

# Metal Accumulation and Metallothionein Response in *Fucus Spiralis*

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**Abstract** - Seaweeds are established sentinels for metal contamination and are utilised for biomonitoring. Metallothionein (MT) is a protein that is induced by metal exposure, and has been widely used as a biomarker for metal pollution. MT has not been reported in spiral wrack (*Fucus spiralis*), but has been identified in bladder wrack (*Fucus vesiculosus*), where it has been suggested to be a protective mechanism against metal exposure. This study aimed to evaluate the potential use of MT in *F. spiralis* as a biomarker for metal pollution for the first time. Samples were collected from Poole Harbour, UK, over a year-long period in 2015. MT and metal concentrations were quantified during winter, spring, summer, and autumn. Linear regression analysis showed few relationships between MT and metal concentrations throughout most seasons. However, during summer, significant positive relationships existed between MT concentrations and iron ( $R^2 = 0.631$ ), nickel ( $R^2 = 0.486$ ), tin ( $R^2 = 0.579$ ), and lead ( $R^2 = 0.415$ ). Furthermore, MT concentrations were significantly higher in summer than in other seasons. It is possible that for most of the year, metal concentrations in Poole Harbour are not high enough to elicit a MT response in *F. spiralis*, as it is a metal tolerant species. This suggests that the use of MT as a sensitive biomarker for metal pollution in *F. spiralis*, at low levels of metal pollution, is limited. However, increased toxicity of metals, and vulnerability of seaweeds to metals, may become pertinent in summer months as metals inhibit photosynthetic processes and growth. This may have caused MT to relate to metal concentrations more closely during summer, as it responds to the increased effects of metals. Therefore, the potential for using MT in *F. spiralis* as a biomarker for metal pollution may be restricted to summer months in temperate regions, at least at low metal concentrations. Further research is required to fully evaluate the use of MT in *Fucus* spp., addressing uncertainties of seasonal variation, and MT response in severely polluted environments.

**Keywords:** Metallothionein, metal toxicity, biomonitoring, brown seaweed, *Fucus spiralis*

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## 1. Introduction

Biomonitoring, whereby organisms are used to measure environmental pollution, has advantages over direct measurements of chemical contaminants in the environment, such as seawater or sediment concentrations. It provides an exclusive measure of the bioavailable pollutants, which have the greatest potential to impact organisms, ecosystems, and human health, and discounts pollutants not taken up by organisms [1]. Furthermore, it allows the measurement of pollutants accumulated over an organism's lifetime, which reduces fluctuations in pollutant concentrations [2]. Previously, the environmental quality of water bodies was assessed using chemical monitoring alone, missing information on ecological impacts and overall water quality [3]. Under the Water Framework Directive (WFD 2000/60/EC), water bodies should be assessed through both chemical and ecological monitoring [4].

Seaweeds are advocated as bioindicators in temperate coastal waters due mainly to their high abundances and immobility [5]. They often dominate metal contaminated habitats [6] as they are resistant to metal pollution [7]. They have an ability to accumulate

dissolved metals from seawater so their intracellular concentrations reflect time-integrated pollution loads in the marine environment [8]. As a consequence, seaweeds are established sentinels for metal contamination and are exploited for biomonitoring [9].

Metallothionein (MT) is a protein of low molecular weight, high heat stability, and high cysteine content [10]. The latter attribute lends itself to be used as a biomarker of metal pollution, as it has a high affinity to bond to metals due to sulphur-containing thiol groups [11]. It is regarded to play a vital role in the detoxification of metals within organisms [11, 12]. This relays a biological response indicating the severity of metal pollution to the organism. Many organisms have been employed as a MT biomarker species, primarily bivalve species [13-17]. However, MT is also noted to have multiple roles such as maintaining homeostasis by regulating essential metals, and as a defence against reactive oxygen species [18, 19]. Factors that contribute to natural variation of MT include tissue weight [20], reproductive stage [21], temperature [22], and salinity [23]. This limits the use of MT as a tool in biomonitoring, as concentrations may alter independently of metal exposure, particularly in bivalve species.

Literature on the MT response in marine algae to metal exposure is limited, compared to other organisms. MT in spiral wrack (*Fucus spiralis*) has never been reported. The MT gene has been identified in bladder wrack (*Fucus vesiculosus*) by Morris et al. [24], which suggested that a protective mechanism against metal exposure exists for this species. Further studies suggest induction in this species following Cu exposure [9], as well as an ability to bind to As, Cd, and Zn [25, 26]. This shows potential for MT to be developed as a biomarker in brown seaweeds.

*F. spiralis* would offer a cosmopolitan bioindicator species for dissolved metal pollution, if MT is shown to be a reliable biomarker in this species. It is geographically widely available and easy to sample, suggesting it is a promising candidate. It may also be less susceptible to natural variation compared to traditional MT biomarker species. However, despite the potential for *F. spiralis* to be used as a MT biomarker species, its use has not been developed, and no study of its MT response to metals has been conducted in the field. Therefore, this study aimed to investigate the potential for MT in *F. spiralis* to be used as a biomarker for metal pollution.

## 2. Methodology

### 2.1. Sample collection

Seaweed samples were collected from four sites in Poole Harbour, UK: Holes Bay (north), Holes Bay (south), Poole Quay, and Sandbanks (Figure 1). Samples were carried out in January, April, August, and October, which are referred to as winter, spring, summer, and autumn. Samples were kept in storage at -20 °C before analysis, as advised by Oaten, et al. [27].

### 2.2. MT analysis

MT concentrations were measured using the UV-spectrophotometric method devised by Viarengo, et al. [28], with modifications by Aly, et al. [29]. Before analysis, approximately 3 cm of the frond tips of seaweed were dissected. This was then homogenised using a ceramic blade and a pestle and mortar (to avoid metal contamination before metal analysis). Three replicates of each sample were measured. Concentrations are reported in µg/g (wet weight).

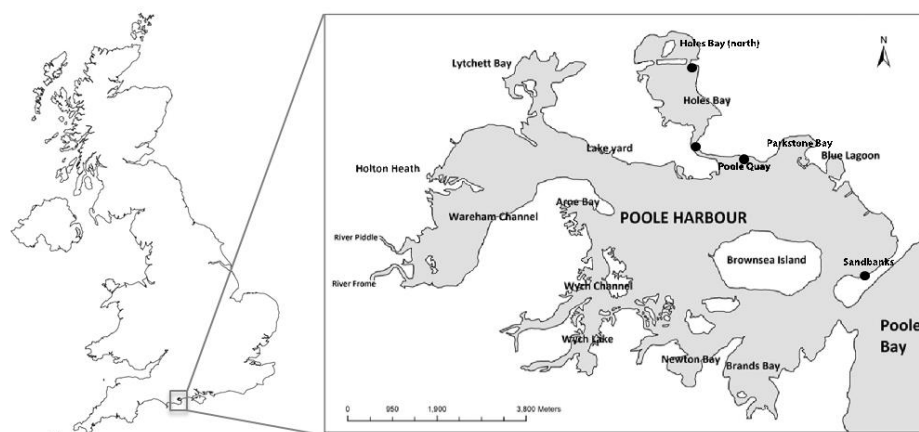


Figure 1. Site map of Poole Harbour, UK, and sampling locations.

### 2.3. Metal analysis

Before analysis, previously homogenised samples (as per MT analysis) were freeze-dried for 72 hours. Accurately weighed samples of approximately 10 mg of dried, ground sample were placed in 7 ml Teflon sealable pots. Blank samples consisting of empty Teflon pots were also prepared. Samples were digested in *Aqua Regia* on a hot plate. Additions of trace metal grade hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were made to oxidize organic matter. Samples were dried, resuspended, and completed with 3% trace metal grade, redistilled, nitric acid ( $\text{HNO}_3$ ), containing 5 ppb In/Re and 20 ppb Be as internal standards to correct for matrix effects and instrument drift. Analysis by inductively coupled plasma mass spectrometry (ICP-MS) was carried out. A mussel reference material (European Reference Materials – CE278k) was measured as a bivalve comparator and concentrations were adjusted according to the recovery rate. Concentrations are reported as  $\mu\text{g/g}$  (dry weight).

### 2.4. Statistical analysis

All statistical analysis was completed using IBM SPSS Statistic v21. Tests for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test) were completed and data was tested parametrically (one-way ANOVA) or non-parametrically (Kruskal-Wallis test), accordingly. Linear regression was used to determine the effects of metal exposure on MT concentrations in *F. spiralis*. Statistical significance was established at  $P = 0.05$ .

## 3. Results

MT concentrations in *F. spiralis* varied greatly throughout the sampling year (Figure 2a, Table 1). In winter, MT concentrations were significantly higher in Holes Bay (north), compared to Holes Bay (south), Poole Quay, and Sandbanks (*post-hoc* Scheffe,  $P = 0.015$ ,  $P < 0.001$ ,  $P < 0.001$ , respectively). Holes Bay (south) was also significantly higher than Poole Quay and Sandbanks (*post-hoc* Scheffe,  $P = 0.004$ ,  $P = 0.048$ , respectively). Concentrations of MT in *F. spiralis* from Sandbanks increased in spring, and became highest in summer and autumn. In spring, MT concentrations were higher in Holes Bay (north) compared to Poole Quay (*post-hoc* Tukey,  $P = 0.04$ ). In summer, the concentration of MT in *F. spiralis* from Sandbanks was

significantly higher than Poole Quay (*post-hoc* Scheffe,  $P = 0.026$ ). During autumn, significant differences in MT concentrations in *F. spiralis* did not exist between sites ( $F = 2.835$ ,  $P = 0.106$ ). Across seasons, mean MT concentrations in winter were significantly lower than concentrations in spring (*post-hoc* Scheffe,  $P = 0.047$ ), summer (*post-hoc* Scheffe,  $P = 0.016$ ), and autumn (*post-hoc* Scheffe,  $P = 0.015$ ) (Table 1).

Metal concentrations in *F. spiralis* also varied greatly throughout the sampling year, and were inconsistent in each season (Figure 2b – j, Table 1). For Fe, Ag and Cd, highest concentrations were generally found at Holes Bay (north) throughout the year. For Sn and Ni concentrations were highest at Sandbanks in winter and summer, and highest at Holes Bay (north) in spring and autumn. For Zn and As, highest concentrations were predominantly found at Holes Bay (south), and for Cu during winter and spring. Pb concentrations were highest in *F. spiralis* at Holes Bay (north), Poole Quay, and Sandbanks in winter, spring, and summer, respectively. Furthermore, concentrations of Cu, Zn, As, Ag, Sn and Cd generally decrease from winter to autumn (Table 1). Across seasons, mean Zn, As, Ag, and Cd concentrations were significantly different (Table 1). Winter As concentrations were significantly higher than concentrations in spring (*post-hoc* Scheffe,  $P = 0.006$ ), summer (*post-hoc* Scheffe,  $P < 0.001$ ) and autumn (*post-hoc* Scheffe,  $P < 0.001$ ). In winter, Zn and Ag concentrations were significantly higher than in summer (*post-hoc* Scheffe,  $P < 0.001$ ,  $P = 0.003$ , respectively) and autumn (*post-hoc* Scheffe,  $P = 0.002$ ,  $P = 0.004$ , respectively). Cd concentrations were significantly higher in winter than summer (*post-hoc* Scheffe,  $P = 0.002$ ).

Linear regression analysis was used to assess the effect of metal exposure on MT concentration. During winter, only Fe tissue concentration showed a significant positive relationship with MT concentration ( $R^2 = 0.792$ ,  $P < 0.001$ ) (Figure 3a). In summer, significant positive relationships were evident, and existed between MT concentrations and Fe ( $R^2 = 0.631$ ,  $P = 0.002$ ) (Figure 3a), Ni ( $R^2 = 0.486$ ,  $P = 0.012$ ) (Figure 3b), Sn ( $R^2 = 0.579$ ,  $P = 0.004$ ) (Figure 3c), and Pb ( $R^2 = 0.415$ ,  $P = 0.024$ ) (Figure 3d). No significant positive relationships existed between MT and any other metals, during any season, and are therefore not shown graphically.

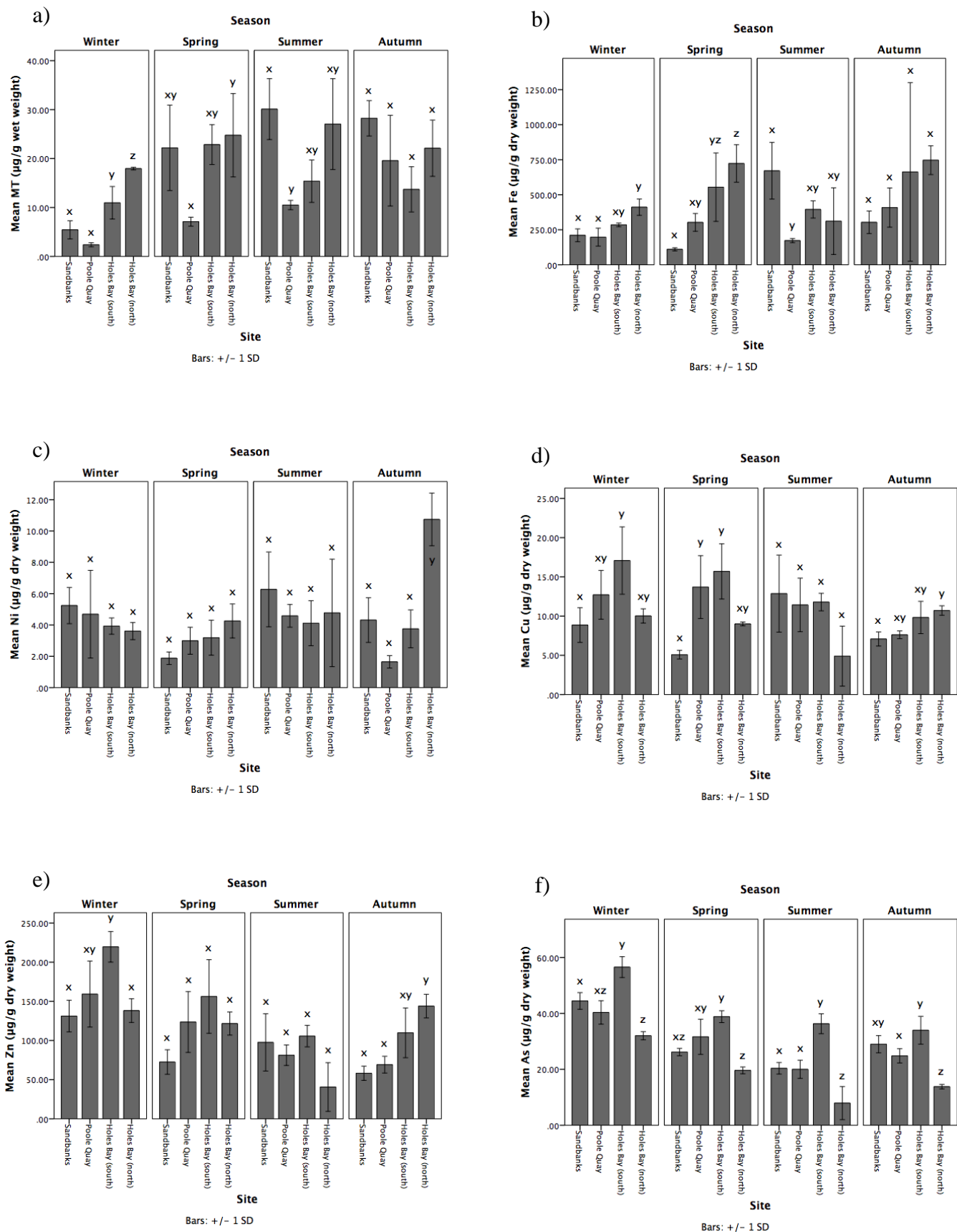


Figure 2. Mean concentrations (µg/g) of a) MT, b) Fe, c) Ni, d) Cu, e) Zn, f) As, g) Ag, h) Cd, i) Sn, and j) Pb in *F. spiralis* from Holes Bay (north), Holes Bay (south), Poole Quay, and Sandbanks in Poole Harbour throughout each season in 2015, with standard deviation (SD) (n = 3). Different letters (x, y, z) indicate significant differences between sites (P = 0.05).

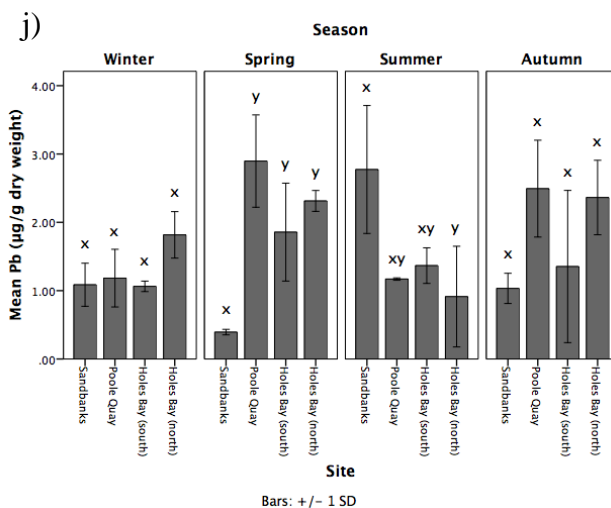
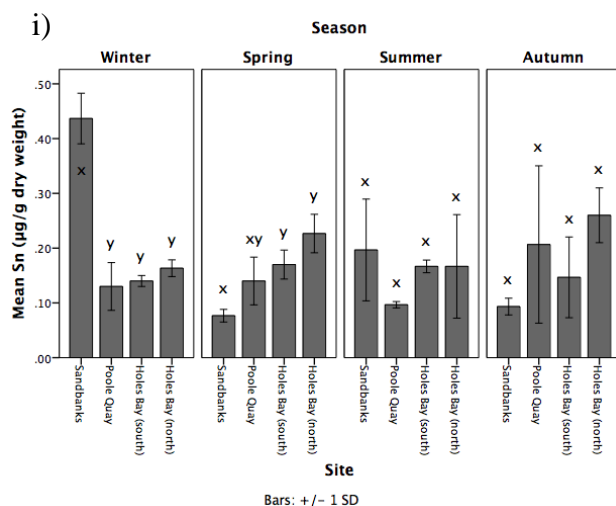
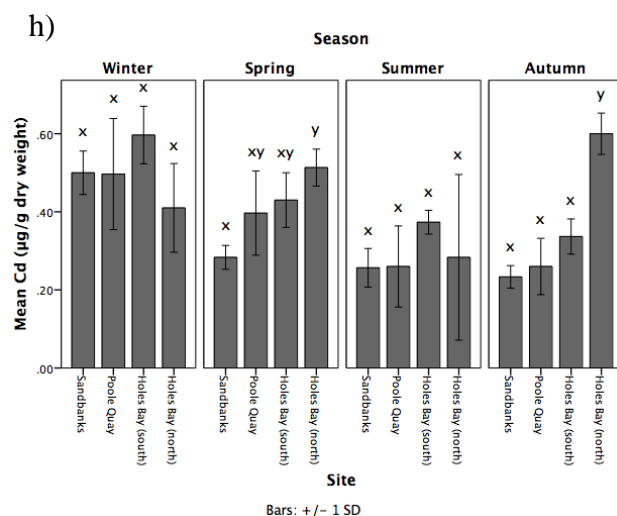
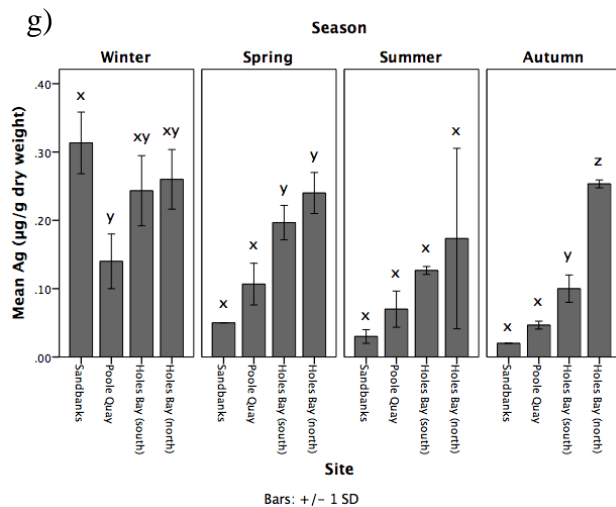


Figure 2 (continued): Mean concentrations ( $\mu\text{g/g}$ ) of a) MT, b) Fe, c) Ni, d) Cu, e) Zn, f) As, g) Ag, h) Cd, i) Sn, and j) Pb in *F. spiralis* from Holes Bay (north), Holes Bay (south), Poole Quay, and Sandbanks in Poole Harbour throughout each season in 2015, with standard deviation (SD) ( $n = 3$ ). Different letters (x, y, z) indicate significant differences between sites ( $P = 0.05$ ).

Table 1. Mean seasonal concentrations of MT ( $\mu\text{g/g}$  wet weight) and metals ( $\mu\text{g/g}$  dry weight) in *F. spiralis* sampled from Holes Bay (north), Holes Bay (south), Poole Quay, and Sandbanks in Poole Harbour in 2015. Different letters (x, y, z) indicate significant differences between seasons ( $P = 0.05$ ).

		Winter	Spring	Summer	Autumn
MT ( $\mu\text{g/g}$ )	Mean	9.18 <sup>x</sup>	19.22 <sup>y</sup>	20.75 <sup>y</sup>	20.90 <sup>y</sup>
	SD	6.40	9.20	9.87	7.58
Fe ( $\mu\text{g/g}$ )	Mean	276.08 <sup>x</sup>	422.60 <sup>x</sup>	387.71 <sup>x</sup>	530.29 <sup>x</sup>
	SD	98.16	273.29	233.55	340.85
Ni ( $\mu\text{g/g}$ )	Mean	4.37 <sup>x</sup>	3.08 <sup>x</sup>	4.94 <sup>x</sup>	5.11 <sup>x</sup>
	SD	1.49	1.18	2.09	1.07
Cu ( $\mu\text{g/g}$ )	Mean	12.17 <sup>x</sup>	10.87 <sup>x</sup>	10.24 <sup>x</sup>	8.81 <sup>x</sup>
	SD	4.13	4.88	4.48	1.87
Zn ( $\mu\text{g/g}$ )	Mean	162.07 <sup>x</sup>	118.52 <sup>xy</sup>	81.28 <sup>y</sup>	95.29 <sup>y</sup>
	SD	42.75	41.66	34.20	39.05
As ( $\mu\text{g/g}$ )	Mean	43.34 <sup>x</sup>	29.07 <sup>y</sup>	21.15 <sup>y</sup>	25.40 <sup>y</sup>
	SD	9.64	7.93	11.06	8.23
Ag ( $\mu\text{g/g}$ )	Mean	0.24 <sup>x</sup>	0.15 <sup>xy</sup>	0.10 <sup>y</sup>	0.11 <sup>y</sup>
	SD	0.08	0.08	0.08	0.09
Cd ( $\mu\text{g/g}$ )	Mean	0.50 <sup>x</sup>	0.41 <sup>xy</sup>	0.29 <sup>y</sup>	0.36 <sup>xy</sup>
	SD	0.11	0.10	0.11	0.16
Sn ( $\mu\text{g/g}$ )	Mean	0.22 <sup>x</sup>	0.15 <sup>x</sup>	0.16 <sup>x</sup>	0.18 <sup>x</sup>
	SD	0.14	0.06	0.07	0.10
Pb ( $\mu\text{g/g}$ )	Mean	1.29 <sup>x</sup>	1.87 <sup>x</sup>	1.56 <sup>x</sup>	1.81 <sup>x</sup>
	SD	0.42	1.06	0.92	0.90

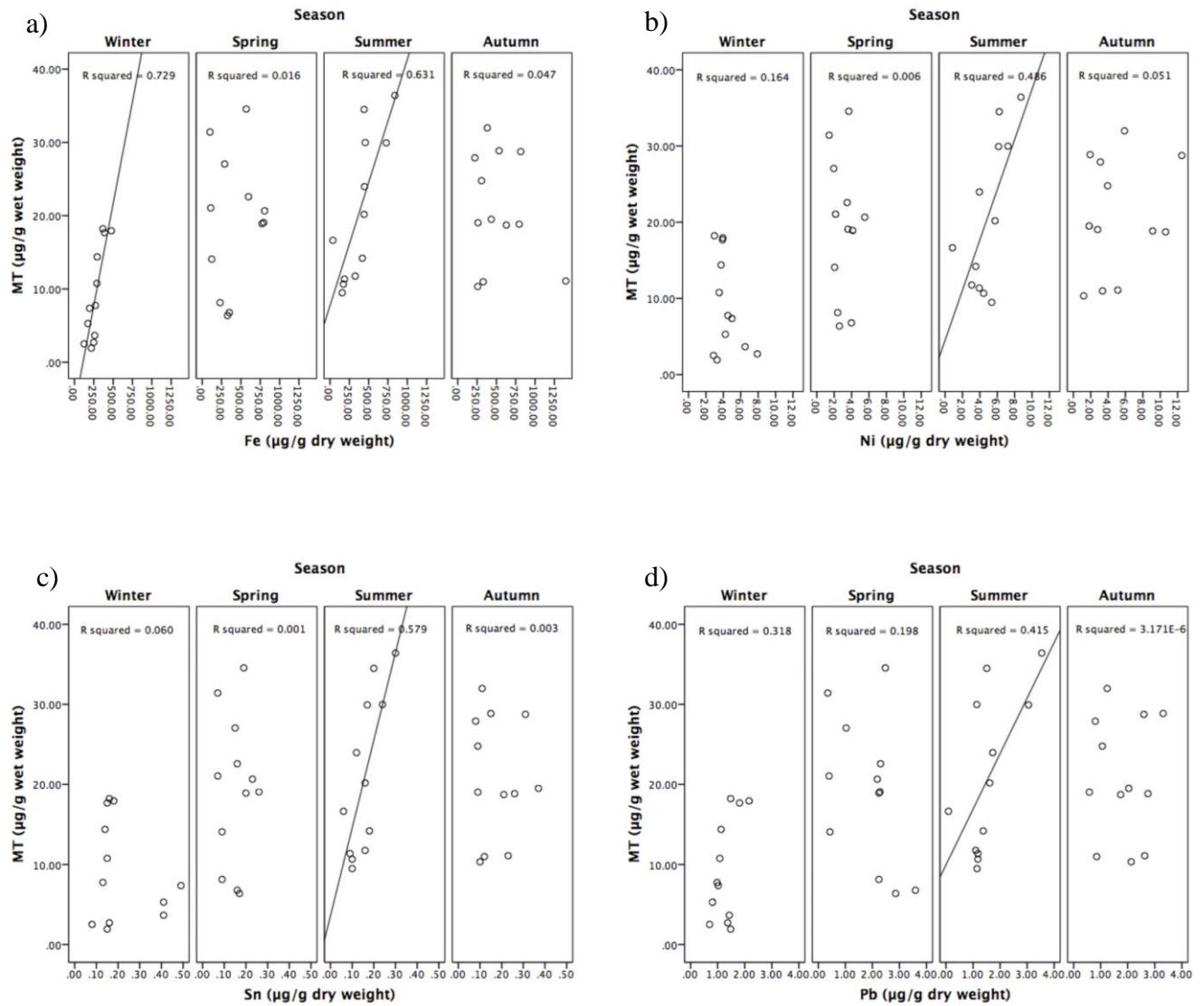


Figure 3. Linear regression between concentrations of MT and a) Fe, b) Ni, c) Sn, and d) Pb in *F. spiralis* sampled from Holes Bay (north), Holes Bay (south), Poole Quay, and Sandbanks in Poole Harbour, across seasons in 2015.

## 4. Discussion

### 4.1. Metal contamination and seasonal variation

Concentrations of metals in *F. spiralis* from Poole Harbour indicate that the most polluted area is Holes Bay (Figure 2b - j). This is in agreement with previous literature on metal pollution in Poole Harbour by Langston et al. [30], Aly et al. [31], and Oaten et al. [32], which reported generally higher metal concentrations within Holes Bay in sediment and seawater, as well as biota. *F. spiralis* metal concentrations at Sandbanks were also relatively high. This may be due to the sewage pumping station near to the site, which periodically discharges storm water. There are also yacht clubs in the vicinity, which may contribute to the metal burden in the area due to sources of metals such as anti-fouling paints on watercraft: higher Sn concentrations in winter could be related to boat maintenance in winter and the removal of old tributyl tin antifoulant.

Seasonal variation for some metal concentrations in *F. spiralis* is apparent. Cu, Zn, As, Ag, Cd, and Sn concentrations tend to reduce from winter to autumn (Table 1). This could be explained by plant growth. Concentrations within seaweeds continue to mount through dormant periods during winter, and dilute as plants grow and reproduce in summer months [33, 34]. Metal bioavailability may also influence seasonal variability of bioaccumulated metal concentrations. For example, dissolved Cd concentration tends to reduce during summer months due to uptake by phytoplankton [35]. Furthermore, higher salinity, which is observed during warmer months due to reduced rainfall and increased evaporation, also causes increased rates of Cd, Zn and Cu binding to chloride ions, thereby reducing dissolved metal bioavailability [36, 37]. Therefore, because seaweeds are predominantly exposed to dissolved metals concentrations within seawater, as they inhabit the water column, Cd, Cu and Zn exposure to seaweeds is reduced during summer. Seasonal variation in brown seaweed metal concentrations has been shown to be particularly evident for Cd and Zn, but less difference is observed for Pb and Ni [38, 39]. This may be explained by an ion-exchange process that actively takes up Pb and Ni, meaning the importance of bioavailability on bioaccumulation of these metals is less [40-42]. Furthermore, the process of phytoplankton metal uptake during summer, and salinity effects to dissolved metal bioavailability is rarely reported for Pb or Ni. This may explain the reduced seasonal variability in Pb and Ni *F. spiralis* concentrations observed in this study (Table 1).

### 4.2. Metal toxicity and MT induction

Generally, the induction of MT is a physiological response to the insult caused by metal exposure [24, 25]. Therefore, toxic metals are more likely to cause MT induction. The order of toxicity of metals to seaweed species is generally  $Hg > Cu > Cd > Ag > Pb > Zn$  [43]. Cu, despite being an essential metal, is the second most toxic metal to seaweeds, the effects of which have been extensively studied due to its use in antifouling paints [43, 44]. It is often cited to inhibit photosynthetic processes and retard growth in seaweed species [45-47]. In addition, inhibition of fertilization and reproduction resulting from Cu exposure has been identified in *F. spiralis* [48]. Pb has also been shown to impact photosynthetic efficiency and growth of red and brown seaweeds, but is less toxic than Cu [45, 49]. Cd can affect growth, pigment content, and carbon assimilation in seaweeds [50]. Zn has also been shown to slow growth in seaweeds [51].

There is limited knowledge on MT response to metal exposure in seaweeds, though few studies exist on *F. vesiculosus*. Morris, et al. [24] noted the MT gene in *F. vesiculosus* to be induced by Cu exposure, and that MT can bind to both Cu and Cd. Further studies confirmed its role as a detoxification mechanism for metals, and reported MT binding abilities to Zn, and As [25, 26]. However, only Owen, et al. [9] confirmed this role in the field *in vivo*. The study found MT to respond to Cu exposure, and this metal was found to be more important for MT induction, due to a stronger and more significant regression coefficient, compared to Zn and Fe. In this study, Cu, Zn, and As seemed not to elicit a MT response in *F. spiralis*. Furthermore, during summer these metals were at minimal concentrations in *F. spiralis* when MT concentrations were at a maximum (Table 1). However, it is important to note that metal body burdens (total mass of metals within the organism) of these metals are likely to be less sensitive to change. This is due to metal uptake increases with increased metabolic rates in response to temperature and light increases, and subsequent growth rate increases, in spring and summer [42, 52]. Fe, Ni, Sn and Pb showed a significant positive relationship with MT in *F. spiralis*, during summer. Previous studies have not reported MT induction in *Fucus* spp. following exposure from these metals, with the exception of Fe [9]. However, these metals are known to induce MT in other species [53, 54]. Otherwise, it is possible these metals are contributing to a combination effect with other more toxic metals, such as Cu, and are cumulatively



above a threshold for MT induction [55]. Another possibility is that these metals are correlated with more toxic metals, not recorded here, that are eliciting a MT response in *F. spiralis*.

#### 4.3. Influences of MT response and variability

MT concentrations in *F. spiralis* were generally low for most of the year and were not related to tissue metal concentrations. This may be due to relatively low levels of metal exposure in Poole Harbour [31]. Seaweed species are very tolerant of metal exposure [7]. As such, the concentrations in Poole Harbour may not be enough to elicit a MT response. A study by Owen, et al. [9] reported *F. vesiculosus* to begin exhibiting the gene for MT when exposed to a concentration of Cu of 30 µg/l. However, Cu concentrations in seawater in Poole Harbour do not exceed 3 µg/l [31]. For comparison, Zn, Fe, and Pb concentrations in *F. vesiculosus* from the Fal Estuary, Cornwall, were as much as an order of magnitude higher, with Cu two orders of magnitudes higher, compared to this study [56]. Owen, et al. [9] did not report tissue concentrations as high in *F. vesiculosus* from the Fal Estuary; perhaps indicating a recovery of contamination levels, but the most polluted site studied was still approximately ten, five, and ten times higher respectively for Cu, Zn and Fe concentrations than in *F. spiralis* in this study.

Aside from low seawater concentrations, low accumulation of metals in seaweeds from Poole Harbour may be the product of low metal concentrations in the tips of fronds, with greater concentrations in the thallus [56]. It has been suggested to dissect the frond at a pre-determined distance from the distal end (10 cm for *F. vesiculosus*) to allow time for new growth to equilibrate with the environment [57, 58]. In this study, tips of seaweeds were analysed in order to select the tissue that reflected the most recent metal concentrations in the surrounding water [46]. However, it may be more suitable to analyse metal exposure and MT response in mature tissue in the thallus, due to potentially higher metal, and likely MT, concentrations. This may also explain the large degree of variation in metal and MT concentrations, evidenced by large standard deviations.

Biological processes may provide insight as to why metals only seem to elicit a MT response in this species during summer. It is known that prolonged exposure to metals can cause damage to growth rates and photosynthetic efficiency in seaweeds [45]. This is

likely due to the redirection of energy for defensive pathways to protect against metals, allowing less energy for growth [45]. The oxidation of photosynthetic pigments by Cu, for example, may also cause a reduction in photosynthetic capability, which also causes production of reactive oxygen species leading to oxidative stress [59, 60]. Furthermore, the substitution of Mg by other metals within chlorophyll can inhibit photosystem II, the reaction centre for capturing photons for photosynthesis, leading to chloroplast dysfunction and photosynthetic processes to cease [47]. Pb, Zn, and Cu can also affect Photosystem II in plants, by inhibiting electron transport and altering the structure of the thylakoid membrane within chloroplasts, whilst Cd and Ni can interact with other metabolic processes [61]. Both photosynthesis and growth rates are at a maximum during summer. Therefore, it is logical to suggest the affect of metals on these processes is exacerbated during summer, causing metal toxicity to seaweeds to increase. MT has been shown to protect against oxidative stress through oxyradical scavenging [18]. MT may therefore follow metal concentrations more closely in summer, due to increased toxicity and oxyradical production caused by metal affects on photosynthesis and growth rates. This could explain the positive relationship between seaweed metal concentrations and MT during summer in this study (Figure 3).

Nutrient concentrations may also impact metal toxicity. Addition of nitrogen and phosphorus has been shown to reduce metal toxicity to Cd, allowing growth rates of red macroalga (*Gracilaria tenuistipitata*) to increase [59, 62]. This may be caused by nutrient complexation with metals, competition of uptake sites with metals, and improved nutrient status of plant organisms [62]. Nutrient concentrations within Poole Harbour towards the end of summer are likely to be minimal [63]. This is due to reduced rainfall and consequent reduction in land based inputs of nitrogen and phosphorus [64]. Increased uptake of nutrients by primary production throughout spring and summer also further reduces nutrient concentrations in the water column [65]. Therefore, low nutrient concentrations in summer may further be increasing the vulnerability of *F. spiralis* to metal toxicity, possibly resulting in a MT response.

## 5. Conclusion

The use of MT in *F. spiralis* as a sensitive biomarker of metal pollution at low concentrations, as

subjected in Poole Harbour, is shown here to be limited, as MT does not appear to be consistently induced by metal exposure. It is possible that for most of the year, metal concentrations in Poole Harbour are not high enough to elicit a MT response in *F. spiralis*, as it is a metal tolerant species. However, during summer, concentrations of MT increase, and linear regression analysis reveals significant positive relationships with Ni, Pb, Sn, and Fe. This may be due to increased toxicity of metals, and vulnerability of seaweeds to metals, as they inhibit photosynthetic processes and growth, which becomes pertinent in summer months. This may have caused MT to relate to metal concentrations more closely, as it responds to the increased effects of metals. This shows evidence for MT in *F. spiralis* to be able to relay the biological impact of metals at low metal concentrations during summer, when important physiological processes are taking place, such as photosynthesis. Therefore, the potential for using MT in *F. spiralis* as a biomarker for metal pollution may be restricted to summer months in temperate regions, at least at low metal concentrations. Further research is required to fully evaluate MT response in *Fucus* spp., addressing uncertainties in frond selection, and seasonal variation and effects. MT response in *Fucus* spp. in severely polluted environments should also be examined in order to establish its relevance as a cosmopolitan bioindicator species.

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