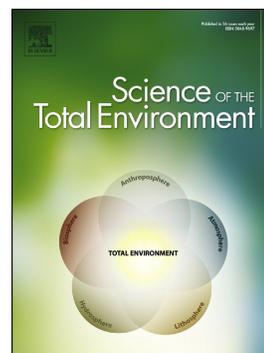


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## Tracing the Natural and Anthropogenic Influence on the Trace Elemental Chemistry of Estuarine Macroalgae and the Implications for Human Consumption

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### Abstract

Macroalgae (seaweed) has been shown to be an effective environmental indicator. We investigate the trace element chemistry of macroalgae samples from locations along the Firth of Forth and Forth Estuary in Scotland. The overall trend in elemental abundance (Os << Re < Ag < U < Cd < Co < Ni < Pb < Cu < As < Zn <<I), and changes along the estuary (seawards: increase As, I, Cd, U, Re, Os; decrease Pb, Cu; mid-estuary peak Zn; based on certain species), are controlled by a number of factors, including: salinity, mixing and macroalgal species differences. Within the same macroalgal species, some elemental abundances (As, I, Pb, Cu, Cd and U) are affected by mixing between freshwater riverine and North Sea marine saltwater. Additional mixing of natural and anthropogenic inputs from the surrounding geology and industry are also observed, affecting Zn, Ni, Co, Re and Os. Macroalgae is also an increasingly popular food, with some species harvested in the Firth of

Forth. Iodine (67-5061 ppm), lead (0.047-4.1 ppm) and cadmium (0.006-0.93 ppm) macroalgal abundances are at safe levels for human consumption (WHO limits). However, many samples exceed the American (3 ppm) and Australian (1 ppm) limits for inorganic arsenic in macroalgae, with values ranging 0-67 ppm. In most of the samples, soaking and cooking the macroalgae reduced the inorganic arsenic content to within the American and Australian limits. However, this has further implications if the macroalgae is used to cook soups (e.g., Dashi), as the leached elements become a significant component of the soup.

### **Key words**

Macroalgae; trace metals; estuary; health; arsenic; iodine

### **1. Introduction**

Macroalgae can reflect the chemistry of the waters in which it lives (Bryan, 1983; Davis et al., 2003; Racionero-Gómez et al., 2016, 2017), and thus can be analysed for a multitude of elements including: trace metals (Balls et al., 1994; Forsberg et al., 1988), rhenium and osmium (Racionero-Gómez et al., 2016, 2017), iodine (Fecher et al., 1998; Teas et al., 2004) and arsenic (Khan et al., 2015; Rose et al., 2007). As such, macroalgae has been shown to be a valuable environmental indicator for marine and estuarine environments (Almela et al., 2006; Bryan, 1983; Fuge and James, 1973; Sproson et al., 2018).

There are a number of different inputs into estuaries such as: riverine freshwater, marine saltwater and anthropogenic discharges from industry and waste (Dobson et al., 2001; Griffiths, 1987; Webb and Metcalfe, 1987). These different inputs carry various elements into the estuary. Analysis of macroalgae can help to understand these inputs, the abundance of the elements within the macroalgae, and the mixing processes (e.g., Bryan, 1983; Francesconi

and Edmonds, 1998; Huang et al., 2013; Sproson et al., 2018).

Some pollutant elements in the aqueous environment, for example Pb and Cd, can become concentrated in macroalgae (Bryan, 1983; Davis et al., 2003; Rose et al., 2007). It is therefore important to understand the metal budget of macroalgae, as it is becoming an increasingly popular food for human consumption (Fecher et al., 1998; Gamallo-Lorenzo et al., 2005; Teas et al., 2004; Mabeau and Fleurence, 1993; van Netten et al., 2000 and references therein). Macroalgae is also used as animal feed; in some coastal regions such as Scotland, sheep commonly graze on seaweed (Bralatei et al., 2017 and references therein). However, there is less control over the chemical quality of macroalgae than for vegetables, which have strict soil quality and pesticide regulations (van Netten et al., 2000). Some of the elements that become concentrated in macroalgae can be toxic and have severe health implications when consumed in high amounts. For example, inorganic As is a carcinogen, Pb causes damage to the central nervous system, Os can affect organ function, and even I (which is essential in the human body) can cause thyroid disorders (e.g., hypo/hyperthyroidism) if consumed excessively (Funari et al., 2016 and references therein; Gamallo-Lorenzo et al., 2005; Khan et al., 2015; Rose et al., 2007; Teas et al., 2004). Therefore, understanding the elemental abundances and inputs into areas in which harvested macroalgae grows is crucial.

Previous studies on the Forth Estuary and Firth of Forth have focussed on the chemistry (e.g., Zn, Al, Cu, Cd, Pb, Ni, Fe, Mn, Ca and nutrients) of the estuarine waters and sediments rather than the macroalgae itself (e.g., Balls et al., 1994, 1997; Balls and Topping, 1987; Dobson et al., 2001; Laslett and Balls, 1995). Sources of pollution into the Firth of Forth and Forth Estuary have decreased over recent decades, with an overall decline in aqueous trace metal and organic inputs (Dobson et al., 2001). It is known that concentrations of trace metals and

nutrients in the waters change along the length of the Firth of Forth and Forth Estuary (Balls and Topping, 1987; Dobson et al., 2001), and trace metal concentrations in the estuarine sediments are higher than in sediments from offshore locations (Balls et al., 1997). These changes are linked to anthropogenic and geological inputs, as well as the mixing between freshwater (west) and North Sea saltwater (east) end members, with salinity playing a role in some dissolved trace element concentrations such as Pb and Cu (Balls et al., 1994; Balls and Topping, 1987; Dobson et al., 2001). Some dissolved trace metals (e.g., Cu, Pb, Ni, Zn, Cd) are also continuously monitored by the Scottish Environmental Protection Agency (SEPA) from three stations/buoys across the Firth of Forth and Forth estuary (SEPA, 2017).

This study utilises macroalgae collected longitudinally along the Forth Estuary and Firth of Forth in Scotland to assess the: 1) elemental abundance change in macroalgae with longitude, 2) mixing of estuarine and freshwater sources, 3) anthropogenic and natural pollutant sources, and 4) implications of elemental abundance in macroalgae harvested for the food industry. To provide a comparison to the latter, we present elemental data for macroalgae, also harvested for the food industry, from a more open marine environment around Hokkaido, Japan.

## **2. Study Area**

The study area comprises an ~80 km stretch of the Forth Estuary and the Firth of Forth in Scotland (referred to collectively as ‘the Forth estuary’), ranging from Kinkardine Bridge in the west to Kingsbarns in the east (Fig. 1).

The Forth Estuary is described as a typical coastal plain estuary, which opens out into the Firth of Forth with significant increases in both width and depth (Webb and Metcalfe, 1987), and is then considered an essentially marine environment as it joins with the North Sea

(Griffiths, 1987). Surrounding the Forth estuary the land consists of a mixture of lowlands and hilly ground, as well as a number of towns/cities and industrialised areas. The geology of the area is reflected in this topography. Prominent features, such as hills, are more resistant plutonic and volcanic Carboniferous-Permian igneous rocks (e.g., Arthurs Seat), whereas the lowlands comprise Devonian-Carboniferous sedimentary units of predominantly sandstone and mudstone (Browne, 1987). The Forth catchment is 4655 km<sup>2</sup> (Dobson et al., 2001), from which a number of rivers and streams drain into the Forth estuary, and the North Sea provides saltwater input. Particularly during low flow periods, there are also significant anthropogenic inputs arising mainly from domestic and trade sources such as coal mining, distilling, oil refining, petrochemicals and domestic dwellings (Griffiths, 1987), although these have declined over recent decades (Dobson et al., 2001). With this mix of fresh water and a tidal influence, salinity is variable its across longitude, ~27 – 34 PSU (SEPA, 2017). Tides are semi-diurnal and are average for the UK (max. range 5 m). Effects of the tides are still seen upstream of Kincardine Bridge (Webb and Metcalfe, 1987), which is as far west as the study area reaches. This means that the whole of the study area is affected by tides to some extent.

### 3. Methodology

#### 3.1 Sample Collection and Preparation

A total of fifty samples of different brown macroalgal species (Phaeophyceae) were collected from 25 locations along the Forth estuary (Fig. 1). *L. digitata* (LD) was selected as this species is commonly harvested and consumed. *F. vesiculosus* (Fv) was found to be the species that is present along almost the entire length of the Forth estuary. *F. serratus* (Fse) and *F. spiralis* (Fsp) represent locations where other macroalgal species were not present. *A. nodosum* (An) was most prevalently observed and collected in the western part of the Forth estuary. Each sample was washed, dried and cut/crushed to <5 mm diameter and stored in

glass vials. Different parts of macroalgae can contain different abundances of elements (e.g., Bryan, 1983; Gall et al., 2004; Racionero-Gómez et al., 2016). Therefore, each sample represents an even as possible mix of tips, blades and vesicles (when present), making up the fronds of the macroalgae. The holdfast and stipe, which typically possess the lowest elemental abundances (Racionero-Gómez et al., 2016), were not included in any sample.

From the Kingsbarns area a large sample of LD was taken and 200 g was powdered in a polycarbonate vessel using a Cryogenic Grinder for use as an 'in house reference material', labelled as, 'RMKB'. From the same locality KB-2 and KB-3 were obtained, representing a washed and unwashed sample, respectively. Additionally, four samples of macroalgae were purchased from the island of Hokkaido, Japan (*L. japonica*, LJ) (HOK 1 to 4), and one from the company Clearspring (JK) (Fig. 2), which we utilise as a reference material (RMJK).

### **3.2 Analytical Details**

Macroalgal samples were analysed for total iodine (I), trace metal abundance (Ag, U, Cd, Co, Ni, Pb, Cu, Zn), arsenic (As), As speciation, rhenium-osmium abundance and isotope composition, using standard published protocol (see Supplementary Material for full details; SM1). In brief, I abundance was determined via alkaline extraction (tetramethylammonium hydroxide; Fecher et al., 1998) and trace metals by 16 N HNO<sub>3</sub> digestion in sealed glass vials, both before quadrupole inductively coupled-plasma mass spectrometry (Q-ICP-MS). For As and As speciation determination two macroalgal species were selected for analysis, Fv as it spans the length of the estuary, and LD, as this is the species that can be harvested. Analysis was carried out using methods described by Bralatei et al. (2017). Total As was measured after microwave-assisted digestion by ICP-MS. Speciation used high performance

liquid chromatography (HPLC) coupled to ICP-MS. Rhenium-Os isotope analysis utilised isotope-dilution negative ion mass spectrometry (Racionero-Gómez et al., 2017).

### ***3.3 Cold, Hot Water, and Soup Experiments***

Each experiment used ~2 g of KB-2, KB-3 and HOK2. In the cold-water, hot water and soup experiments the macroalgae was soaked in room temperature MQ water for 30 mins, put in boiling MQ for 10-20 seconds and soaked in cold MQ for 30 minutes before bringing to boil, respectively. The macroalgae samples were analysed as in sections 3.2. Water samples were analysed directly via ICP-MS.

### ***3.4 Data Evaluation***

Statistical methods used in the evaluation of the data of this study include linear regression ( $R^2$ , p-value) and ANOVA analyses. In parts of the results and discussion, all species and samples are grouped together to show range and average abundance values. This is intended to show the spread of the results, rather than to assume any trend across species. We acknowledge there are multiple differences between species that could affect the abundances of certain elements and therefore the overall trend may not be due to the estuarine location of the particular specimen. ANOVA analysis was used to assess whether different species could have affected the results. Statistical analyses have also been used on individual species for the specific elemental abundances and these results are the main focus of the discussion.

## **4. Results**

### ***4.1 Salinity***

Salinity data provided by SEPA for the Forth estuary is used here. This data has been averaged over days, months and years, and shows a strong positive correlation between

salinity and position along the Forth estuary (SEPA, 2017; Fig. 3; SM4). However, as the salinity data points do not correspond exactly to the macroalgae localities, an equation of the line of best fit (Fig. 3) is used to calculate the corresponding salinities. As a result, the salinity plots onto an exactly straight line. This means when showing element abundance against salinity, the plotted data does not appear to change from that of element abundance against longitude, as salinity (the same as longitude) is just an increasing scale. It is hence considered that the abundance vs. longitude figures discussed (Figs. 4 - 8) also represent abundance vs. salinity, with the x-axis scale ranging from 29 – 36 PSU (practical salinity units).

#### **4.2 Iodine**

Overall iodine abundances are variable (Fig. 4; 67 – 5061 ppm; Average =  $1193 \pm 1676$  ppm, 1SD). Abundances appear to increase seawards across the Forth estuary. However, ANOVA analysis highlights that this trend is likely due to the differences between the species analysed (p-value =  $3.9 \times 10^{-9}$ , f-value = 23). Some individual species though do show an increasing trend seaward, e.g., An (~160 to 490 ppm;  $R^2 = 0.72$ ; p-value = 0.032) and Fsp (~67 to 160 ppm;  $R^2 = 0.79$ ; p-value = 0.043). Fsp has the lowest I abundances (peak of 160 ppm), and LD has the highest but shows no clear trend (fluctuating from ~590 to 5100 ppm; Fig. 4). The species Fv (~120 to 320 ppm) and Fse (~210 to 610 ppm) show a very weak positive trend with longitude (Fig. 4). LJ from Hokkaido show variable I abundances (Fig. 2; 1205 – 4076 ppm; average  $1974 \pm 1717$  ppm, 1SD), similar to Scottish LD samples. RMJK yielded consistent I abundances of ~ 2300 ppm (Average =  $2308 \pm 63.5$  ppm, 1SD, n = 7; SM2c).

#### **4.3 Trace Metals**

Trace metal abundances across all species are variable (see Table 1; Fig. 5; SM3). As with iodine, ANOVA analysis indicates that if all macroalgae species are grouped together, any

trend is likely due to the differences between the species (p-values all <0.023, f-values all >3.3). However, zinc abundances peak mid-estuary and, based on Fv, U abundances increase and Cd abundances show a weak increasing trend seawards (U  $R^2 = 0.79$ , p-value = 0.0013; Cd  $R^2 = 0.36$ , p = 0.085). At 2.85° W, Fv records a Pb abundance (1.8 ppm) and Cu abundance (2.2 ppm) higher than surrounding samples (<0.41 ppm Pb and ~ 1ppm Cu). If these values are removed, a strong decreasing trend in Pb and Cu abundances is observed seawards ( $R^2 = 0.81$ , p-value = 0.0022 and  $R^2 = 0.80$ , p-value = 0.0029, respectively).

Trace metal results for Japanese macroalgal samples are variable (Fig. 2). For example, the highest Zn abundance (21 ppm) is at site HOK-1, whereas the highest Cu abundance (0.69 ppm) is at the RMJK site. LJ correlates well to LD from Scotland, with the exception of Cd, which is elevated to almost ~100x higher in Japanese macroalgae (Fig. 5).

#### **4.4 Arsenic**

##### **4.4.1 Total As**

Total As varies across the Forth estuary (Fig. 6a; 8.0 – 134.5 ppm; average =  $50.64 \pm 34.30$  ppm, 1SD). Fv shows values increasing from 8 ppm (3.7° W) to 35 ppm (2.5° W), ( $R^2 = 0.69$ ; p-value = 0.005). LD does not show a trend ( $R^2 = 0.16$ , p-value = 0.33), but has As abundances much higher than in Fv (41 – 135 ppm). The LJ samples are within the range of the Scottish macroalgal samples (25 – 74 ppm; SM5a).

##### **4.4.2 As speciation**

The highest contributor to total As in LD (41 – 135 ppm) is inorganic As (iAs; 4.21 – 66.86 ppm). The remaining As is comprised of arsenosugars (21.14 – 36.35 ppm) and dimethylarsenic acid (DMA; 0.45 – 3.45 ppm; Fig. 6b; SM5b). No apparent trend in the different As species along the Forth estuary was observed ( $R^2$  all <0.3; p-values all >0.05).

In contrast, Fv contains most of its As content (8.0 – 35 ppm) as arsenosugars (4.39 – 33.31 ppm), with minor contributions by DMA (0.46 – 2.10 ppm) and iAs (0.07 – 0.44 ppm; Fig. 6c). There is an increasing trend in arsenosugars ( $R^2 = 0.61$ ; p-value = 0.013), and weak increasing trend in DMA ( $R^2 = 0.45$ ; p-value = 0.048) seawards along the Forth estuary. For LJ, the main contributor to total As (25 – 74 ppm) is arsenosugars (21 – 49 ppm) then DMA (0.6 – 1.9 ppm), with small amounts of iAs (0 – 0.2 ppm) (Fig. 6d).

#### **4.5 Rhenium-Osmium (Re-Os) Isotope Analysis**

The Re abundance in Fv varies between 15 – 106 ppb along the Forth estuary and between 2.6 – 27 ppb in Japanese LJ (Fig. 7a). The total Os abundances vary between 4.75 – 25.8 ppt in Scotland and 3.7 – 11 ppt in LJ (Fig. 7b). The  $^{187}\text{Re}/^{188}\text{Os}$  values vary between ~10,000 – ~53,000 in Scottish samples, and similarly between ~ 11,000 – ~57,000 in LJ (Fig. 7c).

The  $^{187}\text{Os}/^{188}\text{Os}$  ( $\text{Os}_i$ ) ratio recorded in Fv (Scotland) varies between ~0.80 to 0.92 along the Forth estuary (3.69 – 2.55° W) (Fig. 7d; SM6). Data at mid estuary longitudes (3.3 – 2.9 °W) are sparse due to limited numbers of Fv samples. However, it could be very tentatively inferred that  $\text{Os}_i$ , Re, Os and  $^{187}\text{Re}/^{188}\text{Os}$  values remain roughly constant in the Forth Estuary (3.7 – 3.2° W) before increasing as it opens out into the Firth of Forth (3.2 – 2.5° W). The RMKB sample records two repeatable ratios of 0.80 and 0.81. For the Japanese samples, the  $\text{Os}_i$  values are all ~1 (ranging 0.98 – 1.07; n = 5) (Fig. 7d).

#### **4.6 Soup Experiments**

##### **4.6.1 Trace Metals**

Based on abundances in the macroalgae before and after any experiment, generally the macroalgae contained significantly less Co, Cu, Ag, Pb, Ni, Cd and U, after the extraction by

the cold, hot and soup experiments (percentage decreases of 28 %, 27 %, 28 %, 49 %, 50 %, 26 % and 37 %, respectively) (Fig. 8; SM7a,b). Zinc was less extractable by these methods (10 % decrease). A short hot water treatment generally extracts the least trace metals, whereas the most metals are extracted during the soup treatment.

#### ***4.6.2 Iodine***

In macroalgae, the cold and soup experiments show the greatest loss of I (79-98 % and 85-97 %, respectively), and hot water the least (9-82 %; Fig. 9a; SM7c). The remaining liquid from the experiments generally shows the highest I concentrations in those from the soup experiments. This is with the exception of KB-3 unwashed, where the hot water experiment results in the highest iodine values in the liquid (Fig. 9b; SM7c).

#### ***4.6.3 Arsenic***

In all three extraction experiments, total and inorganic arsenic abundances are lower in the solid sample after treatment (Fig. 9c,d; SM7d). The soup experiment results in the greatest loss of As (87-91 %) and iAs (75-96 %), the hot water experiment resulted in the least loss of As (21-39 %) and iAs (3.4-61 %), and the cold water experiment causes a moderate loss in As (45-91 %) and iAs (65-66 %). The As and iAs abundances in the remaining liquid are the highest in the soup experiment (where the seaweed lost the most As) (Fig. 9e,f, SM7d).

## **5. Discussion**

### ***5.1 Macroalgal elemental change within an estuarine environment***

#### ***5.1.1 Iodine***

The I abundance in seawater (~0.06 ppm) is 16 times that of freshwater (~0.0038 ppm) (Gall et al., 2004; Jones and Truesdale, 1984). This is potentially linked to the change in salinity, which is considered to affect the I abundance in macroalgae (Teas et al., 2004). Abundance vs. salinity can give insights into mixing processes taking place by assuming that macroalgal

element abundance represents relative solute concentrations in the surrounding waters. Utilising this for I (Fig. 4), it is likely that natural mixing between the North Sea in the east (~0.06 ppm) and freshwater riverine inputs in the west (~0.0038 ppm) are a controlling factor on the increase in I abundance seen in some macroalgal species (Fig. 4) along the Forth estuary. Iodine in the ocean is mainly in the form of inorganic iodine, iodide ( $I^-$ ), and iodate ( $IO_3^-$ ) (Gall et al., 2004 and references therein). In macroalgae, I is usually accumulated and stored as inorganic iodine. For example, iodine in LD is ~88 % iodide, 10 % organic iodine and ~1.4 % iodate (Gall et al., 2004; Gamallo-Lorenzo et al., 2005; Teas et al., 2004).

However, as the data are not perfectly linear (Fig. 4), an increase in salinity cannot alone explain all the I abundances observed. Another possible influence is tidal elevation. The more that macroalgae is exposed to air, the more I can be lost through desiccation and oxidation stress (Gall et al., 2004; Huang et al., 2013; Teas et al., 2004). Therefore, samples collected from western localities along the Forth estuary and more exposed by the tide could lose more I than other seaward samples. Further, submerged samples will be able to uptake I from seawater over a larger percentage of the tidal cycle, and hence over their lifetime.

There is also variation in I within individual seaweed samples. More distal parts of a macroalgae blade contain higher I abundances than the more central parts, such as the holdfast (Gall et al., 2004; Teas et al., 2004). Iodine abundances are also higher in juvenile plants due to their higher surface area to volume ratios (Gall et al., 2004). Although during sample preparation (Section 3.1) macroalgal samples included an even as possible mix of the tips, blades and vesicles, excluding the holdfast and stipe, this does not completely rule out sample heterogeneity. For example, the I abundance reproducibility of RMKB was  $3958 \pm 965$  ppm (1SD) prior to undertaking the analysis on powdered aliquots (SM2b,c). This could

account for some variability within individual species, and analyses of more homogenised samples (e.g. powdered) would be needed to establish a firm trend.

### *5.1.2 Trace metals*

The trend in macroalgal trace metal abundance in the Forth estuary was  $Ag < U < Cd < Co < Ni < Pb < Cu < Zn$ , which broadly follows the same trace metal concentrations in the Forth estuary water,  $Cd < Ni \sim Pb \sim Cu < Zn$  (Fig. 10; SEPA, 2017). The concentration of trace metals in the surrounding waters therefore plays a role in the abundance of trace metals found in macroalgae (Bryan, 1969; Forsberg et al., 1988; Fuge and James, 1973). Additionally, the sequence of trace metal abundance may relate to the affinity of macroalgae to different metals. Phaeophyceae uptake mainly bioavailable trace metals as labile divalent cations (Bryan, 1983; Fuge and James, 1973; Vicente-Martorell et al., 2009) from surrounding waters by various processes that include ion exchange (Bryan, 1983; Davis et al., 2003) and metabolic mechanisms (Bryan, 1983), with the metals (that are not required for metabolism) bound to polysaccharides in the cell wall (e.g., alginate, fucoidan and polyphenols; Forsberg et al., 1988). For example, in *Fv*, polyphenols display the following trend in trace metal affinity,  $Cd < Co < Zn < Ni < Pb < Cu$  (Bryan, 1983). Other than Zn, this order follows that of trace metal abundance found in Scottish macroalgae. This may suggest that the affinity of macroalgal components to different trace metals also plays a role in the observed order. The higher Zn abundances found in the Scottish macroalgae could be because Zn is an essential element in macroalgae and also when the metal is bound, very little is then lost (Bryan, 1969). A number of controls on trace metal abundance is supported by the variability of trends in macroalgae and waters shown for other estuaries and coastal environments (e.g., Bryan, 1969; Fosberg et al., 1988; Fuge and James, 1973; Vicente-Martorell et al., 2009).

Trace metal concentrations of the water in the Forth estuary decrease seaward (SEPA, 2017; Fig. 10; SM8). This correlates well to Pb and Cu abundances in macroalgae, but not for Zn and Ni. The seaward decreasing trend in macroalgal Pb and Cu abundances (shown by Fv) could be explained by the mixing of freshwater and seawater end members (Fig. 5f, g; Fig. 10), as both metals have been shown to exhibit changes in concentrations along salinity gradients. Both trace metals are removed from solution at higher salinities (Balls et al., 1994; Balls and Topping, 1987; Vicente-Martorell et al., 2009). Therefore, lower Pb and Cu water concentrations equate to less metal availability for uptake by macroalgae. Copper may also be sourced at low salinities from the breakdown of organic matter (Balls et al., 1994 and references therein), and Pb from runoff of particulate Pb in rivers (Balls et al., 1994), which would be higher in the western end of the Forth estuary. These removal and addition processes indicate potentially non-conservative mixing processes. Further variability in the Forth estuary could be a result of pollutants from sewage or industry. For example, at 2.85° W (Site 23; Fig. 1) both Pb and Cu (and possibly also Ni and Co) abundances in Fv samples show a rise in abundance to >1 ppm. Site 23 is located within an inlet, where the macroalgae is less exposed to seawater, and more exposed to a small stream (Peffer Burn) and the surrounding geology. If Pb and Cu concentrations in the stream water are high, they will not become as diluted by the seawater, potentially leading to the higher abundances recorded in the macroalgae. This point in the Pb and Cu figures (Fig. 5f, g) was therefore removed when calculating  $R^2$  and p-values for these trends. Additionally, most of the trace metal data also shows a significant increase in abundance in An and Fsp at a longitude of ~3.4 – 3.5° W. There are no significant riverine inputs in this area, but there is a potential industrial source. At Rosyth (Site 16) at the time of sample collection, construction of the Queen Elizabeth aircraft carrier ship was taking place. The trace metals contained in the materials and chemicals used in the construction had an easy entrance pathway into the surrounding waters

and could have been taken up by the macroalgae during the six-year construction.

In contrast, Ni and Zn do not correlate with the SEPA trace metal water data. The Ni and Zn in macroalgae show no definitive trend and are relatively constant across longitude, but Zn shows a mid-estuary peak in abundance (Fig. 5e, h). This suggests a mid-estuary source for Zn, perhaps from riverine input or industry (e.g., from Edinburgh or the Waters of Leith). This could also account for mid-estuary peaks in other elements such as Co and Cd. Indeed, sewage discharges are concentrated between Edinburgh and Cockenzie on the south side of the Firth of Forth (Balls and Topping, 1987) and non-conservative addition of Zn and Ni has been noted across the Forth estuary, regardless of salinity (Laslett and Balls, 1995).

The Cd macroalgal data (Fig. 5c) shows a weak increasing trend seaward (based on Fv). This could relate to the increase in seawater component east along the Forth estuary. Studies have shown that in estuarine environments Cd concentrations in solution can increase with higher salinities. An increase in salinity causes the mobilisation of Cd from riverine particulate matter into solution (Balls et al., 1994; Comans and van Dijk, 1988, and references therein). Therefore, with more mobilised Cd in solution at higher salinities (i.e., more seaward), there is more available to be taken up by the macroalgae. Despite the increasing Cd trend in macroalgae, the Cd concentration in the water of the Forth estuary is almost constant (0.01 – 0.02 ppb; SEPA, 2017; Fig. 10). However, this is based on only three localities in the Forth estuary, with no sites at locations that would receive the highest salinities. Moreover, the concentrations in solution are relatively low, so any significant trend may be hard to detect.

Trace metal abundances in the Japanese LJ macroalgal samples (Fig. 2), with the exception of Cd, are similar to those of Scottish LD (Fig. 5). Given that LD in the Forth estuary is present

in more marine conditions less influenced by tidal elevations, and that LJ is sampled offshore Hokkaido, the similar trace element abundances suggest common uptake mechanisms for the species and/or similar seawater chemistry. In contrast, Cd is up to almost 100 times more enriched in LJ. This species may therefore have a stronger uptake affinity for Cd than LD, or perhaps the waters surrounding Hokkaido have a higher Cd concentration than those in Scotland. Other commercially sold and regularly consumed algae products harvested from multiple localities in Japan also show a similar range of Cd abundances (average 0.180 – 1.650 ppm; Kikuchi et al., 2002). This would suggest macroalgae species in Japan are receiving a higher environmental Cd influence than those in Scotland. Three of the five LJ samples that are particularly enriched in Cd (~0.3 to 0.9 ppm) analysed in this study are more closely associated with Quaternary volcanic systems and/or areas of mining. Whereas the other sample sites are more proximal to a coastline composed of clastic sedimentary rocks (Ohta et al., 2005). Stream sediment Cd concentrations around Hokkaido are also highly variable (0.02 – 5.19 ppm), which reflects the streams proximity to these volcanics and mineral deposits (Ohta et al., 2005). This could help cause Cd enrichment in local seawater. The areas surrounding Hokkaido are also likely to have higher salinities than those in the Forth estuary, so any particulate-bound Cd may be more likely to be mobilised into solution (Comans and Dijk, 1988), and could potentially raise Cd concentrations of the water further.

Overall, macroalgal trace metal amounts in the Forth estuary are relatively low compared to other areas of the UK and Europe. For example, Fv from Aberystwyth and Nefyn in Wales (Fuge and James, 1973) and Stockholm in Sweden (Bryan, 1969) record higher Zn, Cu, Ni, Co, Cd and Pb abundances than in Scotland. Specifically in Fv, Zn abundances are ~16 and 23 times higher in Aberystwyth and Stockholm, respectively, than in the Forth estuary. This difference is likely controlled by a number of factors, e.g., local geology and inputs.

### 5.1.3 Arsenic

Total macroalgal As abundances for the Forth estuary (12 – 135 ppm) and for Hokkaido (25 – 74 ppm), are similar to the values previously reported (0.031 – 149 ppm) (Almela et al., 2006; Khan et al., 2015; Koch et al., 2007; Ronan et al., 2017; Rose et al., 2007). In most oxygenated waters, arsenate (iAs(v)) is the dominant arsenic species (Francesconi and Edmonds, 1998; Sharma and Sohn, 2009), although arsenite (iAs(III)) can be formed by biological reduction processes (Francesconi and Edmonds, 1998). In oxygenated waters, iAs(v) is bioavailable to macroalgae and is absorbed as hydrogen arsenate ( $\text{HAsO}_4^{2-}$ ) (Ronan et al., 2017). The majority of the iAs is thought to be rapidly detoxified by methylation and alkylation processes and converted mainly into  $\text{As}_{\text{org}}$  species such as arsenosugars and arsenolipids (Francesconi and Edmonds, 1998; Ronan et al., 2017). Arsenosugars are one of the dominant As species in this study (all samples avg 59 %), but the proportion is less than previously reported in LD and Fv (~85 %) (Sharma and Sohn, 2009 and references therein).

Another significant As species is iAs. iAs is the largest contributor to total As in LD (avg 37 %), whereas Fv has smaller abundances (avg 2 %). These iAs values are comparable to that of LD in Ireland (avg 35 %; Ronan et al., 2017), and Fv sold in Spain (0.72 %; Almela et al., 2006). In contrast, LJ from Hokkaido possesses only an average of 0.3 % iAs, similar to other LJ from Japan (avg 0.74 %; Almela et al., 2006). The iAs abundances measured in seaweed species Fv and LJ are consistent with the majority of seaweeds, which possess less than 20 % iAs of the total As abundance. However, the species LD generally exceeds this (Koch et al., 2007; Laparra et al., 2003, Ronan et al., 2017).

Total seawater As concentration is usually around 2 ppb (Francesconi and Edmonds, 1998),

and in freshwater, total As varies between 0.15 – 0.45 ppb (Sharma and Sohn, 2009). Therefore, the increasing As trend shown in Figure 6a, when compared to salinity (Fig. 3), could be explained by the mixing of seawater and freshwater. It has been noted that As can form a strong positive correlation with salinity, and that As displays conservative behaviour (Larsen and Francesconi, 2003; Waslenchuk and Windom, 1978). However, variability in the data will arise from in-estuary sources of As. Arsenic is found in many rock types and, as it is fairly soluble, can be dissolved out and into streams and rivers, as well entering watercourses from anthropogenic sources such as pesticides, coal burning, and processing of mineral ores (Rose et al., 2007). Due to the many riverine and industrial inputs into the Forth estuary (Fig. 1), there are many potential As pathways. As discussed previously, sample heterogeneity can also impact on As and iAs abundances. Ronan et al. (2017) report that higher iAs abundances recorded in LD were from the decaying distal tips. Therefore, the absolute abundance and safety of macroalgae in regard to As is complicated.

#### ***5.1.4 Rhenium-Osmium***

Fv was selected for Re-Os analysis as it spans the length of the Forth estuary and reliably records Re-Os data in relation to the water mass in which it lives (Racionero-Gómez et al., 2016; Rooney et al., 2016; Racionero-Gómez, 2017; Sproson et al., 2018). Fv contains Re (20 – 95 ppb) and Os (5 – 25 ppt; Fig. 7a, b) abundances within the range of previously published data (Re = 0.1 – 313 ppb; Os = 3.3 – 254.5 ppt) (Racionero-Gómez et al., 2016; 2017; Rooney et al., 2016; Sproson et al., 2018). The higher Re and Os values seaward (Fig. 7) could reflect the higher average concentrations in seawater (0.01 ppt Os, 8.2 ppt Re) compared to riverine water (0.0091 ppt Os, 0.43 ppt Re) (Peucker-Ehrenbrink and Ravizza, 2000). It has been shown in previous studies that Re behaves conservatively in estuarine environments, with higher concentrations at higher salinities (Sproson et al., 2018 and

references therein), and this therefore could be reflected in the macroalgae (Fig. 7a). Osmium has previously been shown to behave non-conservatively (Sproson et al., 2018 and references therein). The Os data in this study would seem to support a conservative nature of Os in estuaries, with lower abundances recorded in the macroalgae at lower salinities (Fig. 7b;  $R^2 = 0.3$ ). Other studies also show the removal of Os from the water column in temperate and arctic estuaries at lower salinities (Sproson et al., 2018 and references therein). However, this is further complicated by competition between Re and Os due to similar uptake mechanisms into the macroalgae. This can cause lower macroalgal Os abundances in waters of higher Re concentrations, and vice versa (Racionero-Gómez et al., 2017; Sproson et al., 2018).

In an estuary, macroalgae will be exposed to varying amounts of seawater (containing higher Re and Os) over a tidal cycle. Therefore, samples that are more commonly submerged would be influenced more by waters of higher Re and Os concentrations than samples further inland (Figs. 7a, b; Sproson et al., 2018). For example, at  $2.52^\circ$  W (Site 25 – south coast near Dunbar) Fv possesses a lower Os abundance (11 ppt) than that of at  $2.54^\circ$  W (Site 4 – Isle of May; 25 ppt). This may be because the River Tyne enters the North Sea very close to Site 25 and could be altering the chemistry of the surrounding waters, affecting the Os abundance.

When compared to the Japanese LJ species, which is more indicative of a marine environment, Os levels are generally in agreement, but the Re levels of Fv from the Forth estuary are up to 10 times higher. This could indicate more localised Re inputs into the Forth estuary and deviation away from a marine setting. However, it must be considered that the Scotland and Japanese macroalgae are of different species, and so the differences may be related to differing species uptake, rather than geographical location.

The range in in  $^{187}\text{Re}/^{188}\text{Os}$  values (10,000 – 50,000) are related to the Os abundance showing little variation (5 – 25 ppt) while the Re abundance (20 – 95 ppb) varies greatly within the Forth estuary. The  $^{187}\text{Re}/^{188}\text{Os}$  values are similar to those previously published (65 – 40,320; Racionero-Gómez et al., 2017; Rooney et al., 2016; Sproson et al., 2018). Typical river (227) and seawater (4270)  $^{187}\text{Re}/^{188}\text{Os}$  values (Peucker-Ehrenbrink and Ravizza, 2000) are much lower than macroalgae. These values support previous research showing that  $^{187}\text{Re}/^{188}\text{Os}$  values are significantly higher in macroalgae than the surrounding environment, but that it does mimic the patterns (i.e., higher  $^{187}\text{Re}/^{188}\text{Os}$  values in macroalgae and seawater at higher salinities) and therefore macroalgae can be used as a qualitative indicator of seawater  $^{187}\text{Re}/^{188}\text{Os}$  (Racionero- Gómez et al., 2017; Sproson et al., 2018).

The  $^{187}\text{Os}/^{188}\text{Os}$  values (0.80 – 0.92; Fig. 7d) fit with previously published data for macroalgae (0.16 – 1.89), but very depending on the environment where the macroalgae lives (Racionero-Gómez et al., 2017; Rooney et al., 2016; Sproson et al., 2018). The  $^{187}\text{Os}/^{188}\text{Os}$  composition represents a ratio between radiogenic continental inputs ( $\sim 1.4$ ) and unradiogenic mantle/hydrothermal inputs ( $\sim 0.12$ ; Peucker-Ehrenbrink and Jahn, 2001; Peucker-Ehrenbrink and Ravizza, 2000). The global riverine  $^{187}\text{Os}/^{188}\text{Os}$  value is  $\sim 1.4$ , with seawater  $^{187}\text{Os}/^{188}\text{Os}$  less radiogenic at  $\sim 1.06$  (Peucker-Ehrenbrink and Ravizza, 2000). However, rivers across the world produce highly variable  $^{187}\text{Os}/^{188}\text{Os}$  ratios due to factors such as the local geology (Sproson et al., 2018 and references therein). As such, the estuarine  $^{187}\text{Os}/^{188}\text{Os}$  composition in the Forth estuary may be less radiogenic than both the average riverine ( $\sim 1.4$ ) and seawater ( $\sim 1.06$ ) values due to the local geology containing a number of mafic igneous bodies that would have a less radiogenic, mantle like  $^{187}\text{Os}/^{188}\text{Os}$  (Fig.1, Fig. 7d). Further, anthropogenic Os inputs, such as sewage and catalytic converters, generally record similar  $^{187}\text{Os}/^{188}\text{Os}$  values to unradiogenic mantle inputs ( $^{187}\text{Os}/^{188}\text{Os} = <0.7$ ), although depending on the source

these values can be variable (e.g., burning of fossil fuels  $^{187}\text{Os}/^{188}\text{Os}$  often  $>1.2$ ) (Funari et al., 2016 and references therein; Peucker-Ehrenbrink and Ravizza, 2000). Therefore, the numerous industrialised areas along the Firth of Forth (e.g., Grangemouth and Edinburgh) could also contribute to unradiogenic  $^{187}\text{Os}/^{188}\text{Os}$  ratios. This, alongside the tidal nature of the Forth estuary, could account for no apparent trend in  $^{187}\text{Os}/^{188}\text{Os}$  compositions (Fig. 7d).

In contrast, LJ from Hokkaido possess more open ocean  $^{187}\text{Os}/^{188}\text{Os}$  compositions ( $\sim 0.98 - 1.09$ ; Fig. 7d), similar to Pacific Ocean values (1.06). The  $^{187}\text{Os}/^{188}\text{Os}$  of 1.09 corresponds to the sample HOK-1, which is the only sample on the west coast of Hokkaido. This value is therefore more indicative of the Sea of Japan and may be slightly more radiogenic due to continental weathering around all sides of the sea.

## 5.2 Implications of macroalgae as a food source

### 5.2.1 Iodine

Iodine is an essential element in the human body (Gamallo-Lorenzo et al., 2005). The recommended daily allowance (RDA) of iodine for adults is 150 – 200  $\mu\text{g}$ , although this amount is rarely reached (Fecher et al., 1998). Seaweeds are therefore a valuable, natural source of iodine. Using the highest I abundance recorded in LD (the harvested species) from the Forth estuary of 5060  $\mu\text{g}/\text{g}$  (ppm), only 0.03 g of the seaweed would need to be consumed per day to reach a daily I consumption of 152  $\mu\text{g}$ . However, as only such small amounts are required, it would be very easy to exceed the RDA of iodine, which can cause thyroid disorders (Gamallo-Lorenzo et al., 2005; Teas et al., 2004). Soaking/washing the macroalgae can reduce the amounts of certain elements (Teas et al., 2004). In this study (Fig. 9a), cooking and cold-water soaking are the most effective methods of reducing the I abundance of macroalgae, although a 30 second blanch in hot water was also fairly effective in the

Scottish samples (KB-2, KB-3). However, if using macroalgae to make soup (e.g., Dashi), the I is readily leached from the macroalgae into the water, producing high I abundances in the resulting soup. From using 2 g of the HOK2 sample, the waters left behind after the soup experiment (highest of all samples) contained 66  $\mu\text{g/g}$  I or 12,700  $\mu\text{g}$  of I (in 192 g) (Fig. 9b). Only 3 g of the liquid soup would provide 198  $\mu\text{g}$  of I and would fulfil the RDA. This is after only 2 g of macroalgae is cooked, so changes in the amount of macroalgae and the volume of water used will also alter the I content in the remaining liquid.

Based on these experiments, I is stored in a form that is readily leached by water. In the macroalgal species LJ, 99.2% of the iodine was found water soluble and lost after multiple leaches (Hou et al., 1997). In this study, results after the cold and hot water leaches show that between 85 and 97 % of I was lost from the macroalgae, so it is likely further leaching would also see a loss of up to 99 %. Inorganic iodine is water soluble, so any iodine left behind after leaching is likely to be organic (Hou et al., 1997). Our leach experiments therefore agree with previous results that iodine in macroalgae is mainly in the form of inorganic I, with lesser amounts of organic I (Gall et al., 2004; Gamallo-Lorenzo et al., 2005; Teas et al., 2004).

### **5.2.2 Trace elements**

For trace metals, the studied macroalgae is within safe limits. The WHO provisional tolerable weekly intake (PTWI) for Pb and Cd is 25  $\mu\text{g/kg}$  and 7  $\mu\text{g/kg}$  body weight, respectively (COT, 2008). Using the highest abundances of Pb (0.94 ppm) and Cd (0.029 ppm) in LD from Scotland, an average 70 kg person would need to eat ~265 g of macroalgae per day to reach the Pb limit, and over 2 kg to reach the Cd limit, which is extremely unlikely. In comparison, using the highest abundances of Pb (0.2 ppm) and Cd (0.9 ppm) in LJ from Hokkaido, a 70 kg person would need to eat ~1.25 kg of macroalgae to reach the Pb limit and

~79 g per day to reach the Cd limit. This is again unlikely but highlights the differences between the Cd and Pb contents of the two seaweed species from different localities.

The leach experiments do not show a dramatic loss in trace metal abundance compared with I (Fig. 8). This may be due to some trace metals binding more tightly to organic compounds (Forsberg et al., 1988, and references therein), whereas iodine is soluble and also easily lost as gas (Huang et al., 2013; Teas et al., 2004). For most trace metals (Cu, Co, Pb, Ag, Ni and U), cooking or cold water soaking the macroalgae does help reduce the abundance. Lead abundance is reduced in all the leach experiments. This ability to reduce the Pb abundance of LD is also shown between the washed KB-2 and unwashed KB-3 samples. The washed KB-2 initial Pb abundance (0.036 ppm) is roughly the same as the unwashed KB-3 value after it has undergone the leaching experiments (~0.025 – 0.036 ppm). This would suggest that the water-soluble portion of Pb is leached from the macroalgae through the simple process of washing, or that some of the Pb may be on the outside of the macroalgae rather than contained within the cells, and therefore an additional length of soaking or temperature makes limited reductions. This is also shown for Co. Zinc shows the greatest variability. In the KB-2 and KB-3 samples, the soup experiments (and hot water experiment in KB-3) show increases in Zn abundance, rather than a decrease. This may be best explained by heterogeneity in the original sample, as separate samples were needed for each of the experiments, (i.e., a separate 2 g of KB-2 was used for each of the hot, cold and soup experiments).

### 5.2.3 Arsenic

It is generally accepted that iAs (arsenate iAs(V) and arsenite iAs(III)), is more toxic than organic As ( $As_{org}$ ; arsobentine and arsenosugars), with iAs(III) being more toxic than iAs(V) (Khan et al., 2015; Rose et al., 2007). iAs(V) Arsenite and arsenate are considered a class 1

carcinogen (Bralatei et al., 2017 and references therein). In the UK there are currently no regulations for total As and iAs in food and macroalgae (Bralatei et al., 2017 and references therein; Petursdottir et al., 2015; Rose et al., 2007), but there are in other countries. The iAs abundances in the studied LD (4.2 – 67 ppm) are in excess of the iAs abundance legislation for macroalgae in Australia and the USA (1 and 3 ppm, respectively; Mabeau and Fleurence, 1993). The tolerable daily intake of iAs is 150 µg for a 70 kg adult (WHO, 1989). As such, a 70 kg adult could only eat 2.25 g of raw LD using the sample with the highest iAs abundance (67 ppm, 2.7° W). This amount would be lower for smaller adults and children. Additionally, although Fv from the Forth estuary possesses iAs abundances (0.07 – 0.53 ppm) below the regulated levels, caution is advised given the high abundance of arsenosugars (4.4 – 33.3 ppm). In the body, arsenosugars can be converted into more toxic forms of As, such as DMA and monomethylarsonic acid (MMA) (Koch et al., 2007; Sharma and Sohn, 2009).

As shown by previous studies (Almela et al., 2005; Laparra et al., 2003; Rose et al., 2007), the total As and iAs in macroalgae (LD and LJ) decreases if pre-soaked or cooked. Soaking or cooking the macroalgae decreases the total As and iAs abundance in most cases by over 50 % (Fig. 9c, d). Following the soup experiment, all three samples (KB-2, KB-3 and HOK2) would pass the USA regulation of 3 ppm iAs in macroalgae, with two samples also passing the Australian regulation of 1 ppm iAs. However, the iAs is then leached into the water, which can be consumed as part of a meal (e.g., Dashi). If an entire soup made from 2 g of macroalgae were to be consumed, between ~40 and 51 µg of iAs could be ingested (Fig. 9e, f). The recipes for making Dashi and other soups containing macroalgae vary considerably in macroalgae amount and volume of water used. Therefore, abundances of iAs remaining in the liquid will be highly variable and could potentially exceed the tolerable daily intake.

## 6. Conclusion

Most of the elements analysed across the Forth Estuary and the Firth of Forth show an increasing trend (As, I, Cd, U, Re, Os), a decreasing trend (Pb, Cu) or a mid-estuary peak (Zn) in macroalgal abundance (based mainly on species Fv). This is controlled by a number of factors including salinity, mixing, and intra- and inter-macroalgal species differences such as affinity for uptake, and atmospheric exposure. Mixing between freshwater riverine inputs and marine inputs from the North Sea affect elemental abundances within the same species. Non-conservative mixing may also play a role, with in-estuary sources and sinks of various elements; e.g., from natural and anthropogenic inputs such as geology, sewage and industrial wastes. Despite pollutant inputs, macroalgae in the Forth estuary generally contains lower trace metal abundances than elsewhere in the UK and Europe. Iodine, lead, and cadmium are within safe limits for consumption, as long as amounts are controlled. However, levels of iAs in the species LD (up to 67 ppm) greatly exceed safe iAs levels in macroalgae set by America (3 ppm) and Australia (1 ppm). Elements (particularly I and iAs) can actually be decreased by cooking and soaking the samples. However, the leached elements are then contained in the resultant soup (e.g., Dashi), which may too exceed recommended levels if ingested.

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**Figure Captions**

**Figure 1** – a) Map of the UK showing the location of the studied area in red, b) Simplified map of the Forth Estuary and Firth of Forth in Scotland, called collectively ‘the Forth estuary,’ showing: the sample sites from which macroalgae was collected; locations of some cities/towns and islands; the main rivers and tributaries draining into Forth estuary; the locations of areas of notable industry. The map also shows a longitudinal scale along the x-axis, which corresponds to the longitudes shown in the graphs of other figures. Map adapted in Inkscape from Browne (1987) and <https://www.google.co.uk/maps>.

**Figure 2** - The abundance, ppm (dw), of trace metals Co, Ni, Cu, Zn, Ag, Cd, Pb, U and other elements As and I in Japanese *LJ* macroalgae samples from the island of Hokkaido  $\pm$  2SE. Values to 2 decimal places, I to 4 significant figures, BD = below detection. Note the elements are not listed in any particular order as order of element abundances vary between different sites. The yellow dots show the locations of the origin of samples HOK 1-4 and RMJK. Image in Inkscape adapted from <https://www.google.co.uk/maps>.

**Figure 3** - Data provided by the Scottish Environment Protection Agency (SEPA) for salinity (PSU – practical salinity units). Data points represent an average of values recorded at that longitude over the years 2013 – 2015. The equation  $y = 5.5754x + 49.62$  corresponds to the line of best fit, with  $R^2 = 0.72611$ .

**Figure 4** - Iodine abundance, ppm dry weight (dw), against longitude across the Forth Estuary and Firth of Forth in Scotland for each macroalgae species plotted on a log (base 10) graph. The dashed line is the approximate longitude at which the Forth Estuary opens out into the Firth of Forth. W = west, E = east. Error bars of 2 x standard error are in most cases these are covered by the markers. FSp = *F. spiralis*, FSe = *F. serratus*, An = *A. nodosum*, Fv = *F. vesiculosus*, LD = *L. digitata*. J represents Japanese *L. japonica* I abundances, which have no relation to longitude and have been added at 2.5° W purely for comparison with the Scottish

samples. This figure also doubles as I abundance vs. salinity, with the x-axis scale ranging from 29 – 36 PSU (practical salinity units). Linear trendlines are removed for clarity.

**Figure 5** - The abundance, ppm (dw), of: a) Ag, b) U, c) Cd, d) Co, e) Ni, f) Pb, g) Cu, h) Zn in each macroalgae species shown against longitude. The dashed line represents the approximate longitude at which the Forth Estuary opens out into the Firth of Forth. W = west, E = east. J represents *L. japonica*, it has no relation to longitude and is added purely for comparison with the Scottish samples. These figures also double as abundance vs. salinity, with the x-axis scale ranging from 29 – 36 PSU (practical salinity units). Linear trendlines are removed for clarity.  $R^2$  values and p-values for the species Fv are: a) 0.014, 0.76; b) 0.79, 0.0013; c) 0.36, 0.085; d) 0.070, 0.49; e) 0.18, 0.26; f) 0.81, 0.0022 (not including 2.85° W); g) 0.80, 0.0029 (not including 2.85° W); h) 0.00, 0.98.

**Figure 6** – a) The abundance, ppm (dw), of arsenic in each macroalgae species *Laminaria digitata* (LD), *Fucus vesiculosus* (Fv) and *Laminaria japonica* (J) shown against longitude across the Forth Estuary and Firth of Forth with a line of best fit ( $R^2 = 0.69$ ) for the species Fv. W = west, E = east. The abundances of J represent the Japanese samples and do not relate in any way to the longitudinal scale, but are just added for comparison. This figure also doubles as As abundance vs. salinity, with the x-axis scale ranging from 29 – 36 PSU (practical salinity units). Next, the major As species components, arsenosugars (As-sug), dimethylarsinic acid (DMA), inorganic As (As(v)) and the total As (total digest), of macroalgae samples b) *L. digitata*, c) *F. vesiculosus*, and d) *L. japonica*. The Scottish samples (b and c) are compared against a longitudinal scale. The Japanese samples (d) are not related to longitude and are against an arbitrary x-axis, samples 1-3 are repeats of RMJK and sample 4 is HOK2. Linear trendlines are removed for clarity.

**Figure 7** - Re-Os data recorded in *F. vesiculosus* shown against longitude. Error bars represent 2 x standard error (2SE). Data shows: a) Re (ppb), b) Os (ppt), c)  $^{187}\text{Re}/^{188}\text{Os}$ , and

d)  $^{187}\text{Os}/^{188}\text{Os}$ . The dashed line is the approximate longitude at which the Forth Estuary opens out into the Firth of Forth. W = west, E = east. Samples marked J represent Japanese samples HOK1-4 and RMJK. These do not relate to the longitude and are shown for comparison between the Scottish and Japanese samples. These figures also double as abundance vs. salinity, with the x-axis scale ranging from 29 – 36 PSU (practical salinity units). Linear trendlines are removed for clarity.

**Figure 8** - The abundance of trace metals Co, Cu, Ag, Pb, Ni, Zn, Cd, U (ppm dw) in the original solid macroalgae samples and remaining in the same sample after the hot water, cold water and soup leaching experiments. Symbol size is greater than analytical uncertainty.

**Figure 9** - a) Iodine abundance (ppm) of the solid seaweed in the leach experiments of three samples. The original samples are shown alongside the abundances of iodine remaining after the hot water, cold water and soup experiments. b) Iodine abundance (ppm) of the remaining liquid in the leach experiments of three samples. The abundances of iodine remaining after the hot water, cold water and soup experiments are shown. c) Total and d) inorganic arsenic abundance (ppm) of the solid seaweed sample in the leach experiments of three samples. The original samples are shown alongside the abundances of total As and iAs remaining after the hot water, cold water and soup experiments. Followed by e) Total and f) inorganic arsenic abundance (ppm) of the liquid remains in the leach experiments of three samples. The abundances of arsenic and iAs are shown remaining after the hot water, cold water and soup experiments. Symbol size is greater than analytical uncertainty.

**Figure 10** - Data provided by the Scottish Environment Protection Agency (SEPA) for trace metal concentration of the waters (ppb). Data points represent an average of values recorded at that longitude over the years 2013 – 2015.

Trace metal	Range	Average $\pm$ 1SD
<b>Ag</b>	0.0038 - 0.20	0.069 $\pm$ 0.056
<b>U</b>	0.097 - 0.81	0.38 $\pm$ 0.22
<b>Cd</b>	0.0061 - 0.92	0.14 $\pm$ 0.19
<b>Co</b>	0.044 - 1.6	0.36 $\pm$ 0.36
<b>Ni</b>	0.16 - 3.1	0.86 $\pm$ 0.71
<b>Pb</b>	0.046 - 4.1	0.77 $\pm$ 0.1
<b>Cu</b>	0.47 - 7.2	1.4 $\pm$ 1.3
<b>Zn</b>	6.5 - 45	17 $\pm$ 9.2

Table 1: Range and average abundances (all ppm) for trace metals across all Scottish macroalgae studied (n=40).

**Highlights**

- Estuarine macroalgae elemental abundance controlled by a number of factors
- Conservative and non-conservative mixing processes affect element enrichment
- Anthropogenic and geological inputs are observed
- Inorganic arsenic levels in macroalgae exceed American and Australian limits
- Levels of arsenic, iodine and others reduced in macroalgae when soaked and cooked

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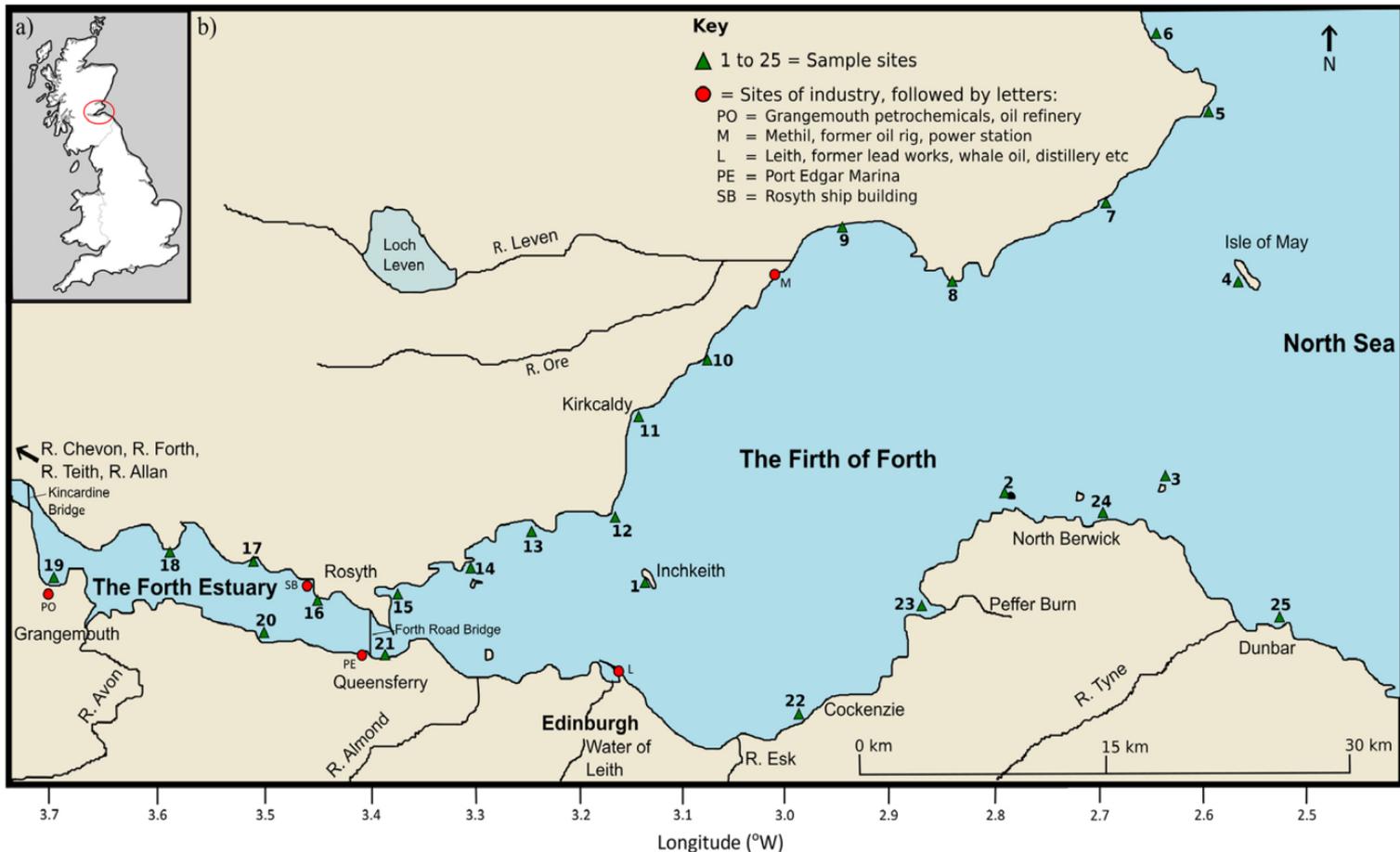


Figure 1

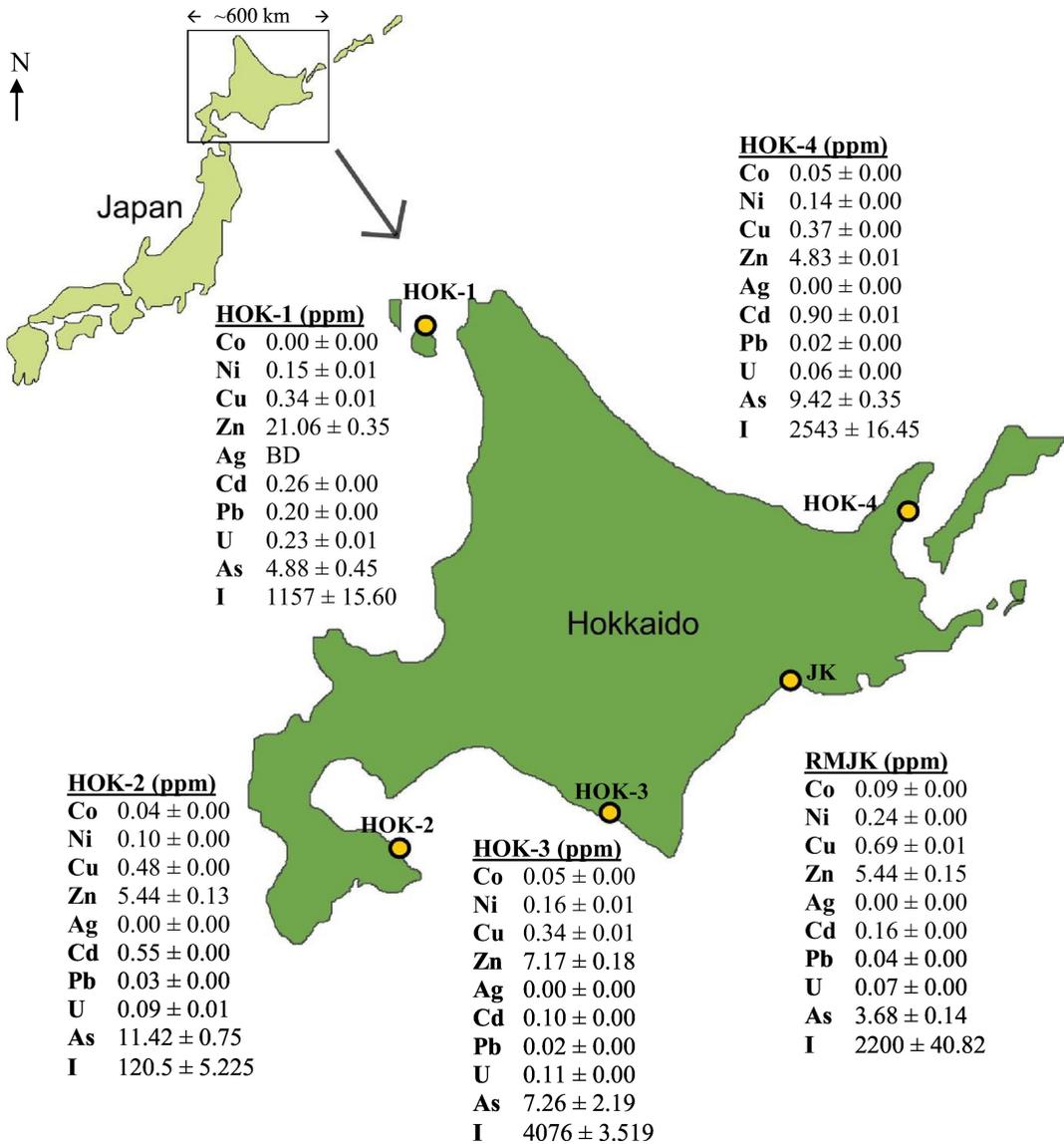


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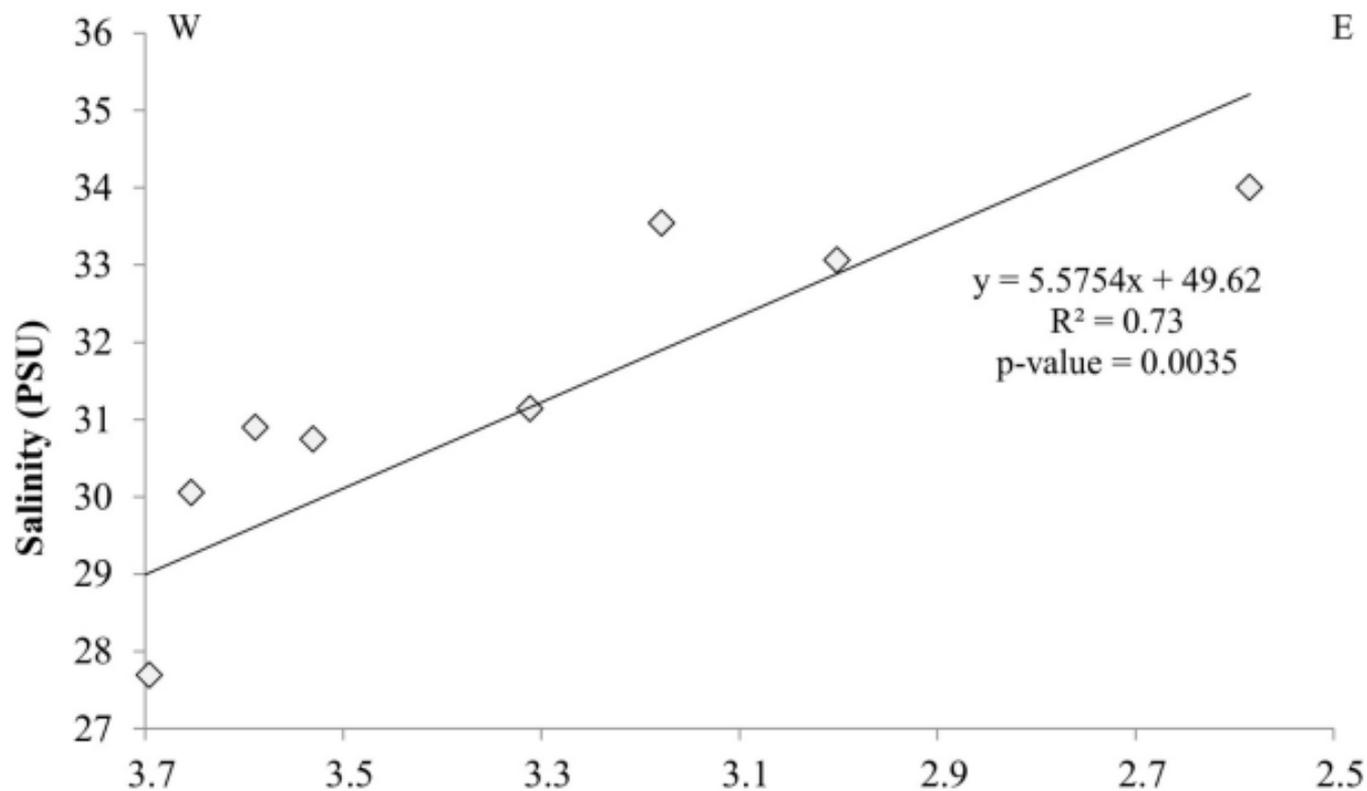


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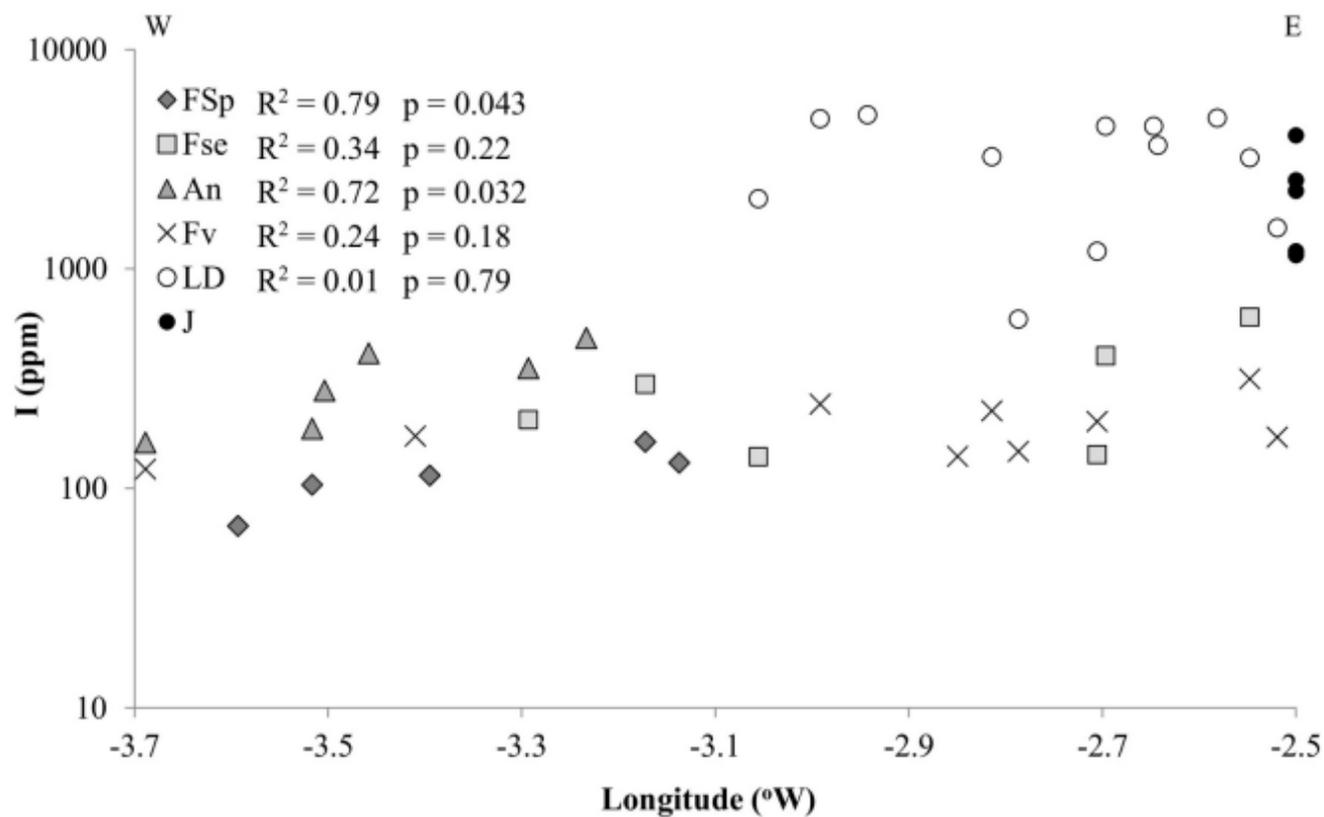


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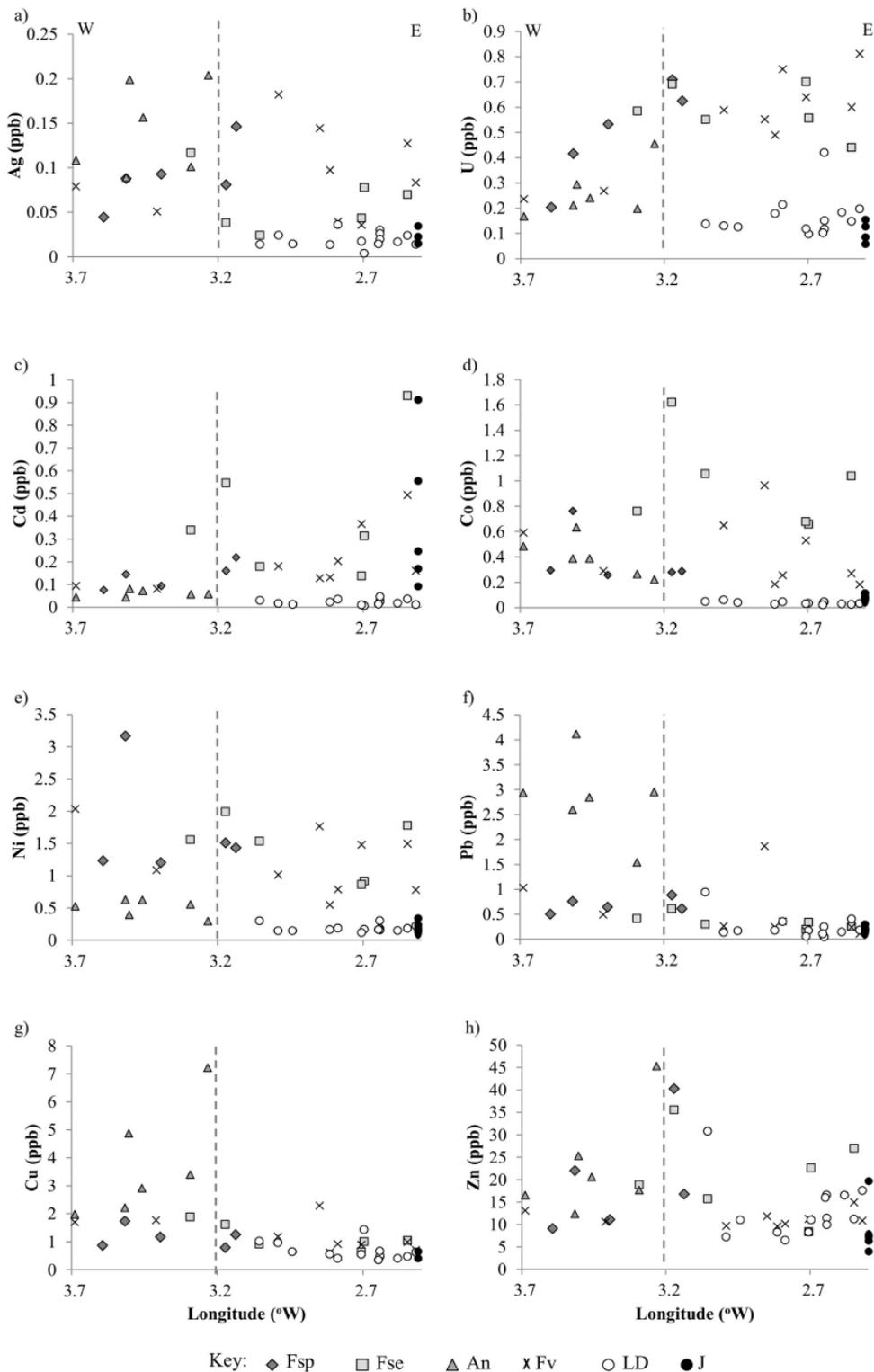


Figure 5

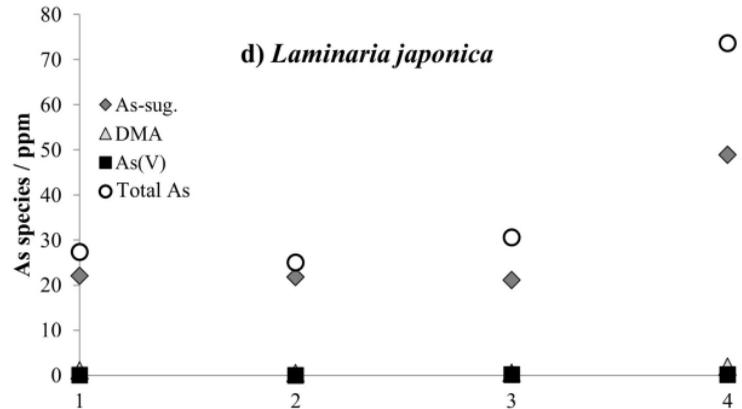
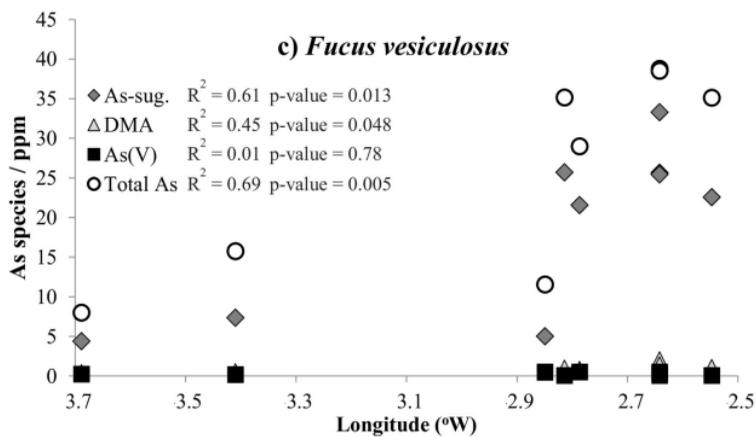
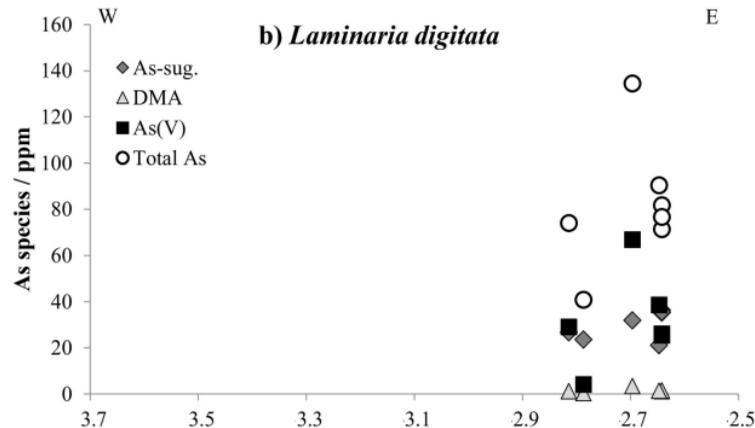
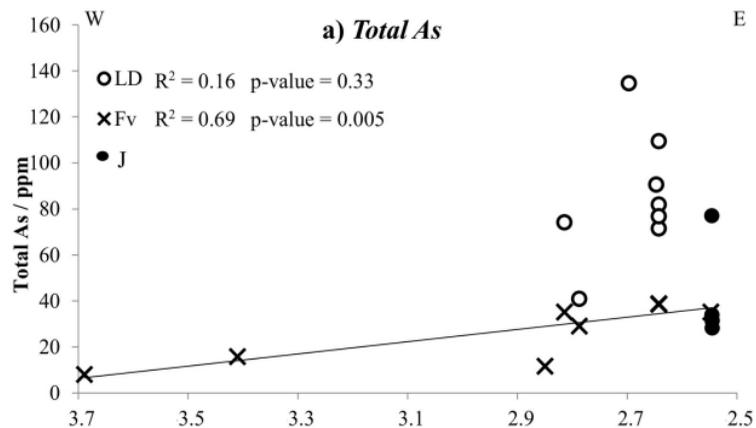


Figure 6

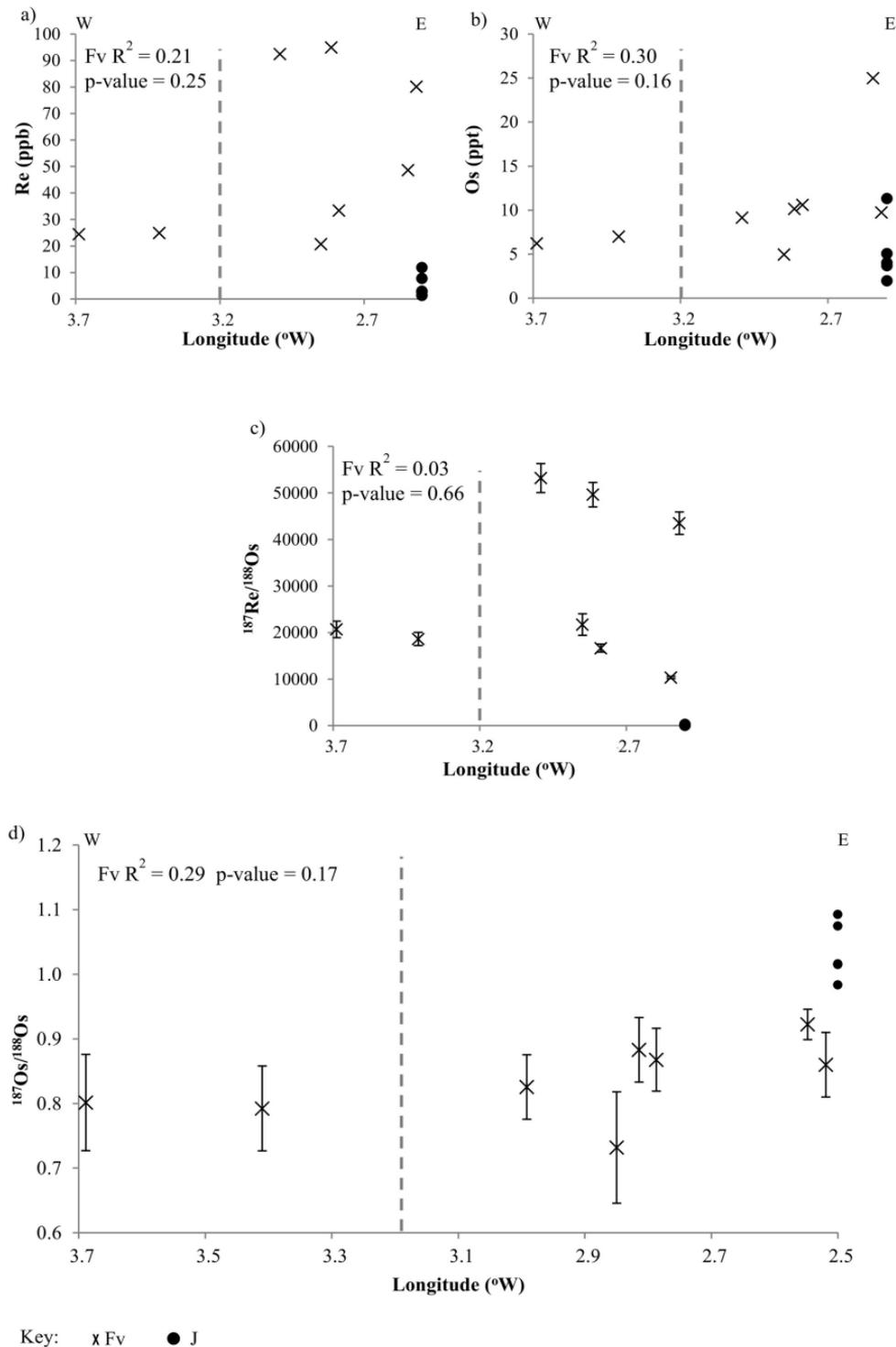


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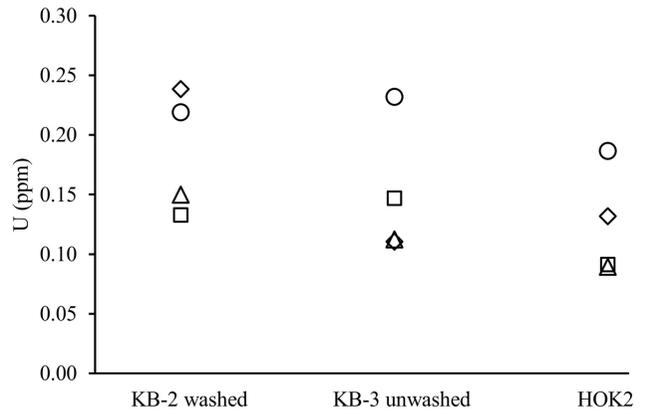
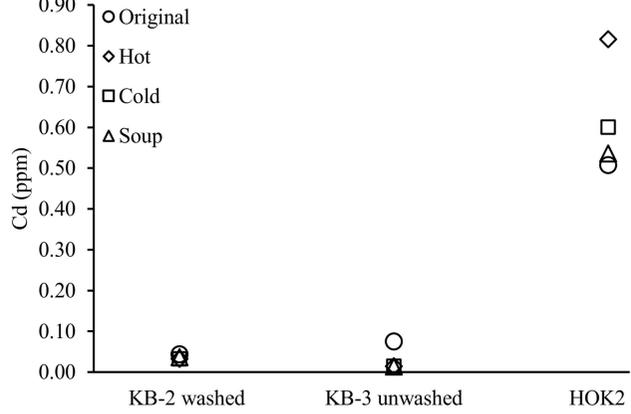
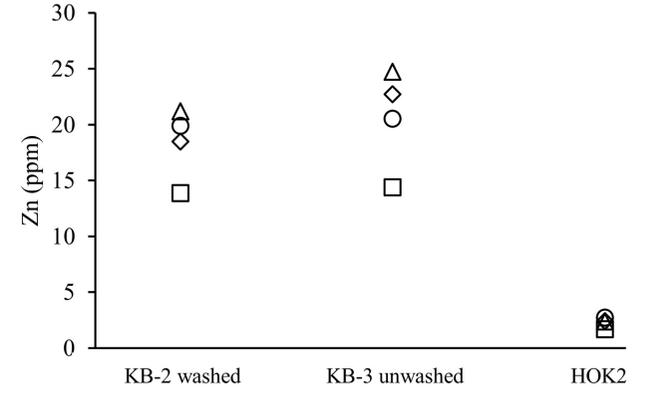
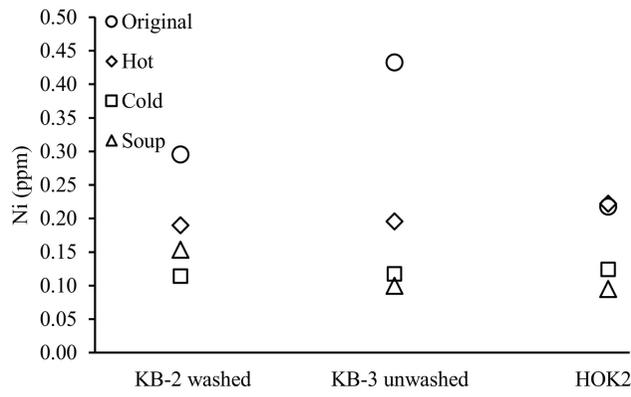
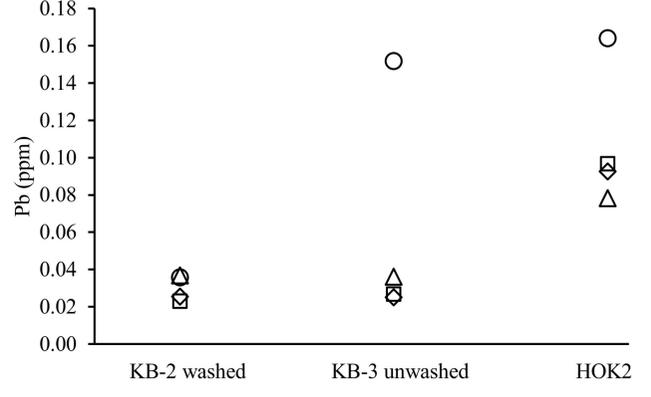
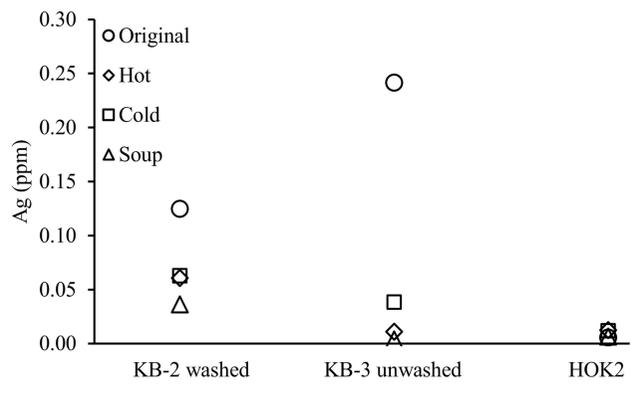
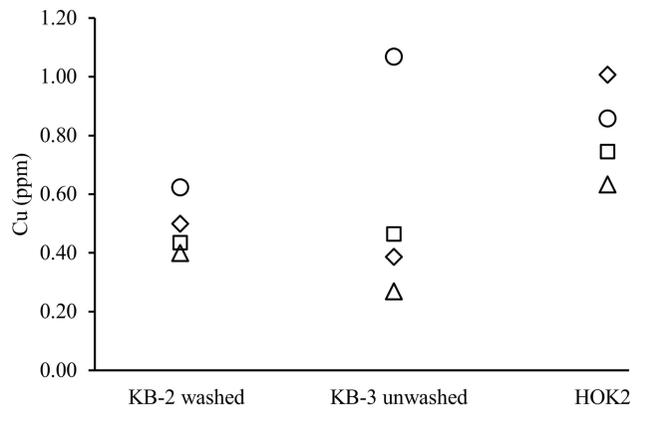
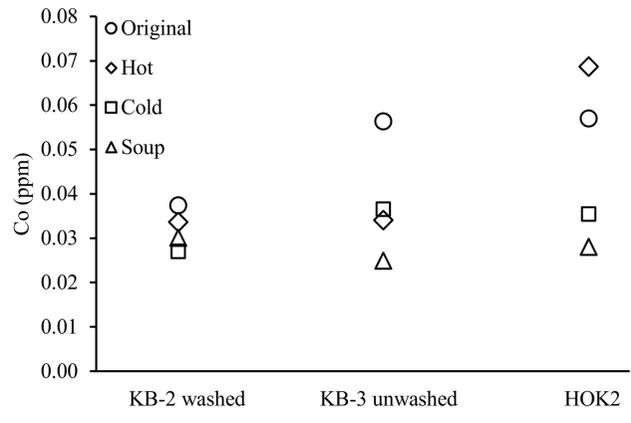


Figure 8

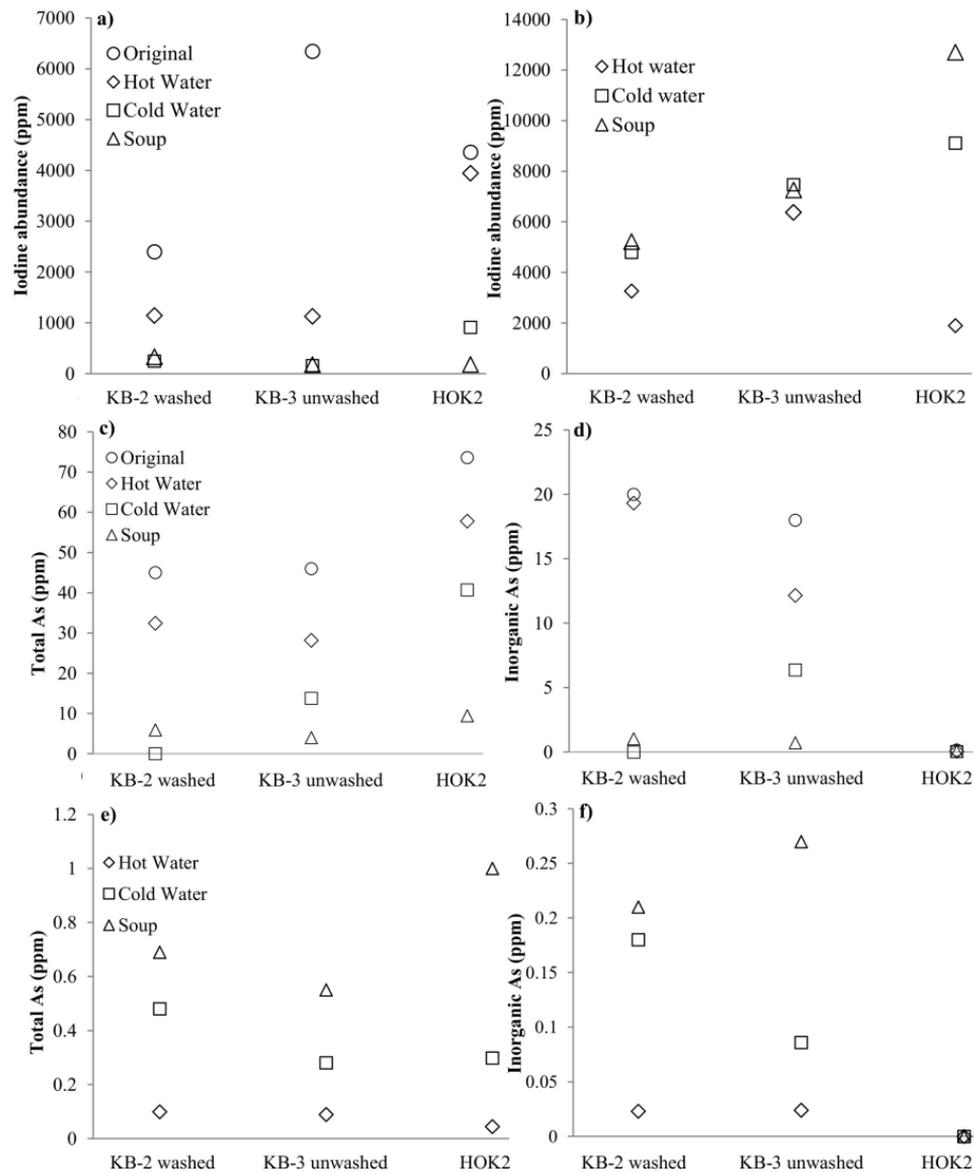


Figure 9

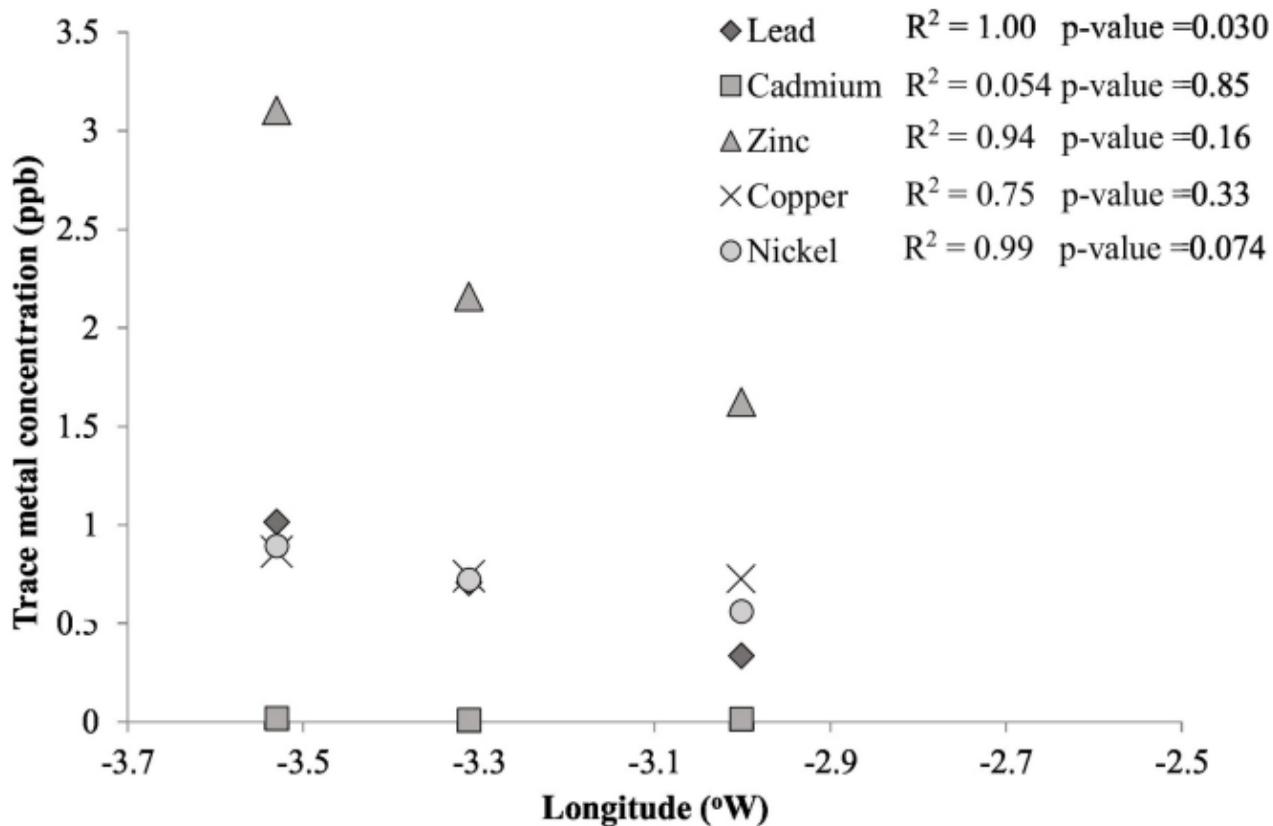


Figure 10