

# 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 as 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, from January to October 2015. MT and metal concentrations were quantified during winter, spring, summer, and autumn seasons. Linear regression analysis showed few relationships between MT and metal concentrations, apart from in summer. 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 ( $R^2 = 0.415$ ). 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, rates of photosynthesis and growth increase, which may increase metal toxicity, due to the inhibition of photosynthesis and growth. Thus, MT may be induced in order to prevent disruption. This study suggests that the use of MT as a biomarker for metal pollution in *F. spiralis* may not be a sensitive biomarker at low levels of metal pollution. However, MT concentrations in *F. spiralis* may respond to metal exposure when natural processes are vulnerable to pollution. The potential for MT to be used as a biomarker in *Fucus* spp. has been highlighted, warranting further research to develop a promising cosmopolitan bioindicator for metal pollution.

**Keywords:** Metallothionein induction – metal toxicity – biomonitoring – brown seaweed

## 1. Introduction

Seaweeds are advocated as bioindicators in temperate coastal waters due mainly to their high abundances and immobility [1]. They often dominate metal contaminated habitats [2] and are resistant to metal pollution [3]. They have an ability to accumulate dissolved metals from seawater so their intracellular concentrations reflect time-integrated pollution loads in the marine environment [4]. As a consequence, seaweeds are established sentinels for metal contamination and are exploited for biomonitoring [5].

Metallothionein (MT) is a protein of low molecular weight, high heat stability, and high cysteine content [6]. 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 [7]. It is regarded to play a vital role in the detoxification of metals within organisms [7, 8]. 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 [9-13]. 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 [14, 15]. Factors that contribute to natural variation of MT include tissue weight [16], reproductive stage [17], temperature [18], and salinity [19]. 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 of marine alga 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. [20], which suggested that a protective mechanism against metal exposure exists for this species. Further studies suggest induction in this species following Cu exposure [5], as well as an ability to bind to As, Cd, and Zn [21, 22]. 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. [23].

### 2.2. MT analysis

MT concentrations were measured using the UV-spectrophotometric method devised by Viarengo et al. [24], with modifications by Aly et al. [25]. 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).

### 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 (H<sub>2</sub>O<sub>2</sub>) were made to oxidize organic matter. Samples were dried, resuspended, and completed with 3% trace metal grade, redistilled, nitric acid (HNO<sub>3</sub>), 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 µg/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 (Kruskall-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.

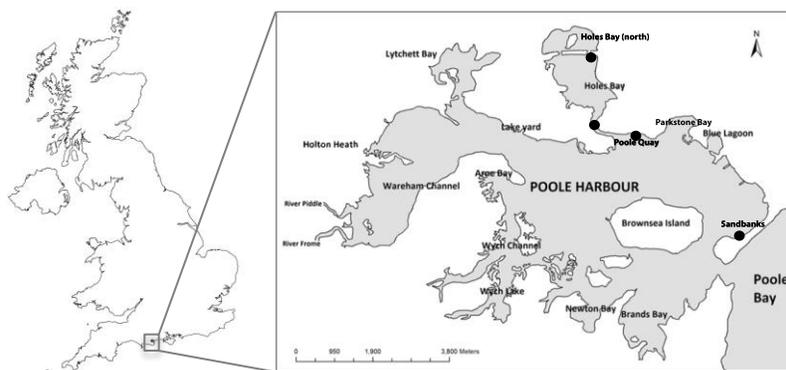


Fig. 1: Site map of Poole Harbour, UK, and sampling locations.

### 3. Results

MT concentrations in *F. spiralis* varied greatly throughout the sampling year (Figure 2a). 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 ( $F = 2.835$ ,  $P = 0.106$ ).

Metal concentrations in *F. spiralis* also varied greatly throughout the sampling year, and were inconsistent in each season (Figure 2b – k). For Fe, 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 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, and Cd generally decrease from winter to autumn.

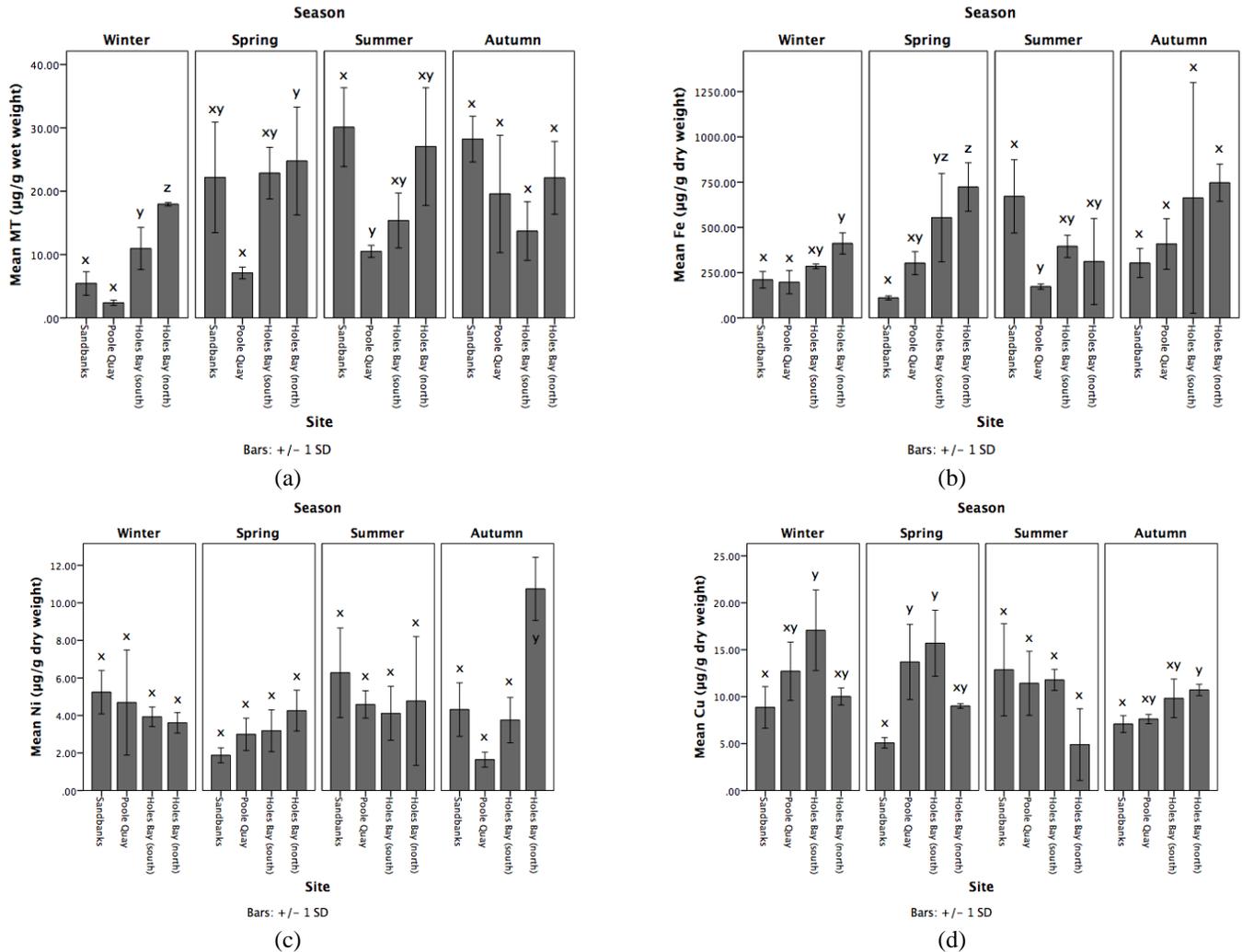


Fig. 1: Concentrations ( $\mu\text{g/g}$ ) of a) MT, b) Fe, c) Ni, d) Cu, e) Zn, f) Cd, g) Sn, h) Pb in *F. spiralis* from Poole Harbour throughout each season in 2015, with standard deviation (SD) ( $n = 3$ ). Different letters (x, y, z) indicate significant differences between sites ( $P = 0.05$ ).

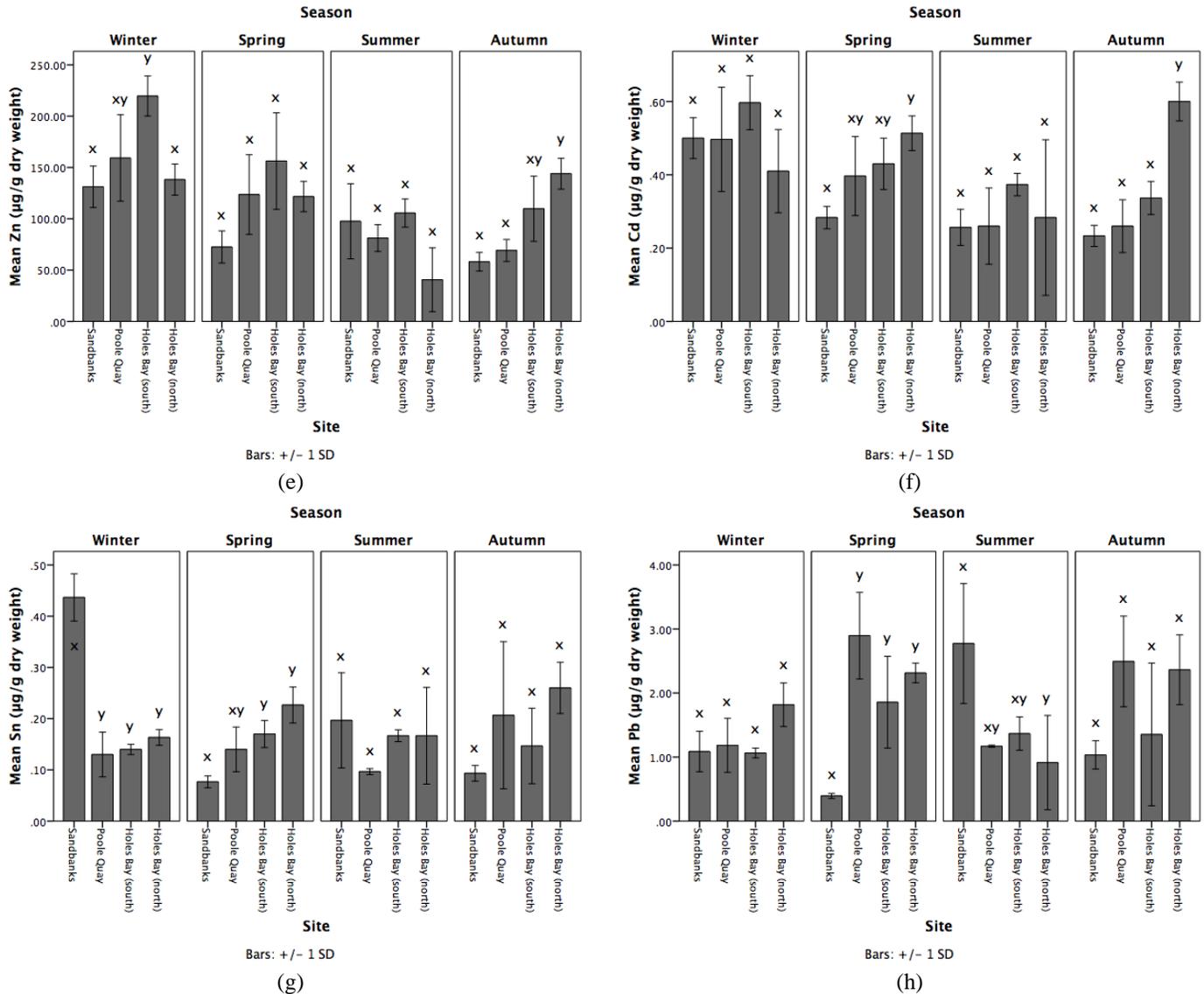


Fig. 2 cont: Concentrations (µg/g) of a) MT, b) Fe, c) Ni, d) Cu, e) Zn, f) Cd, g) Sn, h) Pb in *F. spiralis* from Poole Harbour throughout each season in 2015, with standard deviation (SD) (n = 3). Different letters (x, y, z) indicate significant differences between sites (P = 0.05).

Linear regression analysis was used to assess the effect of metal exposure on MT concentration (Figure 3). During winter, only Fe tissue concentration showed a significant positive relationship with MT concentration ( $R^2 = 0.792$ ,  $P < 0.001$ ). No significant positive relationships existed during spring or autumn. In summer, significant positive relationships were evident, and existed between MT concentrations and Fe ( $R^2 = 0.631$ ,  $P = 0.002$ ), Ni ( $R^2 = 0.486$ ,  $P = 0.012$ ), Sn ( $R^2 = 0.579$ ,  $P = 0.004$ ), and Pb ( $R^2 = 0.415$ ,  $P = 0.024$ ).

## 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. This is in agreement with previous literature on metal contamination in Poole Harbour [26, 27]. *F. spiralis* metal concentrations at Sandbanks were also relatively high. This may be due to the sewage pumping station near 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.

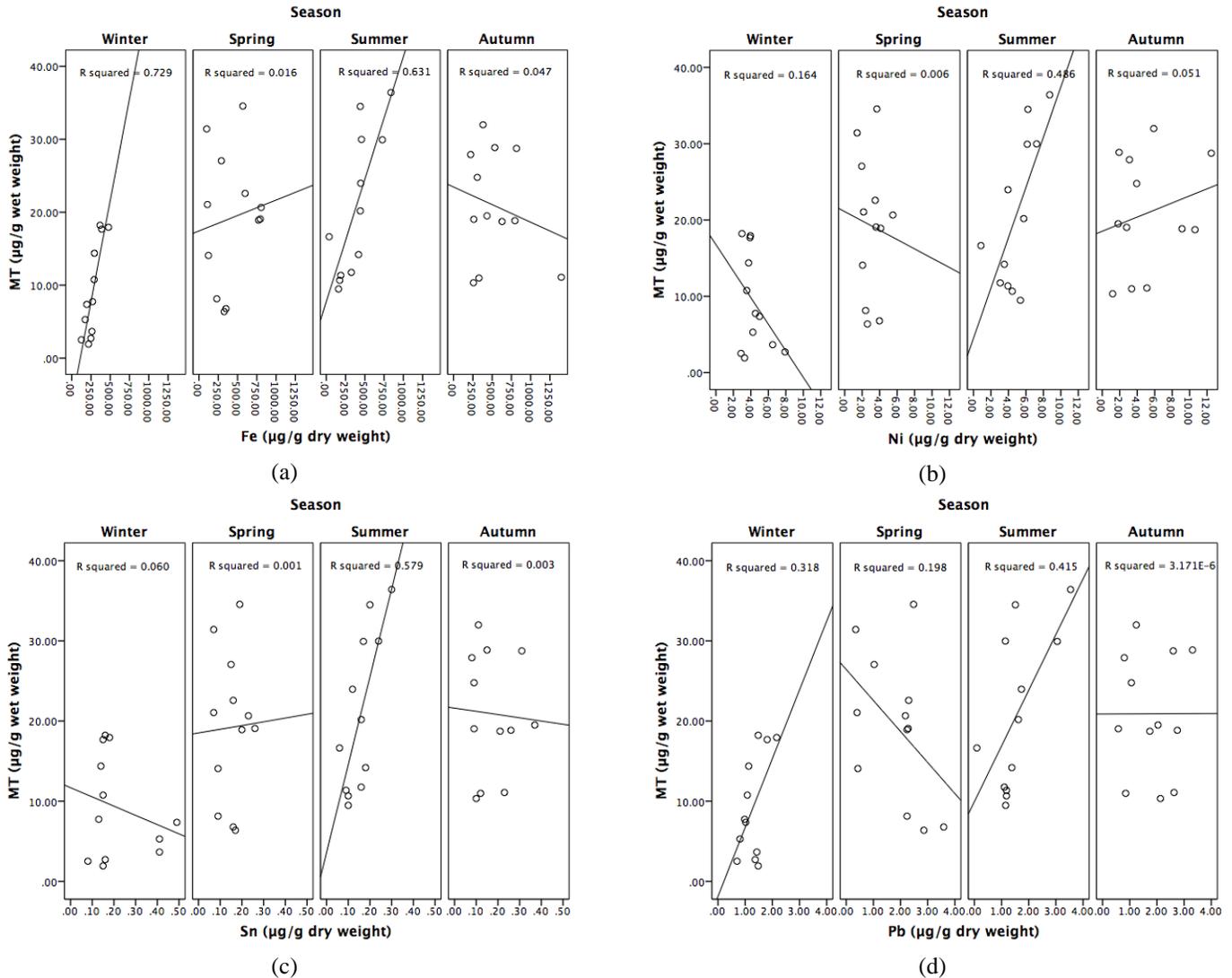


Fig. 2: Linear regression between concentrations of MT and a) Fe, b) Ni, c) Sn, d) Pb in *F. spiralis* from Poole Harbour across seasons in 2015.

Seasonal variation for some metal concentrations in *F. spiralis* is apparent, and metal concentrations tend to reduce from winter to autumn. This could be explained by plant growth. Concentrations could continue to mount through dormant periods during winter, and dilute as plants grow and reproduce in summer months [28, 29]. Other factors may also influence seasonal variability between metals such as reduced bioavailability of certain dissolved metals to seaweeds, such as cadmium, due to uptake by phytoplankton in summer months [30]. Seasonal variation may be particularly relevant for Cd and Zn, but less difference is observed for Pb [31, 32]. This is in agreement with this study. An ion-exchange process may be involved in Pb uptake, and could explain fewer differences in concentrations between seasons [33].

#### 4.2. Metal toxicity and MT induction

The induction of MT is a physiological response to the insult caused by metal exposure [20, 21]. 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 [34]. 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 [34, 35]. It is often cited to inhibit photosynthetic processes and retard growth in seaweed species [36-38]. In addition, inhibition of fertilization and reproduction resulting from Cu exposure has been identified in *F. spiralis* [39]. Pb has also been shown to impact photosynthetic efficiency and growth, but is less toxic than Cu [36, 40]. Cd can affect growth, pigment content, and carbon assimilation [41]. Zn has also been shown to slow growth in seaweeds [42].

There is limited knowledge on MT response to metal exposure in seaweeds, though few studies exist on *F. vesiculosus*. Morris et al. [20] noted the MT gene in *F. vesiculosus* to be induced by Cu exposure, and 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 [21, 22]. However, only Owen et al. [5] 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 elicit a MT response in *F. spiralis*. 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 [5]. However, these metals are known to induce MT in other species [43, 44]. 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 [45]. 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. Seaweed species are very tolerant of metal exposure [3]. As such, the concentrations in Poole Harbour may not be enough to elicit a MT response. A study by Owen et al. [5] 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 [26]. 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 [46]. Owen et al. [5] 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 [46]. 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 [47, 48]. 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 [37]. 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 [36]. This is likely due to the redirection of energy for defensive pathways, as well as the oxidation of photosynthetic pigments [49, 50]. It may also be related to the substitution of Mg within chlorophyll inhibiting photosystem II leading to chloroplast dysfunction [38]. The seasonality of these processes, with maximums in summer, could suggest that metal toxicity to seaweeds is greater during these periods. Therefore, detoxification processes, such as the induction of MT, may increase in summer.

#### 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. However, during summer, concentrations of MT increase, and linear regression analysis reveals significant positive relationships with certain metals. This may be due to increased toxicity of metals as they inhibit photosynthetic processes and growth, which becomes pertinent in summer months. Consequently, MT in *F. spiralis* may be able to relay the biological impact of metals at low environmental concentrations during periods when important physiological processes are taking place, such as seasonal growth. This

research demonstrates the potential for using MT in *F. spiralis* as a biomarker for metal pollution, for the first time. Further research is needed to fully evaluate its potential in brown seaweeds, and develop them as cosmopolitan bioindicator species. Care should be taken to address uncertainties in frond selection and seasonal effects.

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