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In situ determination of trace elements in *Fucus* spp. by field-portable-XRF

Andrew Turner*, Hiu Poon, Alex Taylor & Murray T. Brown[1]

School of Geography, Earth and Environmental Sciences and [1]School of Biological and Marine Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK

*Corresponding author. Tel: +44 1752 584570; Fax: +44 1752 584710; e-mail: aturner@plymouth.ac.uk

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Abstract

Fresh and freeze-dried sample sections of the coastal macroalgae, *Fucus serratus* and *F. vesiculosus*, and the brackish water macroalga, *F. ceranoides*, have been analysed for trace elements by field-portable-x-ray fluorescence (FP-XRF) spectrometry using a Niton XL3t in a low density mode with thickness correction. When analysed fresh in a laboratory accessory stand for a period of 200 seconds, As, Br, Fe and Zn were registered in the apex, mid-frond and lower stipe of all species, with detection limits of a few $\mu g\ g^{-1}$ (As) or a few tens of $\mu g\ g^{-1}$ (Br, Fe, Zn); when analysed dry under the same conditions, concentrations returned were systematically higher and Cu and Pb were detected in a number of *F. ceranoides* sections. Concentrations arising from both approaches on a dry weight basis were highly correlated, with deviations from unit slope attributed to the absorption of fluorescent x-rays by internal and surficial water when analysed fresh. With algorithms correcting for the effects of water on mass and x-ray absorption, sections of *F. vesiculosus* and *F. ceranoides* were analysed in situ with the XRF connected to a mobile stand and laptop. Dry weight concentrations returned for As and Zn were significantly correlated with respective concentrations subsequently determined by ICP-MS following acid digestion and with a slope close to unity; lower concentrations of Fe returned by ICP were attributed to the incomplete acid digestion of silt particles that evaded an initial cleaning step, while Br concentrations could not be verified independently because of loss of volatile forms during digestion. The in situ determination of trace elements in fucoids by FP-XRF provides a rapid and non-destructive means of monitoring environmental quality and identifying hot-spots of contamination, and enables a research strategy to be developed iteratively that is informed by immediate results.

Keywords: macroalgae; fucoids; FP-XRF; monitoring; trace elements; ICP-MS

1. Introduction

Marine macroalgae represent a large and diverse group of primary-producing organisms in the coastal zone that create habitat structure, provide food, promote biodiversity and serve as a carbon sink (Duarte et al., 2013; Matias et al., 2015). Macroalgae also have an economic value, acting as a source of food, nutrients and medicines for human consumption and offering a means of bioremediation and a potential bioenergy resource (Bruhn et al., 2011; Tabarsa et al., 2012). Being sessile, macroalgae are influenced directly by ambient environmental conditions, and in this respect the distribution and occurrence of certain species may often reflect local water quality (Guinda et al., 2008). Moreover, because of their thick cell walls and high polysaccharide content, macroalgae are also able to accumulate many aqueous contaminants, and in particular trace metals and metalloids, to concentrations several thousand times higher than the ambient water column (Zbikowski et al., 2006). Consequently, many species act as vehicles for the transfer of contaminants up the food chain (Chan et al., 2003; Mulholland and Turner, 2011) and serve as useful biomonitors that provide a direct and integrated assessment of bioavailable contaminants over a period of time (Cairrão et al., 2007; Boubonari et al., 2008). The littoral brown fucoids are particularly useful in the latter respect because of their extensive distribution, ease of identification and sampling, tolerance of wide variations of temperature and salinity, abundance all year round and limited ability to regulate contaminant concentrations (Martin et al., 1997; Rainbow, 2006; Sondergaard et al., 2014). Accordingly, *Fucus* spp. have been selected for inclusion in the Environmental Specimen Banks (ECBs) of several European countries in order to monitor long-term changes in anthropogenic contamination (Viana et al., 2010; Rüdel et al., 2010).

As commonly employed biomonitor organisms, there is a requirement for the routine determination of trace elements in macroalgae. Conventionally, analysis is performed on

dried samples that have been digested in hot, concentrated acid by, for example, atomic absorption spectrometry (Reis et al., 2014) or inductively coupled plasma (ICP) spectrometry (Brito et al., 2012). This approach can, however, be time-consuming, labour-intensive and costly, and the destruction of samples has implications for the long-term viability of archived specimen banks. Recently, we investigated the feasibility of field portable-x-ray fluorescence (FP-XRF) spectrometry as a rapid, non-destructive means of determining trace elements in various species of macroalgae (Bull et al., 2017). Specifically, we employed a Niton XL3t spectrometer configured in a low density, 'plastics' mode and with a corrective algorithm for sample thickness to measure dried samples housed in a laboratory accessory stand. For the elements that were detected, there was a significant correlation between concentrations measured directly and those returned independently by ICP-mass spectrometry following HNO_3 digestion, with relationships satisfying the EPA definitive level criterion for As and quantitative screening level for Cu and Zn (Environmental Protection Agency, 2007).

In order to further the XRF approach for measurements of trace elements in macroalgae in situ, the effects of water, as a contributor to both sample mass and x-ray absorption, need to be accounted for and factored in to any calibration. To this end, the present study compares trace element concentrations returned by FP-XRF for different species of macroalgae analysed both in the fresh and freeze-dried states. Specifically, fucoids were selected because of their importance in coastal biomonitoring and their relatively high thickness (for x-ray absorption) compared with other species of macroalgae (Bull et al., 2017). With the effects of water empirically quantified, the practicalities and challenges of deploying the XRF in the field for the direct measurement of trace elements in macroalgae are discussed.

2. Materials and methods

2.1. Sampling and sample preparation

Whole samples of fucoid were handpicked at low tide in July 2016 from two sites within 25 minutes' driving distance of the laboratory at Plymouth University (Figure 1). At Firestone Bay, a small, pebble-sand beach in the coastal embayment of Plymouth Sound, five specimens of two coastal fucoids, *F. serratus* and *F. vesiculosus*, were collected from the rocky substrates of the littoral zone and stored in a cool box in a series of zip-lock polyethylene bags. From the intertidal mudflats of the upper Tavy Estuary, a tidal tributary of the Tamar Estuary and an environment impacted by historical mining activities, ten specimens of *F. ceranoides*, a brackish water fucoid, were collected and stored likewise. In the laboratory, samples of *F. serratus* and *F. vesiculosus* were divided and subjected to two different methods of clearing sediment and epiphytes from the surface; thus, one half was cleaned in Millipore Milli-Q water (MQW) with the aid of a Nylon brush and subsequently scraped with a plastic spatula after applying a 10% solution of ethanol to the tissue surface, while the other half was cleaned with MQW only. After blotting dry with 3-ply hygiene roll, all plants were dissected on a plastic tray using a stainless steel blade, with ~ 5 cm sections of the apex, mid-frond and lower stipe (just above the holdfast) from each plant retained and stored in individual specimen bags. Because of the smaller size of *F. ceranoides* and results arising from the cleaning methods of the two coastal macroalgae, samples of the brackish water fucoid were cleaned in MQW only before being dissected likewise.

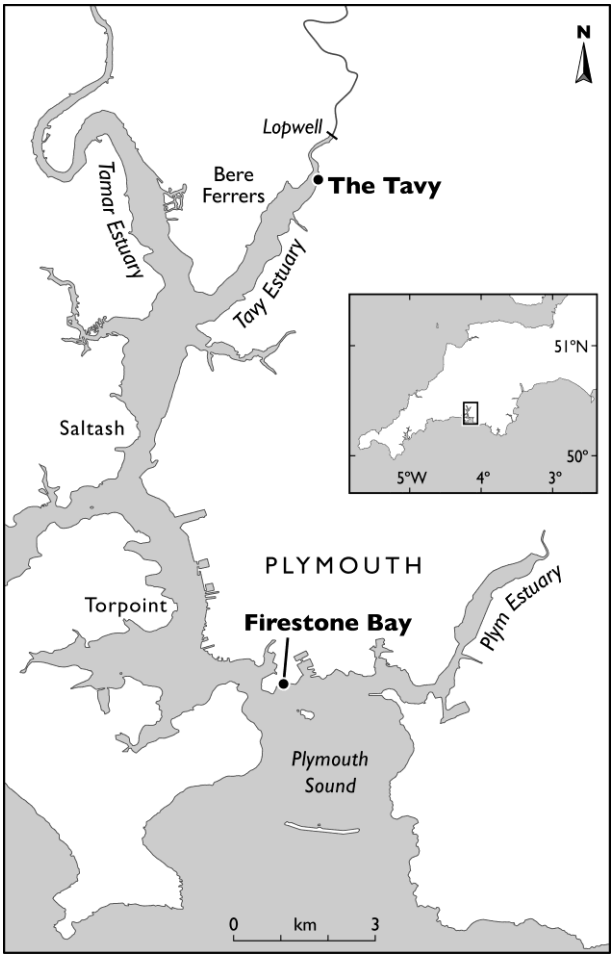


Figure 1: The sampling locations for the fucoid macroalgae.

2.2. FP-XRF analysis

Sample sections processed in the laboratory (n = 90) were analysed for trace elements (As, Br, Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn) directly and without drying by energy dispersive FP-XRF using a battery-powered, 1.3 kg Niton analyser (model XL3t 950 He GOLDD+) housed in a ThermoScientific accessory stand of steel construction and tungsten-plastic shielding (PN 420-017; weight ~ 10 kg, chamber volume = 4000 cm^3). Analysis was performed in a low density mode that uses a fundamental parameters-based alpha coefficient correction model (Turner and Solman, 2016). Because the intensity of fluorescence generated by low density and weakly absorbing samples is dependent on the thickness of material, a corrective algorithm (down to 50 μm) was also applied after section thickness had been measured in mm and to two decimal places using digital callipers. With plastic tweezers, samples were

placed onto a SpectraCertified Mylar polyester 3.6 μm film, which was then positioned carefully such that the smoothest and flattest part of the macroalgal section lay directly and centrally above the 8 mm XRF detector window. After closing the accessory stand lid, the XRF was activated remotely and via USB using a Fujitsu laptop computer. Analysis was tested for a variety of conditions of which a collimation of 8 mm and a counting period of 200 seconds, comprising 150 seconds at 50 kV and 40 μA and 50 seconds at 20 kV and 100 μA, appeared to be optimal in terms of detection, error and sample throughput. To check the performance of the XRF and as an analytical quality control, Niton polyethylene reference discs impregnated with known concentrations of various trace elements (PN 180-619, LOT#T-18 and PN 180-554, batch SN PE-071-N) were analysed throughout each measurement session. On completion of measurements, spectra and elemental concentrations (in $\mu g\ g^{-1}$ and with a counting error of 2σ) were downloaded to the laptop using Niton Data Transfer PC software.

Immediately after sample measurement, individual macroalgal sections were weighed using a five-figure Sartorius analytical balance before being returned to their original specimen bags and freeze-dried for 48 h using an Edwards Super Modulyo. Dried sections were then re-analysed by XRF under the operating conditions described above and after appropriate (dry) thickness correction, before being re-weighed, returned to their specimen bags and stored under desiccation pending acid digestion (see below).

2.3. Macroalgae digestion and analysis by ICP-MS

As an independent measure of trace elements in the macroalgae, all freeze-dried sample sections were subsequently acid-digested and analysed by inductively coupled plasma-mass spectrometry (ICP-MS). Thus, samples of about 0.1 g were accurately weighed into individual Teflon tubes to which 2.5 ml aliquots of HNO_3 (Fisher Chemical TraceMetal™

Grade) were added. The contents were digested in a CEM MARS 5 XPRESS microwave at 1600 W for 45 min before being allowed to cool to room temperature. Digests were then washed into individual 10 ml volumetric flasks and diluted to mark with ultra-pure Millipore Milli-Q water. For an assessment of digestion efficacy and analytical accuracy, a fucoid reference material (*Fucus vesiculosus*, ERM-CD200; certified for As, Br, Cd, Cu, Fe, Hg, Pb, Se and Zn) was digested in triplicate likewise.

Digests were analysed for elements that had been detected by XRF using a collision cell-ICP-MS (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}, 0.72 L Ar min^{-1} and 3.5 mL 7% H_2 in He min^{-1}, respectively. The instrument was calibrated externally using four mixed standards prepared by dilutions of a QC 26 multi-element solution (CPI International, Amsterdam) in 0.1 M HNO_3, and internally by the addition of 100 μg L^{-1} of In and Ir to all samples and standards. Data were acquired over a dwell period of 10 ms, with 50 sweeps per reading and three replicates.

Aqueous concentrations derived from ICP-MS were converted to dry weight concentrations (in μg g^{-1}) from the volume of diluted digest and mass of macroalga dissolved in acid. Limits of detection on this basis were < 2.5 μg g^{-1} for all trace elements analysed, and measured concentrations in the reference macroalga were within 15% of published values with the exception of Br and Fe (recoveries of about 50% and 70%, respectively).

2.4. Statistical analysis

Correlation analysis was performed on paired data series using the Data Analysis Toolpak in Excel 2016, with the strength of association reported as Pearson's moment correlation

coefficient (r) and the significance of the relationship as the probability of r not being different from zero (p, and where $\alpha = 0.05$). One-way ANOVA and Tukey's post-hoc test were used in Minitab 17 to identify significant differences ($\alpha = 0.05$) in mean elemental concentrations and water contnets among macroalgae and parts thereof, and in mean elemental concentrations arising from the three analytical methods.

3. Results and Discussion

3.1. Macroalgal water content and thickness

Quantification of the water content of the macroalgal sections is critical for converting elemental concentrations from a fresh weight basis to a dry weight basis and for evaluating the impact of the fluid on x-ray behaviour and intensity (mainly through photoelectric absorption, but also via Compton scattering and internal reflections; Parsons et al., 2013). Mean percentage water, calculated from the fresh and dry weights of each section and shown in Table 1, ranged from about 50% to nearly 90%, and for all species the order of descending water content was: apex > mid-frond > lower stipe. There was no statistical difference in water content between common sections of *F. vesiculosus* and *F. serratus*, but the water content of sections of *F. ceranoides* were significantly greater than corresponding sections of the former two species. The method of tissue cleaning made a difference to mean water content that was significant only for the lower-stipe of *F. vesiculosus* from Firestone Bay. Thus, here, cleaning in MQW resulted in a higher percentage compared with sections having undergone additional cleaning with ethanol

Fucoid section thickness, measured for XRF data correction, did not display a clear dependency on species, location with respect to the frond or means of tissue cleaning. On average, however, sections were thicker while wet (1.02 ± 0.15 mm) than when freeze-dried (0.85 ± 0.19 mm).

Table 1: Percentage water content of the fucoid macroalgal sections undergoing cleaning in Milli-Q water (MQW) and ethanol, and/or MQW only. The mean and standard deviation of *n* measurements is given in each case.

	F. serratus (*n*=5)		*F. vesiculosus* (*n*=5)		*F. ceranoides* (*n*=10)
	MQW	MQW+ethanol	MQW	MQW+ethanol	MQW
apex	81.2±1.8	76.3±2.4	77.7±2.2	73.1±1.7	86.7±1.9
mid-frond	67.7±4.0	63.7±5.1	62.6±3.7	60.3±2.8	76.9±4.8
lower stipe	61.1±1.7	54.2±5.3	61.8±1.3	51.8±2.1	70.5±3.6

3.2. XRF detection limits for trace elements in macroalgae

XRF detection limits for trace elements in the fucoids, defined as three counting errors for a 200-second counting time, are presented in Table 2. Here, limits for all species, sectional locations and cleaning methods have been pooled and are shown for samples analysed in both the fresh state and after freeze-drying; with regard to the former, limits are shown on a fresh weight basis and, after correction for water content, a dry weight basis. Note that for some elements (Cd, Cr, Cu, Hg, Ni, Pb) limits have been averaged from at least fifteen measurements in which the element was not detected by the instrument but a value of 3σ was returned directly; where less than fifteen sample sections were undetectable (As, Br, Fe, Zn), limits were based on the values of 2σ returned on detection and after multiplication by 1.5.

Mean detection limits are generally lower when samples are analysed fresh than when freeze-dried, presumably because the greater flexibility of wet macroalgal sections allows them to be placed closer to the detector window of the instrument. However, when wet weight concentrations are converted to a dry weight basis, detection limits are higher than samples analysed dry. Here, we surmise that the effects of water on elemental dilution and x-

ray absorption and scattering outweigh the benefits of increased proximity to the detector. Overall, mean detection limits are lowest and average less than 10 μg g^{-1} (on both a dry weight and wet weight basis) for As and Pb and are less than 25 μg g^{-1} for Br, Cu, Hg, Ni and Zn, and are similar to corresponding limits reported for dried sections of *F. serratus* reported by Bull et al. (2017). Within these constraints, As and Fe were detected in all fucoid section analyses performed in the present study (n = 180), while Br and Zn were detected in 178 and 172 cases, respectively, with non-detection always associated with the analysis of fresh samples. Note that although Cu and Pb were detected in some samples of *F. ceranoides*, the number of cases (n = 7 and n = 5, respectively) was too few for establishing relationships between the different analytical approaches and differences among the three sectional components of the macroalga.

Table 2: A summary (as mean ± one standard deviation; n > 15) of the Niton XRF detection limits for trace elements in fucoid macroalgae analysed fresh and dry and for a 200-second counting time (dw = dry weight; fw = fresh weight).

	As	Br	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Zn
dry, μg g^{-1} dw	4.7±1.0	17.9±4.6	26.3±2.3	6.1±2.2	16.2±4.2	24.5±6.7	12.3±2.8	14.4±3.8	5.9±1.0	11.7±2.7
fresh, μg g^{-1} fw	1.6±0.2	4.7±1.2	14.4±2.4	7.9±2.0	5.3±1.0	6.8±1.3	5.0±1.2	5.0±1.0	2.3±0.5	3.5±1.1
fresh, μg g^{-1} dw	6.6±2.0	20.1±9.5	61.0±23.4	32.3±11.9	22.2±8.5	28.2±10.1	21.2±9.0	21.0±8.9	9.7±3.8	14.3±5.5

3.3. Comparison of elemental concentrations when analysed wet and dry

Figure 2 compares the dry weight concentrations of readily detectable elements (As, Br, Fe, Zn) in the fucoids that were returned by the Niton XRF when analysed dry, [XRF-*dry*], and when analysed fresh and individually corrected for water content, [XRF-*fresh*]. Note that here, data for each element are not discriminated by species, location on the frond or means of tissue cleaning. Also shown are the best-fit equations (forced through the origin) that define each element, along with corresponding Pearson's moment correlation coefficients

and the line signifying unit slope. In all cases, and despite changes in thickness and morphology incurred by freeze-drying, elemental concentrations arising from both approaches were highly correlated ($p < 0.01$) with gradients exceeding unit value; that is, concentrations returned when analysed dry were, on average, higher than concentrations returned when analysed fresh but dry-weight corrected. This suggests that the presence of internal and surficial water suppresses the strength of fluorescent x-rays reaching the detector window of the FP-XRF through absorption and scattering. Consistent with this assertion, deviation from unit slope is greatest for Fe, whose characteristic x-rays are of low energy (K_α = 6.405 keV; L_α = 0.705 keV) and relatively easily absorbed by water, and least for Br, whose characteristic x-rays are of higher energy (K_α = 11.924 keV; L_α = 1.481 keV) and, therefore, less easily absorbed.

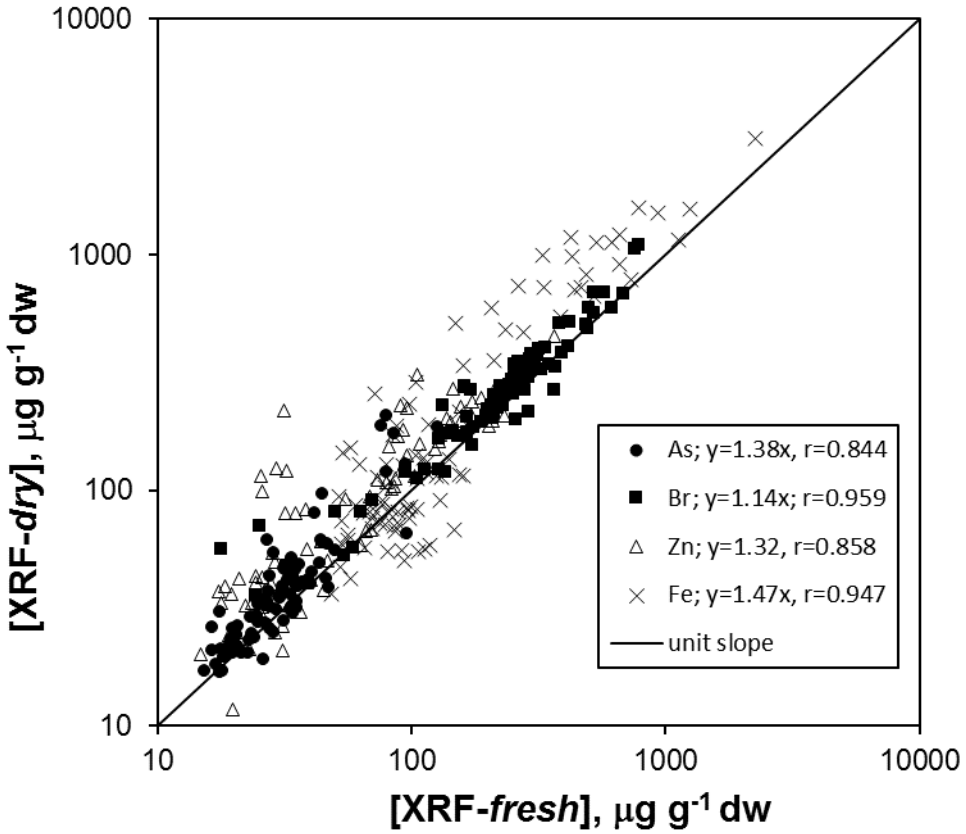


Figure 2: Dry weight elemental concentrations in the coastal and brackish water fucoid macroalgae returned by the Niton XRF when analysed dry and fresh. Shown inset for each element are equations of best fit when forced through the origin.

3.4. Inter- and intra-species variations in elemental concentrations and comparison with ICP-MS

Figures 3 to 6 show the dry-weight concentrations of As, Br, Fe and Zn in the different parts of each species of fucoid and as determined by the two XRF approaches (that is, analysis of fresh sections versus analysis of freeze-dried sections) and by ICP-MS following acid digestion. Note that all data presented are for tissues cleaned in MQW only and that results arising from samples subjected to additional cleaning with ethanol were very similar. Regarding As, mean concentrations were not statistically different among the different methods of determination with the exception of the lower stipe in *F. serratus*, where concentrations were lower when analysed by XRF in the fresh state than by ICP, and the apex in *F. ceranoides*, where concentrations were higher when analysed dry by XRF. Among the different parts of the frond, mean concentrations were generally higher in the apex than the mid-frond and lower stipe, an effect that was evident from each analytical approach in at least one species of fucoid. Overall, absolute concentrations of As were greatest in the apex of *F. ceranoides*, and concentrations were significantly greater in the mid-frond and lower stipe of *F. ceranoides* than in corresponding parts of both *F. serratus* and *F. vesiculosus* according to at least one analytical approach.

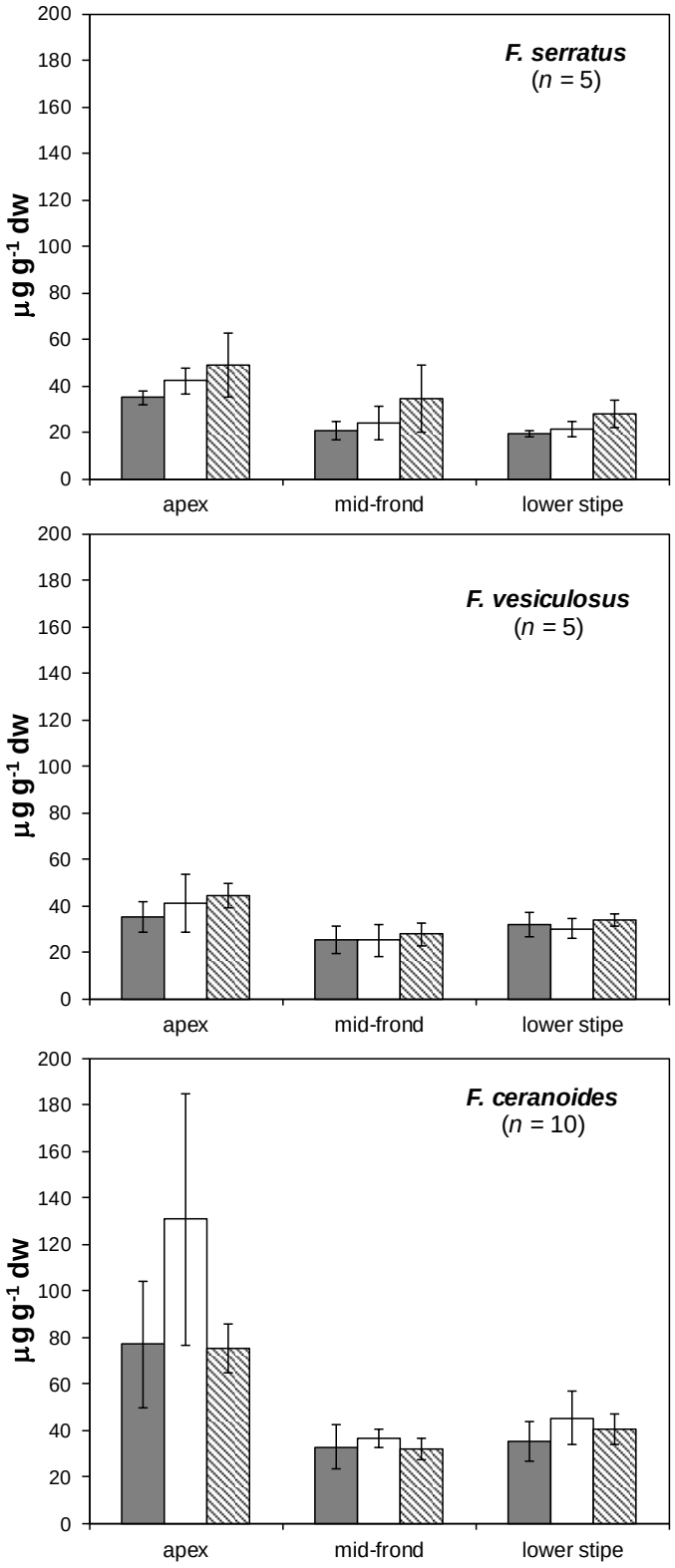
With respect to Br, results arising from ICP-MS analysis have been neglected due to loss of volatile forms (e.g. HBr and Br_2) during acid-oxidizing digestion, an effect that is often significant when opening the digestion vessel at the end of the mineralisation process (Di Narda et al., 2001). Mean concentrations of the halogen were never statistically different between the two XRF approaches and concentrations were not different among the three sectional components of *F. serratus.* Concentrations were, however, significantly lower in

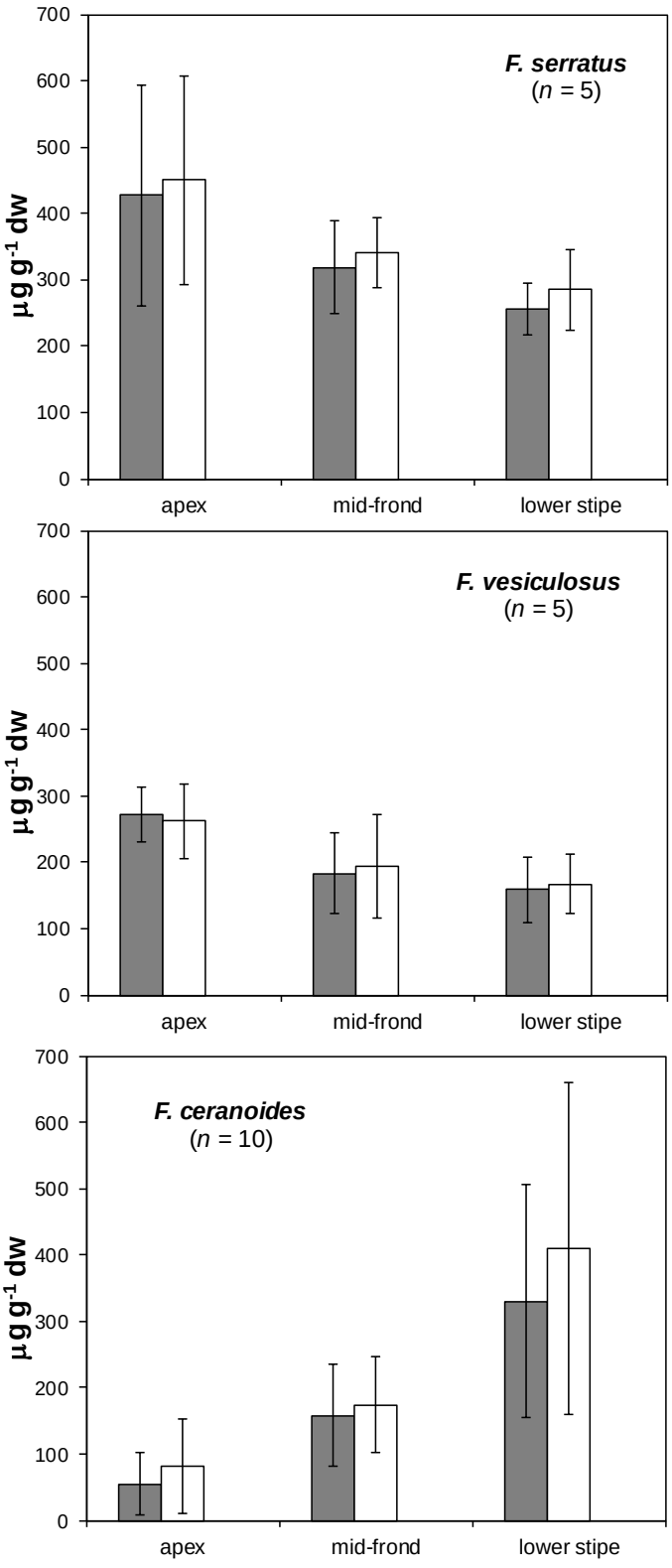
the stipe of *F. vesiculosus* than in its apex, and significantly higher in the stipe of *F. ceranoides* than in the apex where the lowest overall mean concentrations were observed.

Among the elements readily detected, concentrations of Fe were most variable among replicates. Consequently, there were no statistical differences observed between the two XRF approaches, despite mean concentrations returned being double when analysed dry in some cases. Determination by ICP-MS returned significantly lower concentrations than one or both XRF approaches (and by factors up to an order of magnitude) for the mid-fronds of both *F. serratus* and *F. ceranoides* and the apex and lower stipe of the latter.

Statistical differences in the mean concentrations of Zn were observed among the three analytical approaches only for the lower stipe of *F. vesiculosus* (lower by XRF after section drying), and the apex of *F. serratus* and apex and mid-frond of *F. ceranoides* (higher when analysed by XRF after drying than by both other approaches). With the exception of the apex analysed by XRF when fresh, mean concentrations of Zn were always statistically higher in *F. ceranoides* than corresponding concentrations in *F. vesiculosus*. In fewer cases, mean concentrations were higher in *F. ceranoides* than in *F. serratus* and in *F. serratus* than in *F. vesiculosus*.

Figure 3: Dry weight concentrations of As in the different parts of the fucoid species and as returned by FP-XRF analysis of fresh sections (grey bars) and dry sections (open bars) and by ICP-MS analysis following acid digestion (hatched bars). Errors represent the standard deviation about the mean of *n* measurements.





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337 Figure 4: Dry weight concentrations of Br in the different parts of the fucoid species and as

338 returned by FP-XRF analysis of fresh sections (grey bars) and dry sections (open bars).

339 Errors represent the standard deviation about the mean of *n* measurements.

Figure 5: Dry weight concentrations of Fe in the different parts of the fucoid species and as returned by FP-XRF analysis of fresh sections (grey bars) and dry sections (open bars) and by ICP-MS analysis following acid digestion (hatched bars). Errors represent the standard deviation about the mean of *n* measurements.

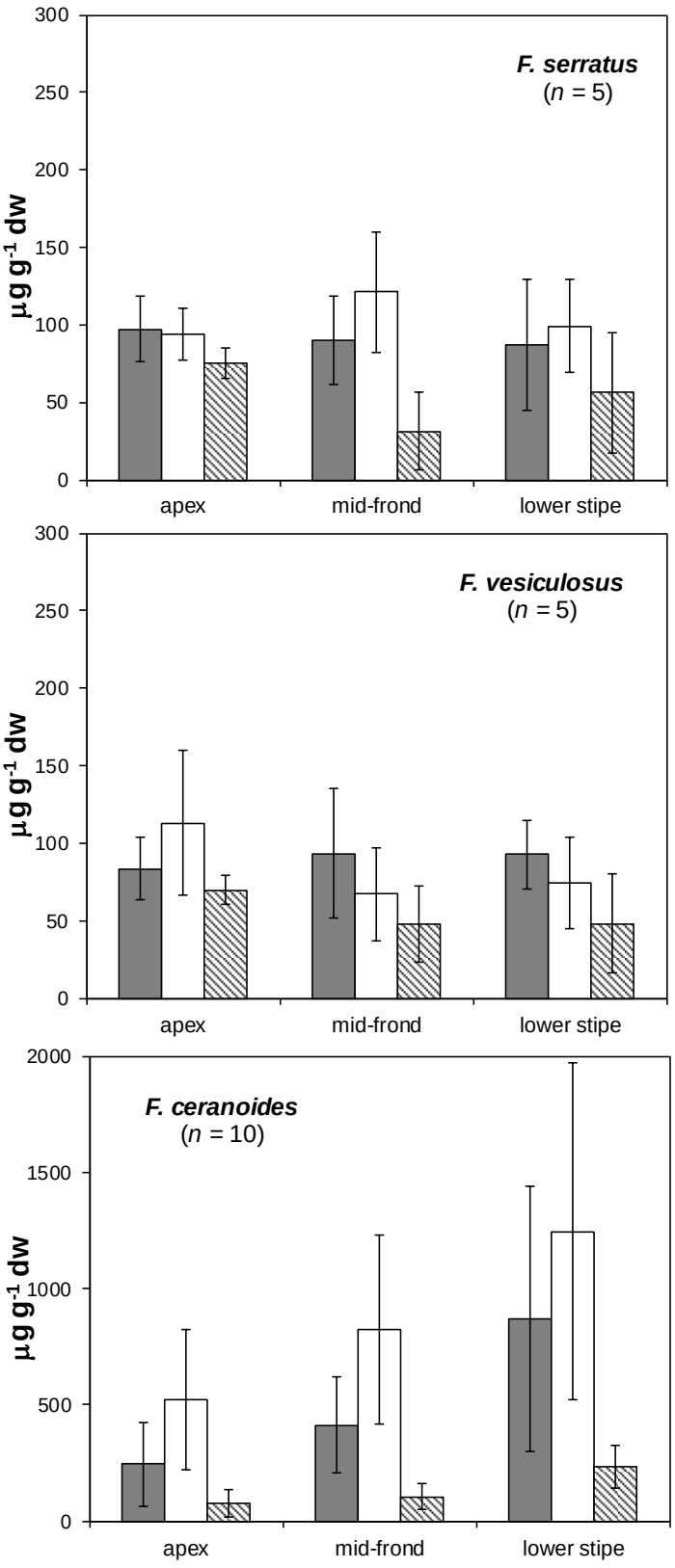
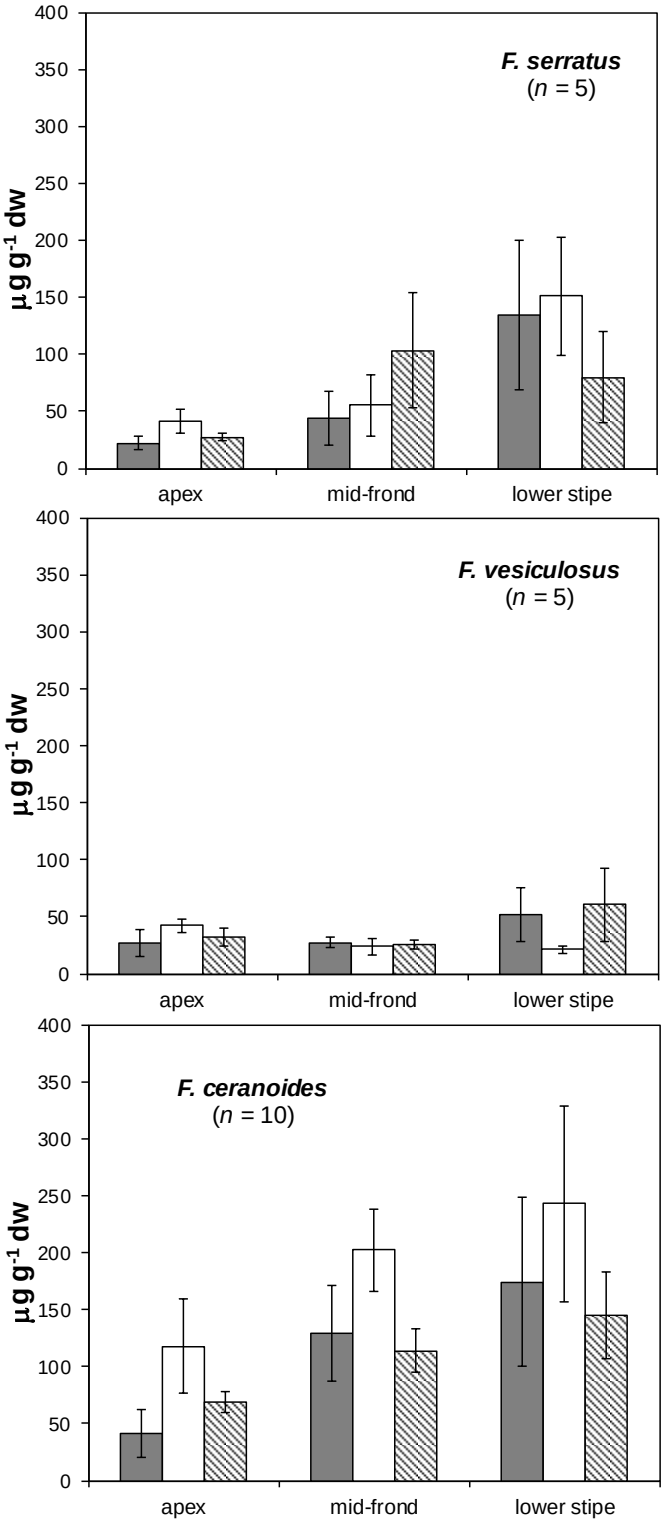


Figure 6: Dry weight concentrations of Zn in the different parts of the fucoid species and as returned by FP-XRF analysis of fresh sections (grey bars) and dry sections (open bars) and by ICP-MS analysis following acid digestion (hatched bars). Errors represent the standard deviation about the mean of *n* measurements.



371 *3.5. Summary and implications of findings*

372 In a previous article, we demonstrated the potential of FP-XRF for determining trace element
373 concentrations in different species of dried coastal macroalgae in a laboratory accessory
374 stand (Bull et al., 2017). The technique has distinct advantages over conventional methods
375 involving sample digestion that include reduced time and costs, non-destruction of material
376 (of particular significance to archived specimen banks), increased sample throughput,
377 minimal operator training, capability of exploring tissue spatial variability and avoidance of
378 hazardous wastes. Because monitoring in situ requires direct analysis without drying,
379 however, the present study evaluated the effects of the presence of internal and surficial
380 water on elemental concentrations returned for various fucoids. Thus, a comparison of
381 results arising from the analysis of fresh sections that had been dry-weight normalised and
382 the analysis of sections that had been subsequently freeze-dried revealed a greater sensitivity
383 of the latter approach but results that were highly correlated for all elements considered.
384 Lower dry-weight concentrations returned when analysed fresh are attributed to the
385 absorption of characteristic x-rays by water contained within or at the surface of the
386 macroalga.

387

388 Since variations in the percentage water in a given section of fucoid were small, with
389 relative standard deviations of less than 5% in most cases, instantaneous, quantitative
390 correction for macroalgal water content may be readily accomplished through species- and
391 section-specific algorithms; alternatively, it is possible that the fluorescence of Cl (K_α = 2.62
392 keV, K_β = 2.82 keV) could be used as a direct proxy for water content if local salinity is
393 known (Tjallingii et al., 2007). Additional, element-specific corrections for x-ray absorption
394 by water based on the gradients of the relationships between samples analysed fresh and dry
395 (Figure 2) would also be required for complete quantification of concentrations on a dry
396 weight basis. In practice, corrections for the effects of water may be stored in the Niton XRF

software as alternative calibrations in the low density mode by adding appropriate slopes and, if necessary, intercepts.

In most cases, dry-weight concentrations of As and Zn obtained by the analysis of fresh and dried sections of fucoids by FP-XRF were not statistically different to corresponding concentations derived independently by ICP-MS following acid digestion. For Fe in the estuarine macroalga, *F. ceranoides*, however, we attribute significantly lower results arising from ICP analysis to the incomplete release of Fe from the macroalga and to the presence of silt particles on the tissue surface that evaded cleaning and that were detected by the XRF but not completely digested by HNO_3. Among the elements analysed, the latter effect would be most significant for Fe given its high concentration in fine sediment from the Tavy Estuary (about 60,000 $\mu g\ g^{-1}$ determined on dried, intertidal silt by FP-XRF in a higher density, 'mining' mode, and compared with As and Zn concentrations of 90 and 250 $\mu g\ g^{-1}$, respectively). The heterogeneous dispersion of silt on the tissue surface would also account for the relatively high variability of Fe concentrations measured by XRF among replicates of the same sample section.

3.4. Deployment of the XRF in situ

With the effects of macroalgal water evaluated and quantified, the feasibility of employing the Niton FP-XRF spectrometer in situ was tested. Thus, the Tavy Estuary was revisited and sections from *F. ceranoides* and *F. vesiculosus* analysed under the operating conditions described above (instrument mode, counting time, energy ranges) after cleaning in MQW, dissection, blotting dry and thickness measurement with callipers. Initial attempts using the XRF handheld against sections placed on a solid but smooth surface (e.g. a plastic tray on a flat rock) and activated manually via the tilting touchscreen proved unsuccessful for a number of reasons. For example, positioning the XRF window such that it covered the

macroalgal section completely was difficult, despite the aid of live video-footage generated by a colour charge-coupled device camera and sampling imaging system adjacent to the detector; moreover, once positioning had been accomplished, holding the instrument still for a suitable length of time against the slimy, fucoid surface was not possible. A moving x-ray source over a low density, irregular sample also poses a safety hazard to the operator though radiation scattering; although this hazard could be minimised by using a backscatter collar-shield around the nose of the instrument (Figure 7a), the additional size and weight of equipment further inhibited accurate and steady positioning of the detector window.

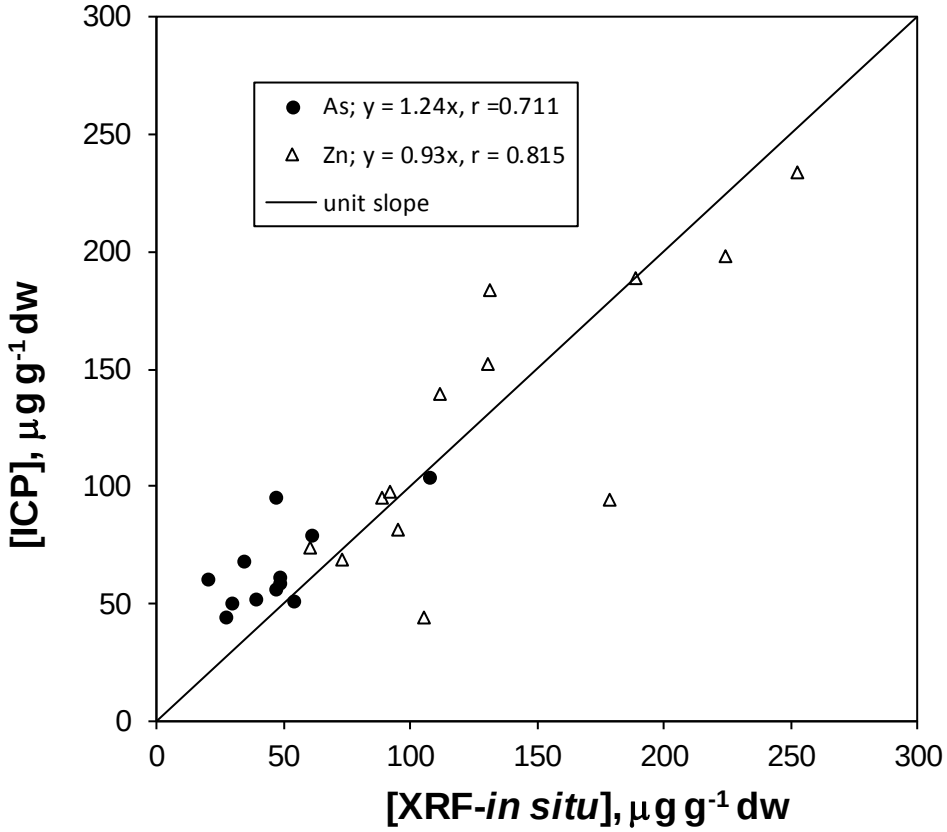
Successful application of the XRF in the field was, however, accomplished when coupled to a lightweight (~ 2.5 kg) and small-volume (300 cm^3) mobile test-stand (ThermoScientific, PN 430-032) and laptop (Figure 7b). Here, the test-stand was placed on a level, stable surface and the instrument subsequently securely fixed to the steel baseplate with the nose pointing upwards. Individual sample sections were placed on polyester film and positioned centrally over the detector window with the aid of plastic tweezers and, if necessary, held flat and in place with weights (e.g. small stones) at each end (Figure 7c). Once the shielded (tungsten-plastic) stand lid was closed, measurements were activated remotely using the laptop and via USB.

Essentially, this is the same approach as that employed in the laboratory using the accessory-stand. Additional benefits of performing measurements in situ, however, include the development of a strategy or focus that is iterative or directly informed by immediate results, identification of contamination hot-spots, elements of concern or the effects of a pollution incident, determination of which samples to return to the laboratory for further characterisation, and little or no degradation of macroalgae should transport to the laboratory be otherwise time-consuming. With three people in the field and working concurrently on

449 separate tasks (sampling, sample processing and analysis), algal section throughput for a 200
450 second counting time was about 15 per hour, and with a single, fully-charged battery, the
451 XRF could be deployed for a period of up to six hours. Given the weight of equipment
452 involved (about 15 kg for the XRF, stand, laptop and cases), set up and measurement are
453 also possible with a single operator, although throughput would be significantly reduced
454 because of the requirement for an individual to conduct multiple tasks successively or
455 concurrently.
456
457 For different sections analysed in situ, concentrations measured directly were converted to
458 dry weight concentrations using the average (generic) percentage water for a given type of
459 section of a particular species and subsequently corrected for x-ray water absorption by
460 applying the element-specific gradients defining the relationship between samples analysed
461 fresh and dry (Figure 2). In Figure 8, results for As and Zn derived accordingly, [XRF-*in*
462 *situ*], are shown for samples in which concentrations were subsequently determined by ICP-
463 MS following drying and acid digestion. For both elements, correlations were significant (p
464 < 0.05) with r values exceeding the US EPA quantitative screening criterion of 0.7
465 (Environmental Protection Agency, 2007). For Fe, concentrations derived in situ were
466 significantly correlated with but considerably higher than those derived independently by
467 ICP-MS for reasons outlined above. With respect to *F. vesiculosus* in the Tavy, mean
468 concentrations of As and Zn derived in situ (67 and 200 $\mu g\ g^{-1}$) are also similar to mean
469 values reported in the literature for the upper estuary (86 and 382 $\mu g\ g^{-1}$ respectively;
470 Rainbow et al., 2011).
471
472
473
474

Figure 7: (a) The Niton XL3t plus backscatter shield; (b) configuration of the instrument in situ and coupled to the portable stand and laptop; (c) a fucoid section placed above the detector window and within the stand.

Figure 8: Relationship between As and Zn concentrations in *F. ceranoides* and *F. vesiculosus* determined by ICP-MS following acid digestion and by FP-XRF deployed in situ and after correction for the content and x-ray absorption of water.



4. Conclusions

Although FP-XRF does not have the capability of sub-part per million analyses to replace atomic or mass spectrometry, this study has shown that the Niton XL3t provides a rapid, cost-effective and non-destructive means of measuring various trace elements in both fresh and dry fucoid species of macroalgae, provided that a low density mode with thickness correction is employed. The analytical conditions described (mode of application, collimation, counting time, energy ranges) allow the ready quantification of As to dry weight concentrations down to a few µg g^{-1} and Br, Fe and Zn to concentrations of a few tens of µg g^{-1}; measurement of Cu and Pb in fucoids is also possible in moderately to highly contaminated sites. Coupled to a mobile test-stand and laptop, the instrument can be

deployed in situ for rapid diagnostic and strategic purposes and to evaluate intra- and inter-specific concentration variations, with full quantification possible after empirical adjustment of data for the effects of water on sample weight and x-ray absorption.

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