP-MS).
146 Thus, samples of about 0.1 g were accurately weighed into individual Teflon tubes to which
147 2 ml aliquots of HNO₃ (Fisher Chemical TraceMetal™ Grade) were added. The contents
148 were digested in a CCEM MARS 5 XPRESS microwave at 1600 W for 45 min before being
149 allowed to cool. Digests were then washed into individual 10 ml volumetric flasks and
150 diluted to mark with ultra-pure Millipore Milli-Q water. For an assessment of digestion
151 efficacy and analytical accuracy, a seaweed reference material (*Fucus vesiculosus*, ERM-
152 CD200; certified for As, Cd, Cu, Hg, Pb, Se and Zn) was digested in triplicate likewise.

153

154 Seaweed digests were analysed for elements that had been detected by XRF using a collision
155 cell-ICP-MS (Thermo X-series II, Thermoelemental, Winsford, UK) with a concentric glass
156 nebuliser and conical spray chamber. RF power was set at 1400 W and coolant, auxiliary,
157 nebuliser and collision cell gas flows rates were 13 L Ar min⁻¹, 0.70 L Ar min⁻¹, 0.72 L Ar
158 min⁻¹ and 3.5 mL 7% H₂ in He min⁻¹, respectively. The instrument was calibrated externally
159 using four standards prepared by dilutions of a QC 26 multi-element solution (CPI
160 International, Amsterdam) in 0.1 M HNO₃, and internally by the addition of 100 µg L⁻¹ of In
161 and Ir to all samples and standards. Data were acquired over a dwell period of 10 ms, with
162 50 sweeps per reading and three replicates.

163

164 2.4. Presentation, quality and analysis of data

165 Spectra arising from the XRF analyses were quantified by fundamental parameter
166 coefficients to yield elemental concentrations on a dry weight basis (in µg g⁻¹) and with a
167 counting error of 2σ (95% confidence) that were downloaded from the instrument to the
168 laptop using Niton data transfer (NDT) PC software. As a performance check of the FP-XRF

169 in plastics mode, a Niton reference polyethylene disc that had been impregnated with As,
170 Cd, Cr, Hg, Pb, Sb and Se at concentrations up to about 300 $\mu\text{g g}^{-1}$ (PN 180-619, LOT#T-18;
171 diameter = 31 mm, thickness = 13 mm) was analysed in triplicate. Measured concentrations
172 were within 10% of reference values for all elements present with the exception of Pb (15%).
173
174 Aqueous concentrations derived from the ICP-MS were converted to dry weight
175 concentrations (in $\mu\text{g g}^{-1}$) from the volume of diluted digest and mass of macroalga digested.
176 Limits of detection on this basis were $< 0.5 \mu\text{g g}^{-1}$ for all trace elements analysed, and
177 measured concentrations of elements certified in the reference macroalga were within 15%
178 of published values.

179
180 Minitab 17 was employed to establish differences in dry weight elemental concentrations
181 arising from the two methods of drying via a non-parametric Wilcoxon signed rank test, and
182 to determine differences in concentrations resulting from addition of elements in the exposed
183 experiments by one-way ANOVA. Correlations and linear regressions establishing
184 relationships between the two analytical approaches were performed in Microsoft Excel
185 2010.

186

187 **3. Results**

188 *3.1. FP-XRF detection limits*

189 The Niton XLT3t series of FP-XRF analysers calculates element-specific limits of detection
190 (LODs) that are dependent on the characteristics of the sample (e.g. composition and
191 thickness), counting time and mode of instrument application from counting errors
192 multiplied by 1.5 (that is, $2\sigma \times 1.5$, or 99.7% confidence interval). Indicative LODs for the
193 three species of seaweed, shown in Table 1, are based on mean counting errors arising from
194 the 200-second analysis of the oven-dried and freeze-dried baseline samples. Among the

195 algae, LODs are highest for *U. lactuca* and lowest for *F. serratus*, reflecting the sequence of
 196 increasing measured thickness and, presumably, primary x-ray absorption and secondary x-
 197 ray fluorescence. Among the trace elements, LODs are lowest for As, Cr and Pb and highest
 198 for Cd, Ni, Sb and Sn.

199

200 Table 1: Mean detection limits ($\mu\text{g g}^{-1}$ dry weight; $n = 6$) of the Niton FP-XRF employed in
 201 plastics mode and for a total counting time of 200 s for trace elements in the three species of
 202 macroalga.

macroalga	As	Cd	Cr	Cu	Hg	Ni	Pb	Sb	Se	Sn	Zn
<i>F. serratus</i>	3.1	28.8	8.2	11.8	9.8	16.1	4.6	56.8	7.7	37.0	7.9
<i>P. palmata</i>	3.2	31.8	5.7	19.3	14.3	24.8	6.7	66.2	11.8	44.8	12.6
<i>U. lactuca</i>	8.0	44.2	12.5	48.6	35.4	58.9	13.4	87.1	25.4	57.1	23.8

203

204

205 3.2. FP-XRF determination of trace elements in baseline macroalgae

206 Table 2 summarises the dry weight concentrations of trace elements in the baseline samples
 207 as returned by the XRF. (Note that both here and with exposed material, concentrations in
 208 the oven- and freeze-dried samples were statistically indistinguishable ($p > 0.05$), despite the
 209 latter being flatter, smoother and less curled, and the data for each species of alga have,
 210 therefore, been pooled.) Thus, under the operating conditions of the instrument, As was
 211 detected in all samples of *F. serratus* and *P. palmata* but in no samples of *U. lactuca*, Cu and
 212 Zn were detected in all seaweed species but not always in each replicate, and Pb was only
 213 detectable in one or two replicates of each species. Although the XRF occasionally returned
 214 concentrations of Cr in *U. lactuca* that were well above the detection limit for the metal, it
 215 was subsequently established that this was the result of an analytical artefact related to
 216 sample thickness (see explanation below).

217

218 Table 2: Number of cases detected and mean and standard deviation of trace element

219 concentrations in baseline macroalgae ($\mu\text{g g}^{-1}$ dry weight) as returned by the Niton FP-XRF

220 in plastics mode and for a total counting time of 200 s.

macroalga	As			Cu			Pb			Zn		
	<i>n</i>	mean	sd	<i>n</i>	mean	sd	<i>n</i>	mean	sd	<i>n</i>	mean	sd
<i>F. serratus</i>	6	31.6	5.8	3	18.6	5.9	2	6.6	0.6	6	38.0	11.1
<i>P. palmata</i>	6	7.3	1.8	4	28.4	7.0	1	7.2		4	31.0	24.1
<i>U. lactuca</i>	0			2	42.6	10.2	2	13.4	1.9	6	56.7	16.9

221

222

223 3.3. FP-XRF determination of trace elements in exposed macroalgae

224 Based on the observations above, individuals of each species were exposed to As, Cu and Zn

225 for a period of seven days in order explore the performance of the XRF over a wider range of

226 trace element concentrations. Note that Pb was not included in the exposures because its

227 fluorescent L_{α} peak overlaps with the K_{α} peak of As with the consequence that

228 concentrations of the latter cannot be effectively calculated in the presence of relatively high

229 concentrations of Pb (Parsons et al., 2013). Figure 1 shows the dry weight concentrations of

230 As, Cu and Zn in each algal species returned by the XRF as a function of added aqueous

231 concentration. Here, control concentrations of trace elements in the algae are similar to those

232 determined in the baseline samples and as reported in Table 2. Addition of aqueous As

233 resulted in a non-significant increase in mean concentration in *F. serratus* and *P. palmata*

234 relative to the corresponding controls but only at the highest added concentration for the

235 former species; addition of the metalloid to *U. lactuca*, however, failed to elicit detectable

236 accumulation. Addition of aqueous Cu resulted in significant ($p < 0.05$) increases in mean

237 concentrations relative to the corresponding controls for *P. palmata* and *U. lactuca*, but a

238 significant increase in *F. serratus* was observed only at the highest added concentration of

239 the metal. Addition of aqueous Zn resulted in significant increases in mean concentration

240 relative to the controls for all species of seaweed, and for *F. serratus* incremental additions
241 of metal were accompanied by successive, significant increases in accumulation.

242

243 3.4. A comparison of trace element concentrations returned by FP-XRF and ICP-MS

244 As an independent and more sensitive method of trace element determination in the
245 macroalgae, all samples analysed by FP-XRF ($n = 90$) were subsequently digested in
246 concentrated HNO₃ and analysed by ICP-MS. The results revealed no false negatives among
247 the samples and for all elements considered (as listed in Table 1); that is, lack of detection by
248 the XRF was not accompanied by a measurement by ICP that exceeded the corresponding
249 LOD of the XRF. False positives were returned for Cr in all samples of *U. lactuca*; thus,
250 here, the XRF reported concentrations of Cr (up to 100 $\mu\text{g g}^{-1}$) that greatly exceeded
251 concentrations returned by the ICP ($< 1.5 \mu\text{g g}^{-1}$). We attribute this discrepancy to the
252 difficulty in obtaining an accurate thickness of the green seaweed and the high sensitivity of
253 Cr determinations to the thickness correction algorithm in the plastics mode of the Niton
254 XL3t (Turner and Solman, 2016).

255

256 Direct comparisons of the two approaches for the trace elements detected by XRF (As, Cu,
257 Pb and Zn) are illustrated in Figure 2 and statistical summaries defining the datasets are
258 presented in Table 3. Overall, data points for As are close to and are equally distributed
259 either side of unit slope; for Cu, Pb and Zn, however, most (or all) data points lie above but
260 within an order of magnitude of unit slope. XRF-ICP relationships for all elements displayed
261 a correlation coefficient, r , above 0.8 that was significant ($p < 0.05$), and linear regression
262 analysis revealed lines with slopes, m , when forced through the origin, ranging from about 1
263 for As to over 2 for Cu and Pb. With respect to individual algal species, all relationships
264 were significant with variation among line slopes except for As in *P. palamata*; here, data

265 points were clustered around a relatively small range in concentration and a relationship
 266 could only be defined with a positive intercept.

267

268 Table 3: Statistical definitions of the XRF-ICP relationships shown in Figure 2. Note that all
 269 relationships forced through the origin were significant ($p < 0.05$) except for As in *P.*

270 *palmata* (m = slope, r = correlation coefficient, ns = not significant).

macroalga	As			Cu			Pb			Zn		
	n	m	r	n	m	r	n	m	r	n	m	r
<i>F. serratus</i>	30	0.959	0.832	25	1.610	0.522	2			30	1.370	0.959
<i>P. palmata</i>	25	1.052	0.108 (ns)	28	1.630	0.335	1			27	1.650	0.909
<i>U. lactuca</i>	0			26	2.360	0.792	2			27	2.690	0.887
all	55	0.962	0.956	79	2.260	0.893	5	2.443	0.849	84	1.690	0.827

271

272

273 4. Discussion

274 Although more sensitive, conventional (laboratory-based) XRF techniques have been
 275 employed to determine trace elements in seaweeds and other biological materials after
 276 complete sample digestion or chemical treatment-pelletisation (Vlachos et al., 1998; Ferreira
 277 et al., 2012; McComb et al., 2014), the present study appears to be the first to report the
 278 direct application of a field-portable instrument in this respect. Advantages of a portable
 279 instrument that incorporate a low density mode and when used in a laboratory accessory
 280 stand include minimal sample preparation (e.g. cleaning and drying), non-destruction of
 281 material, rapid, multi-element analysis, avoidance of hazardous waste generation and
 282 minimal operator training.

283

284 The Niton XL3t FP-XRF configured in a plastics mode and with thickness correction is able
 285 to detect a number of elements in various species of algae collected from a protected coastal
 286 site down to dry weight concentrations of a few $\mu\text{g g}^{-1}$. That overall and species-specific
 287 relationships between XRF and ICP are, in all but one case, significant, indicates the counts
 288 from the FP-XRF analysis are converted via fundamental parameters into concentrations that

289 are directly proportional to those returned by the independent digestion-ICP approach. In the
290 case of As, an overall slope close to unit value suggests that the plastics mode of the Niton
291 XL3t instrument is suitable for direct determinations in macroalgae. Where slopes exceed
292 unit value and the XRF over-estimates elemental concentrations, however, data require
293 empirical adjustment. This may be achieved by applying element- and, perhaps, algal-
294 specific corrections to measurements obtained by the XRF. Alternatively, the instrument
295 allows the operator to edit and store up to four alternative calibrations per mode for a suite of
296 elements by adding slopes and, if necessary, intercepts to both main and low energy ranges.
297 Because of the significant proportionality between concentrations returned by the FP-XRF
298 and those delivered by an independent method whose accuracy has been verified, the method
299 meets US EPA validation guidelines; specifically, As analyses meet the definitive level
300 criterion ($r > 0.9$) and Cu, Pb and Zn the quantitative screening level criterion ($r > 0.7$)
301 (Environmental Protection Agency, 2007).

302

303 The portable XRF also has potential for the direct monitoring of the spatial and temporal
304 distribution of trace elements in macroalgae in situ. Here, implementation of additional
305 safety features would be necessary, such as a back scatter radiation shield or a portable test
306 stand. Field measurements would require suitable water protection of the detector window
307 and would entail analysing samples without drying and stacking but after appropriate
308 cleaning and thickness measurement. Sensitivity, error and, possibly, accuracy would be
309 compromised by the presence of water through its contribution to density and its propensity
310 to scatter and photoelectrically absorb radiation (Parsons et al., 2013). Algal water content
311 would also have to be factored in for dry weight concentrations to be determined through
312 element- and species-specific wet-to-dry weight algorithms, although it is possible that
313 measurements of Cl in the low energy range ($K_{\alpha} = 2.62$ keV, $K_{\beta} = 2.82$ keV) could be used

314 as a proxy for sea water content (Tjallingii et al., 2007). The feasibility of in situ screening
315 for trace elements in a variety of coastal macroalgae is currently being investigated.

331

332

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337

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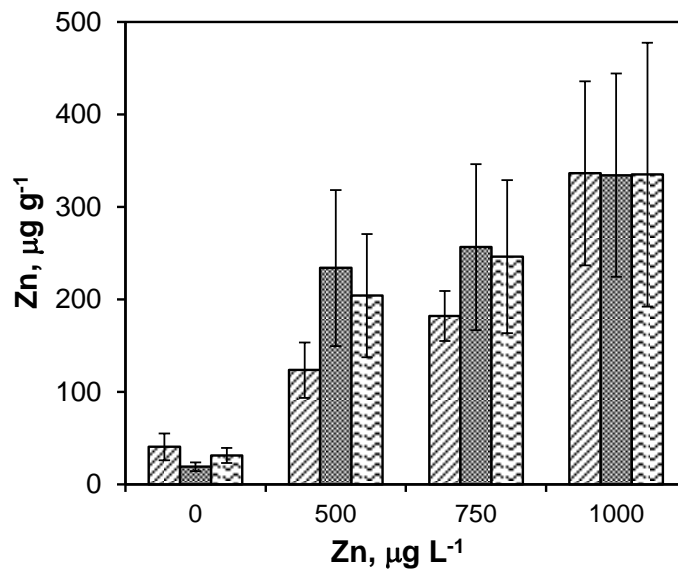
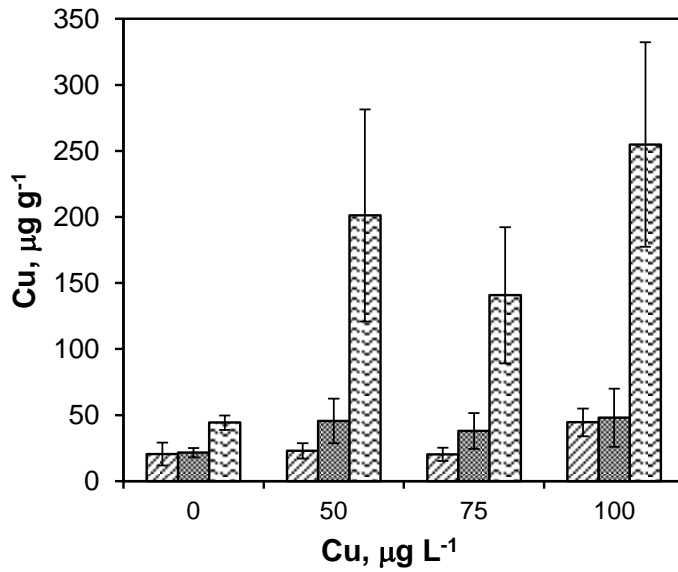
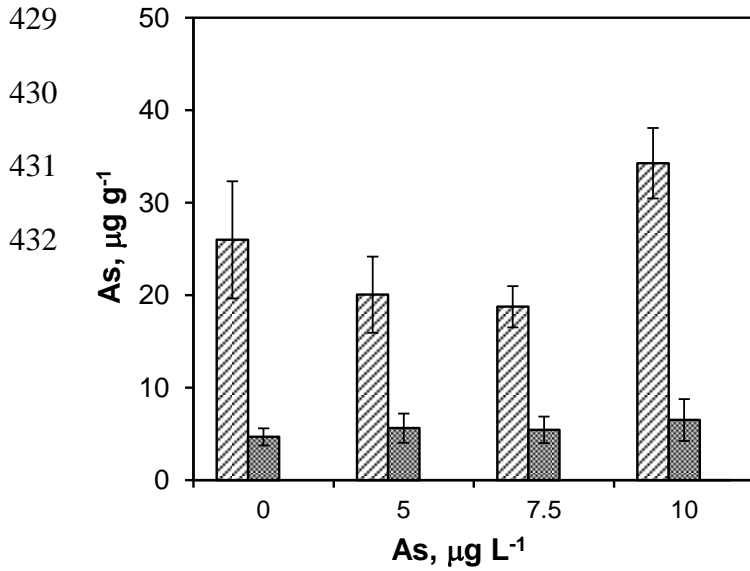
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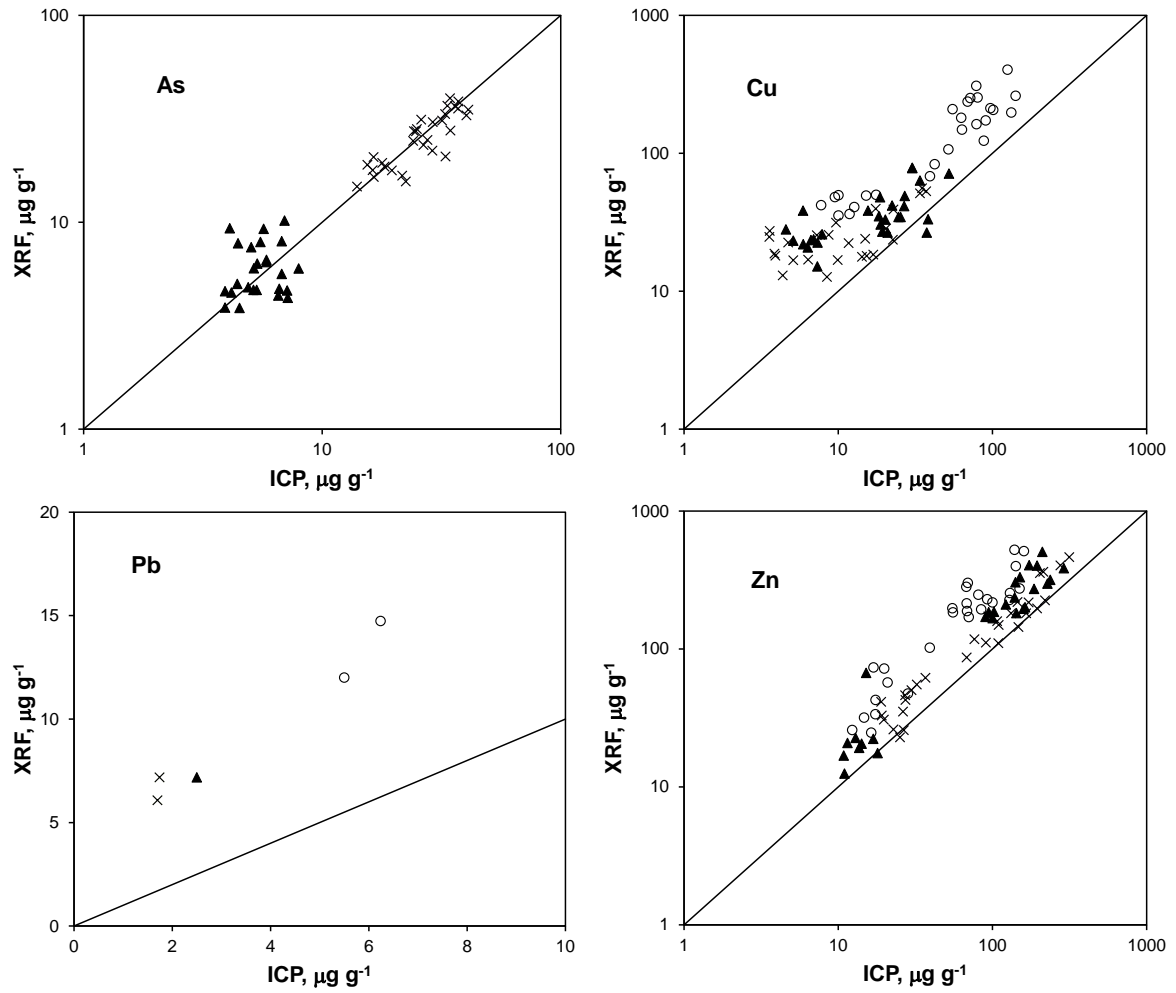
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425 Figure 1: Dry weight concentrations of As, Cu and Zn in *Fucus serratus* (hatched), *Palmaria palmata*
 426 (stippled) and *Ulva lactuca* (zig-zag) as returned by the Niton FP-XRF and following aqueous addition of
 427 different concentrations of each trace element. Note that errors denote one standard deviation about the mean of
 428 up to six independent determinations, and that As was never detected in *U. lactuca*.



433 Figure 2: A comparison of dry weight concentrations of As, Cu, Pb and Zn in *Fucus serratus* (X),
434 *Palmaria palmata* (▲) and *Ulva lactuca* (o) returned by the Niton FP-XRF and by ICP-MS
435 following acid digestion. Statistical parameters defining each dataset are given in Table 3 and solid
436 lines denote unit slope.



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