Macroalgae as spatial and temporal bioindicators of coastal metal pollution following remediation and diversion of acid mine drainage

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Abstract

Acid mine drainage (AMD) is a significant contributor of metal pollution leading to ecosystem damage. Bioindicator organisms such as intertidal brown macroalgae have an important role in quantifying the risks of metal bioaccumulation in coastal locations exposed to AMD contamination. Measurement of As, Cd, Cu, Fe, Pb, and Zn accumulation was performed in *Fucus serratus*, *Fucus vesiculosus* and *Ascophyllum nodosum* sampled from two marine locations near to an abandoned Cu mine in Anglesey, Wales, UK. Transect samples were taken from a coastal location (Amlwch) that has seen a substantial increase in AMD contamination over 15 years, in comparison to a nearby estuarine location (Dulas Estuary leading to Dulas Bay) with a historic legacy of pollution. These were compared with samples from the same sites taken 30 years earlier. Some of the Dulas macroalgae samples had Cd, Cu and Zn concentrations that were above background but in general indicated a non-polluted estuary in comparison to substantial pollution over previous decades. In contrast, *Fucus* samples collected from directly below an AMD outflow at Amlwch showed extremely elevated metal bioaccumulation (> 250 mg Fe g^{-1}, > 6 mg Cu g^{-1}, > 2 mg Zn g^{-1}, > 190 µg As g^{-1}) and evidence of macroalgae toxicity, indicating severe pollution at this site. However, the pollution dispersed within 200 m of the outflow source. This study has demonstrated the efficiency of three brown macroalgae species as indicators for metal bioavailability at high spatial resolution and over time.

Keywords: Acid mine drainage, Bioaccumulation, Bioindicator, Macroalgae, Marine pollution, Trace metals

1. Introduction

Mining for minerals such as Cu is linked with the discharge of acid mine drainage (AMD), an effluent produced when sulfide-containing rocks are exposed to water and oxygen, leading to acid-induced mobilisation of toxic trace metals (Azapagic, 2004). The highly acidic, metal polluted run-off enters nearby freshwaters and has substantial adverse effects on aquatic biodiversity, reducing the value of the water for agricultural, recreational or industrial uses, and rendering it unsafe for human consumption (Akcil and Koldas, 2006; Tripole et al., 2006). For mine sites near to the sea, AMD may also be an important route for coastal and marine pollution, the consequences of which are less well studied. Despite adsorption reactions and geochemical precipitation that disperse and dissolve metals once they enter the sea (Foster et al., 1978), the unique hydrodynamics of estuarine systems and their action as sinks for pollutants makes them prone to a build-up of metal compounds in sediments that increases the risks of bioaccumulation in biota and subsequent trophic transfer (Simons et al., 2011). Use of bioindicators to detect metal pollution and determine these risks of bioaccumulation are therefore important.

Macroalgae are ideal candidates as biological indicators for metal pollution monitoring due to their wide distribution, and their ability to tolerate, accumulate and concentrate metals with respect to the corresponding seawater concentration (Bryan and Langston, 1992; Conti and Cecchetti, 2003; García-Seoane et al., 2018; Volterra and Conti, 2000). Furthermore, since macroalgae often cannot move away from the pollutant, their sessile nature make them excellent indicators of the pollution source (García-Seoane et al., 2018). Intertidal brown macroalgae such as *Fucus* spp. act as a good indicator of the bioavailable forms of metals (Giusti, 2001; Ryan et al., 2012; Sánchez-Quiles et al., 2017). For example, elevated metal concentrations in *Fucus vesiculosus* are indicative of trace metal contamination (Barnett and Ashcroft, 1985; Forsberg et al., 1988; Stengel et al., 2005). Likewise, *Fucus serratus* has been frequently used to detect trace metal effluent plumes in estuaries caused by industrial activities (Fuge and James, 1973; Klumpp and Peterson, 1979). In addition, other brown macroalgae species including *Ascophyllum nodosum* have been shown to be suitable bioindicators for metal contamination (Foster, 1976; Ryan et al., 2012; Stengel et al., 2005). When using organisms as biological indicators potential problems in interpreting the results of metal bioavailability can occur due to seasonal and environmental variation (such as salinity and sea currents) during sampling. Factors such as the age of an organism and variation in metal partitioning in different tissues are also potential sources of error. To account for such variation more than one macroalgal species over multiple sampling sites and tidal positions should be adopted (Conti and Cecchetti, 2003; García-Seoane et al., 2018).

This investigation makes use of a study site in Anglesey, Wales, UK (Fig. 1A), an area long known for Cu mining. The Parys Mountain mine was the largest single producer of Cu in the 18th century but mining at the site ceased in 1911 (Coupland and Johnson, 2004). The continual discharge of this metal-rich water from the mine adits and spoil heaps enters the Irish Sea via two rivers: the Northern and Southern Afon Goch, which allow significant outflow of Fe, Cu, Cd and Zn into the sea (Mayes et al., 2010). Ochre precipitates are formed when metal-rich and acidic water enters and mixes with neutral water of the sea. These precipitates are periodically flushed out of the Afon Goch system under high discharge, with metal precipitates from the Southern Afon Goch settling in the shallow Dulas Estuary before flow into the sea at Dulas Bay at high tide (Parkman et al., 1996). Less is known regarding the behaviour of the metals released via the Northern Afon Goch at Amlwch although metal dispersion at this part of the coast is likely to be more rapid as the discharge is directly into the sea.

In 2003 an underground dam at the Parys Mountain mine was dismantled as part of drainage and flood risk management, which caused mine effluent to be redirected into the Northern Afon Goch, whereas the Southern Afon Goch now only receives contamination through spoil leachate runoff (Coupland and Johnson, 2004; Dean et al., 2013; Johnston et al., 2008; Younger and Potter, 2012). The resulting shift in contamination allowed for down-stream biotic recovery in the Southern Afon Goch that has seen a 3-fold improvement in benthic invertebrate diversity (Dean et al., 2013). However, evidence of high metal bioconcentration within tissues of the crustacean *Talitrus saltator* at Dulas Bay (Fialkowski et al., 2009) suggests that the effluent plume discharged by the mine via the Southern Afon Goch still enters the food chain. The ecological consequences of higher pollution load along the Northern Afon Goch are unclear, but elevated trace metal/metalloid levels within marine biota at Amlwch would be predicted.

Therefore this investigation assessed the degree of AMD pollution entering the Irish Sea along the northeast coast of Anglesey, Wales adjacent to Dulas Bay and Amlwch, resulting from the Parys Mountain Cu mine. The aim of this study was to assess the efficiency of brown macroalgae to be used as indicators for metal contamination and bioavailability due to coastal AMD pollution at high spatial resolution and over time. This was achieved by measuring trace metal/metalloid concentrations in the macroalgae *F. serratus*, *F. vesiculosus* and *A. nodosum* along intertidal and estuarine transects, and by comparing present trace metal concentration in *F. serratus* and *F. vesiculosus* with past concentration levels collected at the same sites. Through the use of such bioindicator data, an assessment of risk of metal pollution transfer into the marine food chain at specific locations can be gained, which would allow a determination as to whether remediation is needed.

2. Materials and Methods

2.1. Study sites and macroalgae sampling regime

To evaluate the marine pollution resulting from AMD from the Parys Mountain mine on Anglesey, in north-west Wales, UK, two coastal areas that are currently or historically associated with AMD from this mine were selected; Dulas and Amlwch on the north-east coast of Anglesey (Fig. 1A). The Southern Afon Goch has allowed AMD to flow into Dulas Estuary and then into the Irish Sea at Dulas Bay. A spit of land divides Dulas Estuary; this prevents rapid dilution and dispersion of AMD beyond the estuary. At low tide seawater retreats completely and only the narrow channel of the Southern Afon Goch is visible. Six sites (DE1 – DE6) were chosen within Dulas Estuary for the collection of brown macroalgae at rocky areas within the high tidal zone. One of the chosen sites (DE1) was on the land-side of a spit of land and close to the river while sites DE2 – DE5 were along a transect on the coastal side of the spit, and site DE6 was closest to Dulas Bay (Fig. 1B). *F. vesiculosus* was the only *Fucus* sp. that was abundant throughout the estuary so for comparison samples of *A. nodosum* were also collected from these sites. Twelve sites (DB1 – DB12) were chosen along the coastline of Dulas Bay, split into two intertidal transects running either side of the river mouth to identify a possible gradient in metal concentration. Two sites (DB6 and DB7) were either side of the river mouth, sites DB1 – DB5 ranged between 100 m and 1600 m to the north of the river outflow and sites DB8 – DB12 ranged between 100 m and 2000 m to the south of the river outflow (Fig. 1E). All sites at Dulas Bay were chosen as having sufficient abundance of both *F. serratus* and *F. vesiculosus*.

The Northern Afon Goch is a canalised stream that flows north where it enters the Irish Sea at Amlwch via a dredge discharge pipe. Twelve sites (A1 – A12) were chosen for the collection of brown macroalgae at rocky areas within the high tidal zone along the coastline near Amlwch, again split into two intertidal transects running either side of the river outflow to identify a possible gradient in metal concentration. One site (A6) was immediately below the outflow, sites A1 – A5 ranged between 100 m and 1700 m to the west of the river outflow and sites A7 – A12 ranged between 100 m and 1200 m to the south of the river outflow (Fig. 1D). While *F. serratus* was abundant at all Amlwch coastal sites, *F. vesiculosus* was only highly abundant at sites A1 and A2 but absent or only present as occasional samples at all other sites.

Two locations on the west coast of Anglesey were chosen as control sites that were not affected by AMD. The Afon Alaw Estuary was used in comparison to Dulas Estuary since it is physically similar to Dulas Estuary. Three sites (AE1 – AE3) were chosen within the Alaw Estuary for the collection of *F. vesiculosus* and *A. nodosum* at rocky areas within the high tidal zone (Fig. 1C). A rocky shore site at Porth Cwyfan (PC1) (Fig. 1A) was used in comparison to Amlwch and Dulas Bay for the collection of *F. serratus* and *F. vesiculosus*. All sites were sampled in 2017 and a single site at Amlwch (A1), Dulas Bay (DB1), Dulas Estuary (DE6) and Porth Cwyfan (PC1) was previously sampled in 1987 to allow for temporal comparison of the AMD pollution impact. Location coordinates for each sample site is shown in Table S1.

Three replicate samples of each macroalgal species were taken from each site during the 2017 sampling and five replicate samples were taken during the 1987 sampling. Standardised measures were employed to minimise problems of variability (García-Seoane et al., 2018). To avoid age-related variation, macroalgae samples collected were of a similar size at a fixed tidal height and were free of surface defects or evidence of predation. Care was also taken to avoid wave-damaged and epiphyte-attached samples. Macroalgae in direct contact with fine sediment was avoided as this hinders the ability of algae to act as an indicator for metal (Rainbow et al., 2002). The samples were washed with seawater to rid samples of surface contamination through adhesion. To avoid cross-contamination, samples were placed in labelled polythene bags and transported to the lab in insulated cooler bags.

2.2. Macroalgae metal analysis

The concentrations of As, Cd, Cu, Fe, Pb and Zn associated with *F. serratus*, *F. vesiculosus* and *A. nodosum* were measured. The macroalgae samples were first rinsed with deionised water after being transported to the lab then placed in cool storage until analysed. Samples were then well rinsed in deionised water three times and brushed to remove any adhered particulates before being cut using stainless steel scissors. A 2 cm long non-damaged, vesicle and epiphyte free area within the older part of the seaweed was collected since metal concentrations in *Fucus* spp. are higher in the older part of the thallus (Forsberg et al., 1988; Riget et al., 1997). Samples where dried at 80°C for 48 h and ground using a pestle and mortar to facilitate digestion, then 0.1 g aliquots of dried material were digested in 5 mL of 70% ultra-pure nitric acid for 2 h at 100°C refluxed on a hotplate. Digests were then diluted to 2% nitric acid in deionized Milli-Q water (Millipore) and filtered (0.45 µm filter) before analysis by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700x (Agilent, Stockport, UK), which was calibrated using a matrix matched serial dilution of Specpure multi element plasma standard solution 4 (Alfa Aesar). A CRM of *Fucus* *vesiculosus* (ERM-CD200; European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium), certified for As, Cd, Cu, Hg, Pb, Se and Zn, was used to test the accuracy of metal extraction and quantification from seaweed. All of the values were >92.9% of the CRM concentration.

2.3. Water sample data

To assess water quality, specifically trace metal pollution (particulate and dissolved) and pH from the point source discharges, historical water quality plus flow rate data for the Northern Afon Goch at Amlwch and the Southern Afon Goch at Dulas Estuary between 1995 and 2017 were obtained from Natural Resources Wales.

2.4. Data analysis

Mean annual metal mass flux values were calculated as t yr^{-1} of total (dissolved and particulate) Cu, Cd, Fe or Zn using discharge data and metal concentration data. A metal pollution index (MPI) was used to aggregate total metal content in macroalgae to facilitate spatial comparison, utilising the following equation (Usero et al., 1996):

$$MPI = (Cf_1 \times Cf_2 \ldots \times Cf_n)^{1/n}$$

where Cf_n = concentration of metal n in a given sample expressed as mg Kg^{-1} dry weight. The critical index limit for MPI is 100, anything beyond this can be considered critically polluted (Prasad and Sangita, 2008). A Kolmogorov-Smirnov test was performed to test for normality within the data set. Statistical comparison of data was performed either using Kruskal-Wallis test or one-way ANOVA ($P < 0.05$), as appropriate and Tukey's multiple comparison post hoc test. Linear regression analyses were performed to compare metal concentrations between two species. All statistical analyses were performed using GraphPad Prism v6.04.

3. Results and Discussion

3.1. Current and historical status of metal pollution entering the Irish Sea via the Southern and Northern Afon Goch

The analysis of long-term water quality data (1995 – 2017) indicates that alterations in the drainage from the Parys Mountain mine in 2003 has re-routed and increased the AMD outflow into the sea (Figs. 2 & 3; Fig. S1). Since 2003 total metal concentrations decreased in the Southern Afon Goch as it enters Dulas Estuary while metal concentrations dramatically increased in the Northern Afon Goch immediately prior to entering the Irish Sea at Amlwch (Fig. 2). The change in the drainage regime also resulted in a marked reduction in pH to ~ pH 3 in the Northern Afon Goch (Fig. 3C). Mean annual discharge data in both rivers did not significantly vary over the years although the median discharge did show inter-annual variation (Fig. 3A). The metal mass flux entering the Irish Sea from the Northern Afon Goch increased markedly following the 2003 re-routing of mine drainage into the river (Fig. 3B). For example, between 2004 – 2008 the mean annual mass flux of metals into the sea increased by 30-fold for Fe, nearly 5-fold for Cu, 3-fold for Zn and nearly 10-fold for Cd compared to the previous five years. Thus metal outflow via the Northern Afon Goch accounts for the largest single marine release of Fe and Cu nationally within the UK, the second largest release of Zn, and is also a major contributor of Cd (Mayes et al., 2010). No other mine site in the country matches the size of Cu release from the Parys Mountain mine, which makes up 71% of total Cu released nationally. In contrast, decreases in metal flux into the Irish Sea via the Southern Afon Goch were observed, which were significantly lower for Cu, Zn and Cd (Fig. 3B). However, the northern river is releasing greater amounts of metals to the sea than at any time from the Southern Afon Goch, and as a consequence the total metal mass flux from Parys Mountain to the Irish Sea is now higher than in the pre-2003 period, particularly for Fe and Cu. A natural wetland situated on the Southern Afon Goch is acting as a sink for metals and has a substantial remedial effect on the AMD pollution entering the system, so further reducing the metal loadings to the Irish Sea via Dulas

Estuary (Aguinaga et al., 2018). The wetland reduced concentrations of metals by around 50% before 2003 and now removes over 95% of the metals (Dean et al., 2013). In contrast, the Northern Afon Goch is largely canalised and the effluent is not subject to passive remediation by vegetation. Thus total metal loading to the Irish Sea from the Parys Mountain mine has increased dramatically, and therefore this site remains a major source of metal pollution to the Irish Sea.

3.2. Bioindicators for coastal metal bioavailability at Dulas Estuary and Dulas Bay

To determine whether reduced AMD outflow into Dulas Estuary and Dulas Bay was associated with low metal bioconcentration at or near background levels within macroalgae at these locations, samples were collected and analysed for metal concentration. It should be noted that this study did not specifically differentiate between internalised and surface bound metal such as by EDTA washing (García-Ríos et al., 2007), although all samples were rigorously washed in deionised water prior to drying and tissue digestion. However, other studies have indicated that for brown macroalgae species including *F. vesiculosus* over 90% of the associated metals were intracellular bound (Ryan et al., 2012), suggesting that these bioindicator species would be suitable to assess bioavailability of metal pollutants.

For the analysis of Dulas Estuary comparison was made with the Afon Alaw Estuary as a control site since it is not exposed to AMD pollution. Furthermore, Dulas Estuary and Afon Alaw Estuary have similar shore and substrate profiles suggesting comparable periods of immersion and habitat types. *F. vesiculosus* was the only *Fucus* sp. that was abundant to a similar extent across both estuaries; therefore comparisons were also made with the equally ubiquitous brown algae *A. nodosum*. At Afon Alaw Estuary Cu, Zn, Cd and As concentrations within *F. vesiculosus* from all sites, and Fe and Pb at two of the sites (AE2 and AE3) were within the typical background range for *Fucus* sp. in unpolluted environments (Riget et al., 1997; Ryan et al., 2012; Tomlinson et al., 1980), and therefore considered as baseline levels, whereas *F. vesiculosus* Fe and Pb concentrations from site AE1 were unexpectedly high (Fig. 4A). Within the Dulas Estuary at all six sample sites, concentrations of Fe, As and Pb in *F. vesiculosus* were also at baseline levels, as were concentrations of Cu, Zn and Cd from sites DE3, DE4, DE5 and DE6. However, for these three metals, the concentrations were significantly higher than baseline concentrations at sites DE1, furthest from the sea (Fig. 1B), and at site DE2 (Fig. 4A). An equivalent metal association profile was seen for *A. nodosum* (Fig. S2), and there was a significant positive correlation between *F. vesiculosus* and *A. nodosum* for all metals, and individually for Zn and Cu (Fig. 4C).

Along the Dulas Bay shoreline transect, both *F. vesiculosus* and *F. serratus* were abundant and so samples were taken from both species. Comparison was made with an unpolluted control location at Porth Cwyfan where concentrations of all six measured metals were at typical background (baseline) levels for both *F. vesiculosus* (Fig. 4B) and *F. serratus* (Fig. S3). At Dulas Bay concentrations of Cd, As and Pb associated with both *Fucus* spp. were also at or below baseline levels at all sites. However, concentrations of Cd, As and Pb in *F. vesiculosus* sampled from the river mouth at sites DB6 and DB7 (Fig. 1E) were significantly higher than in samples taken at many of the sites in both directions further along the beach (Fig. 4B). A similar pattern was also seen for As in *F. serratus* (Fig. S3). For Fe, Cu and Zn, only the *Fucus* samples collected from sites DB6 and/or DB7 had significantly elevated concentrations of these metals, at 2 – 4-fold higher than the baseline levels (Fig. 4B, Fig. S3). There were also subtle but significantly higher concentrations of Fe and Cu in *F. vesiculosus* at site DB4 (Fig. 4B) and in *F. serratus* at site DB8 (Fig. S3). There was also a strong positive correlation between *F. vesiculosus* and *F. serratus*, which was significant for all metals analysed together, and individually for Fe, Zn and Cu (Fig. 4D). While the metal profiles were essentially equivalent for both *Fucus* spp., there was significantly higher Fe associated with *F. serratus* from site DB7 (3411 $\mu g\ g^{-1}$) than with *F. vesiculosus* from this site (1345 $\mu g\ g^{-1}$).

It is interesting that while there was significant elevated bioaccumulation of Fe at the mouth of the Southern Afon Goch on the Dulas Bay shoreline at site DB7, there was no evidence of bioaccumulation above baseline levels within the estuary. The bioavailability of Fe into macroalgae will be reduced by the buffering effect of seawater causing Fe precipitation (Foster, 1976). Nevertheless, it appears that sufficient quantities of Fe had accumulated at the shoreline to mediate transfer into *Fucus* samples. Furthermore, Fe may act as a vector for other metals via adsorption onto suspended iron oxyhydroxide colloidal particulates (Stüben et al., 2003).

3.3. Bioindicators for coastal metal bioavailability at Amlwch

Since AMD outflow into the Irish Sea at Amlwch was found to generate a significantly high metal pollution load over the last 15 years, macroalgae samples were collected and analysed for metal concentration to examine whether there was evidence of high metal bioconcentration close to the source of source and whether there was evidence of metal dispersal. Comparison was again made with the unpolluted control location at Porth Cwyfan. *F. vesiculosus* and *F. serratus* were both present along the shoreline adjacent to Amlwch but *F. serratus* was more abundant and so replicate samples of *F. vesiculosus* could only be taken at sites A1 and A2, which are over 950 m from the outflow (Fig. 1D).

The AMD from the Northern Afon Goch flows into the Irish Sea at site A6 via a drainage pipe (Fig. 5A) then gives rise to a plume of metals that are clearly visible in the sea (Fig. 5B), and which is composed of iron oxides and iron hydroxides, and other precipitated metals and dissolved metals. The concentration of all measured metals (Fe, Cu, Zn, Cd, As, Pb) associated with *F. serratus* biomass at site A6 was significantly higher than the baseline concentrations within the macroalgae from site PC1 (Fig. 5E). However, the Cd concentration within the *F. serratus* is perhaps lower than expected despite concentrations within the Northern Afon Goch outflow typically reaching 30 – 40 μg L^{-1} (Fig. 2). A likely explanation, given the extremely high abundance of Zn is that Cd accumulation is being suppressed as a result of competition with high Zn concentrations for limited binding sites (Giusti, 2001). Though Zn may reduce toxicity of Cd at Amlwch for macroalgae, it is still of concern. These free metal ions could be readily available for bioaccumulation as Cd quickly attaches to sediment, where particle-bound and interstitial Cd may exert toxic effects on benthic animals (Swartz et al., 1986). Another toxic metal that is of concern is Pb found in the outflow at Amwlch at concentrations typically reaching 15 µg L^{-1} (Fig. S1), and accumulating into *F. serratus* to nearly 70 µg g^{-1} here, substantially above baseline levels (Riget et al., 1997; Ryan et al., 2012).

The effect of this AMD exposure was clearly evident by visual inspection of both species of *Fucus*; in contrast to samples from all other sites, the macroalgae consistently showed evidence of discolouration and reduced size of the fronds (Fig. 5C & D). The concentrations of Fe (278.6 mg g^{-1}), Cu (6.3 mg g^{-1}), Zn (2.3 mg g^{-1}) and As (196.0 µg g^{-1}) associated with *F. serratus* from site A6 were extremely high in comparison to the current literature for *Fucus* spp. The Fe concentration was over 100 times higher than previous measurements for *Fucus* spp. (Fuge and James, 1973) and over 10 times higher than the Fe concentration value of 22.7 mg g^{-1} observed in the brown macroalga *Padina pavonica* isolated from the Syrian coast (Al-Masri et al., 2003). Likewise this Cu concentration was over 60 times higher than values seen in *F. vesiculosus* (Stenner and Nickless, 1974). The highest Zn concentrations recorded in this study were substantially higher than the typical concentrations for *Fucus* spp. even under polluted conditions, although equivalently high Zn concentrations (3.6 mg g^{-1} for *F. serratus*; 4.2 mg g^{-1} for *F. vesiculosus*) have previously been seen (Bryan and Gibbs, 1983; Stenner and Nickless, 1974). Finally, the As concentration was slightly higher than previous uppermost recorded value of 147 µg g^{-1} for *F. serratus* (Klumpp and Peterson, 1979) and 160 µg g^{-1} for *F. vesiculosus* (Langston, 2009). This observation, alongside the high concentration of As in the Northern Afon Goch outflow of between 75 – 100 µg L^{-1} (Fig. S1) indicates marine pollution at this site and marine bioavailability of As. A large amount of the As in human diet comes from seafood and various reports have investigated the relationship between As exposure from seafood and carcinogenic diseases (Borak and Hosgood, 2007; Buchet et al., 1994). Arsenate is readily taken up by plants and algae due to its chemical similarity to phosphate (Sanders et al., 1989).

The concentrations of Fe, Cu, Zn, As and Pb in the macroalgae reduced dramatically from sites within both directions of the outflow, indicating rapid dispersal of metals. Thus, the *F. serratus* concentrations of Fe, As, Cd and Pb at all other sites along the coastline (sites A1 – A5 and A7 – A12) were at baseline concentrations (Fig. 5E). However, for Zn there was also significantly higher macroalgae concentration at site A5, which is situated 100 m to the north-west from the outflow, and for Cu there were significantly higher macroalgae concentrations at site A5 and at sites A7 and A8, which are 100 m and 200 m to the south-east from the outflow (Fig. 1D). In *F. vesiculosus* concentrations of Fe, Cd, As and Pb at sites A1 and A2 were not significantly different from the PC1 values, and while Cu and Zn concentrations were significantly higher than the PC1 values (Fig. 5F), they were still close to or within the metal concentration ranges seen in *F. vesiculosus* collected from other clean sites (Giusti, 2001; Pedersen, 1984; Ryan et al., 2012).

3.4. Geographical distribution of present day metal pollution

A detailed spatial analysis is needed when determining the dispersal of a pollutant, which should be therefore informing the sampling design. Many bioindicator studies do not sample at high resolution and indeed many studies do not specify the distance between the sample site and the point source of pollution (García-Seoane et al., 2018). In this study we were able to use the *Fucus* spp. metal concentration values at the known distances to calculate MPI values for each sample site associated with both outflow locations to generate a spatial distribution of pollution risk at Amlwch and Dulas Bay (Fig. 6). MPI values for all sites were < 35 (requiring no treatment) with the exception of site A6 at the mouth of the Northern Afon Goch with a MPI value of 659, which is indicative of a critically polluted site that requires immediate remediation efforts (Tomlinson et al., 1980). There was evidence of a very steep pollution gradient from either direction of the Northern Afon Goch outflow at site A6 (Fig. 6A) indicating rapid dispersal of the metal pollution within 100 m of outflow and consequently a rapid reduction in bioaccumulation at these adjacent sites. At Dulas Bay, which has a legacy of many decades of AMD pollution, there was no evidence for a metal pollution gradient (Fig. 6B). No significant correlation was detected between distance and MPI value.

3.5. Historical comparison of macroalgae bioindicators for coastal metal bioavailability

In addition to the *Fucus* samples collected in 2017, samples had been collected at some of the same sites in 1987 on the Amlwch coast (site A1), Dulas Estuary (site DE6), Dulas Bay (site DB1), and control site Porth Cwyfan (site PC1) and measured for Cu and Zn concentration using the same methods as for the samples in 2017, allowing direct comparison. A comparison of the samples between these 30 year periods would allow an evaluation of the use of macroalgae as temporal bioindicators and determine whether the alteration in direction of drainage outflow in 2003 could be seen on the basis of metal bioaccumulation into the macroalgae. There was no significant difference in Cu and Zn concentration within Porth Cwyfan *Fucus* samples between the two time periods and likewise no significant difference between times within the Dulas Bay samples (Fig. 7). There was a significantly increased Zn concentration in the macroalgae samples collected from site A1 near the Northern Afon Goch outflow in 2017 compared to 1987, while there was a significant, extremely large decrease in Cu concentration in the samples from site DE6 within Dulas Estuary collected in 2017 in comparison to the samples collected at that site in 1987. This observation is consistent with earlier studies that demonstrated that prior to the alteration in drainage from the Parys Mountain mine in 2003 the Dulas Estuary and Dulas Bay were heavily polluted sites that demonstrated high metal bioaccumulation into marine organisms (Al-Thaqafi and White, 1991; Foster, 1976; Foster et al., 1978; Parkman et al., 1996; Rainbow et al., 1999). For example, other past surveys of *F. vesiculosus* at the edge of Dulas Estuary performed in 1997 have measured concentrations of Cu at 246 ± 106 μg g^{-1} (Rainbow et al., 1999), while the Cu values are also substantially higher to the concentrations measured in 2017 but not as high as those observed in 1987.

3.6. Perspectives

While it is clear that the AMD discharge from the Parys Mountain mine site is still a cause of significant levels of trace metal pollution at the Irish Sea coast (Fig. 3), the re-routing of the discharge away from Dulas Estuary is likely to have longer-term benefits to the ecology of this estuary. This reduced AMD flow along the Southern Afon Goch has been further decreased and remediated by the action of the natural wetland, which is approximately 7 km upstream of Dulas Estuary (Aguinaga et al., 2018; Dean et al., 2013). Although there was an increase in metal bioavailability into macroalgae from specific sites at the Southern Afon Goch as it passes through the estuary (sites DE1, DE2), and at the mouth of the river as it enters the bay (sites DB6, DB7), the degree of pollution within Dulas Estuary overall is no longer a concern. This is in stark contrast to the situation at the site in previous decades (Al-Thaqafi and White, 1991; Parkman et al., 1996; Rainbow et al., 1999). However, the upstream wetland has given rise to a build-up of significant amounts of Fe-rich particulate metal sediment (Dean et al., 2013) that appears to be flushed into the estuary and the bay during periodic high discharge events (Fig. 3A). This may explain some of the above-baseline metal accumulation profiles seen in several of the macroalgae samples (Fig. 4). With regard to the AMD outflow at Amlwch, this is now responsible for an overall higher metal flux into the Irish Sea, and this outflow gives rise to extremely high levels of metal bioaccumulation into macroalgae (Fig. 5). However, the direct discharge into the Sea does lead to rapid dispersal and dilution, and thus may be less damaging to the coastal ecosystem. Future studies are needed to evaluate the biodiversity at both sites in more detail in order to conclude whether the changes in AMD discharge from the Parys Mountain mine have had significant beneficial and/or negative consequences.

Although macroalgae are very good bioindicators for dissolved metal bioavailabililty, it is also possible that a macroalgal bioindicator is underestimating the total metal bioavailability risks. Particulate metals will bind to the external surface of macroalgae, which may overestimate bioaccumulation if samples are not sufficiently washed (García-Seoane et al., 2018); however, macroalgae will not efficiently accumulate particulate metals unlike filter feeders such as the barnacle (Rainbow et al., 1999). Therefore, it must be considered that the risks of bioavailability by high accumulation of particulate metals within Dulas Estuary could have been underestimated.

3.7 Conclusions

Overall, we can conclude that three species of brown macroalgae (*F. serratus*, *F. vesiculosus* and *A. nodosum*) can be used as robust bioindicators to allow high resolution mapping of trace metal pollution. Furthermore, the significant positive correlation between the species, suggesting equivalent bioaccumulation mechanisms and characteristics, indicates that they can be used interchangeably as bioindicators at sites with presence for different species. We propose that by making use of macroalgae bioindicators at high spatial resolution wherever possible, analysis can then be directed to further investigate the marine food chain at specific high-risk locations in detail to quantify metal pollution transfer into higher trophic levels and to determine ecological consequences of pollutants such as AMD. The use of macroalgal bioindicators would then also be used to determine whether remediation activities at heavily polluted sites are required. For example, the outcomes from this present study indicate the need to reduce the substantial AMD outflow via the Northern Afon Goch at Amlwch in order to reduce the metal pollution and risks of toxicity to marine biota at this Irish Sea coastal site.

Acknowledgements

We thank Natural Resources Wales for providing historical water quality and flow rate data for the Northern Afon Goch at Amlwch and the Southern Afon Goch at Dulas Estuary. We thank Paul Lythgoe (Manchester Analytical Geochemistry Unit, School of Earth and Environmental Sciences) for ICP-AES analysis.

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Figures

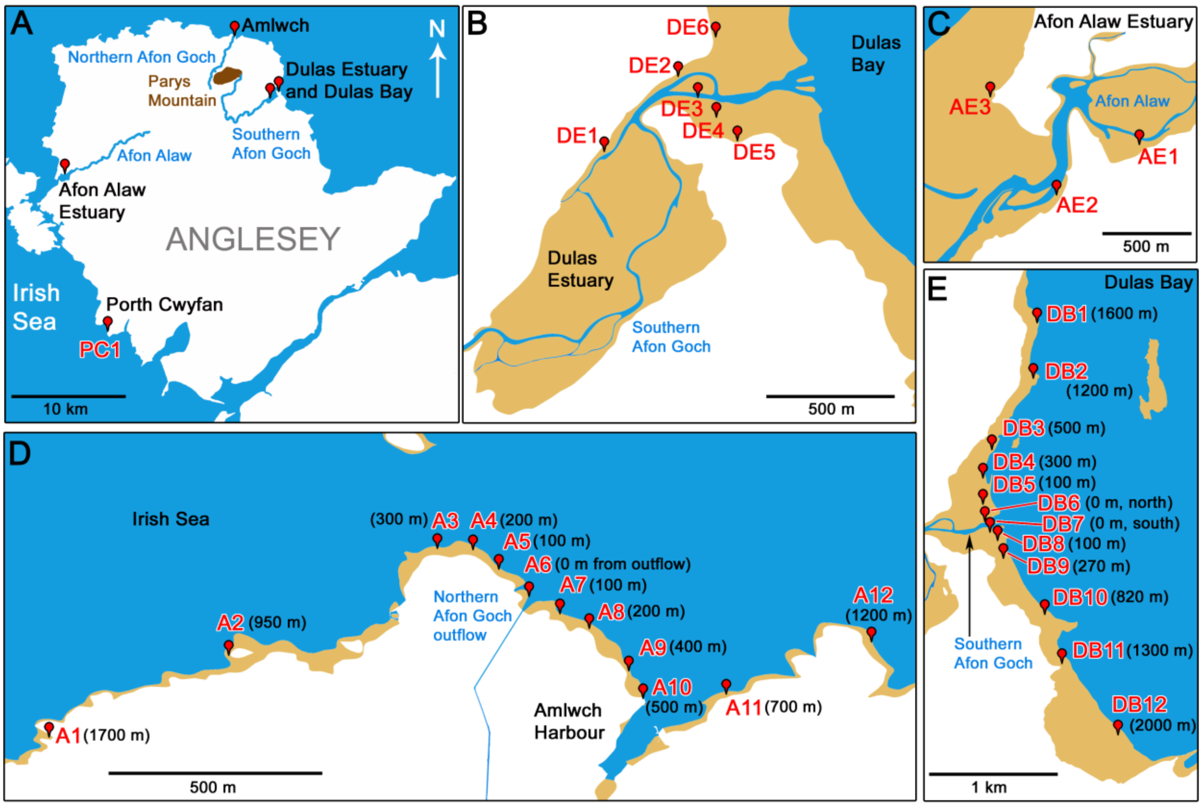

Figure 1. Location of the coastal sample sites and the AMD source at Parys Mountain mine. (A) Map of Anglesey, UK and location of sampling sites at Amlwch, Dulas Estuary, Dulas Bay, Afon Alaw Estuary (non-polluted control site) and Porth Cwyfan (non-polluted control site). The single sample site (PC1) at Porth Cwyfan is indicated. (B) Map of Dulas Estuary and sample sites DE1 – DE6. (C) Map of Afon Alaw Estuary and sample sites AE1 – AE3. (D) Map of Amlwch coast and sample sites A1 – A12. Site A6 is at the source of AMD outflow via the Northern Afon Goch. The distance of all other sample sites from the outflow is indicated. (E) Map of Dulas Bay and sample sites DB1 – DB12. Site DB6 and DB7 are on the north and south banks of the Southern Afon Goch river mouth, which is the source of AMD outflow. The distance of all other sample sites from the outflow is indicated.

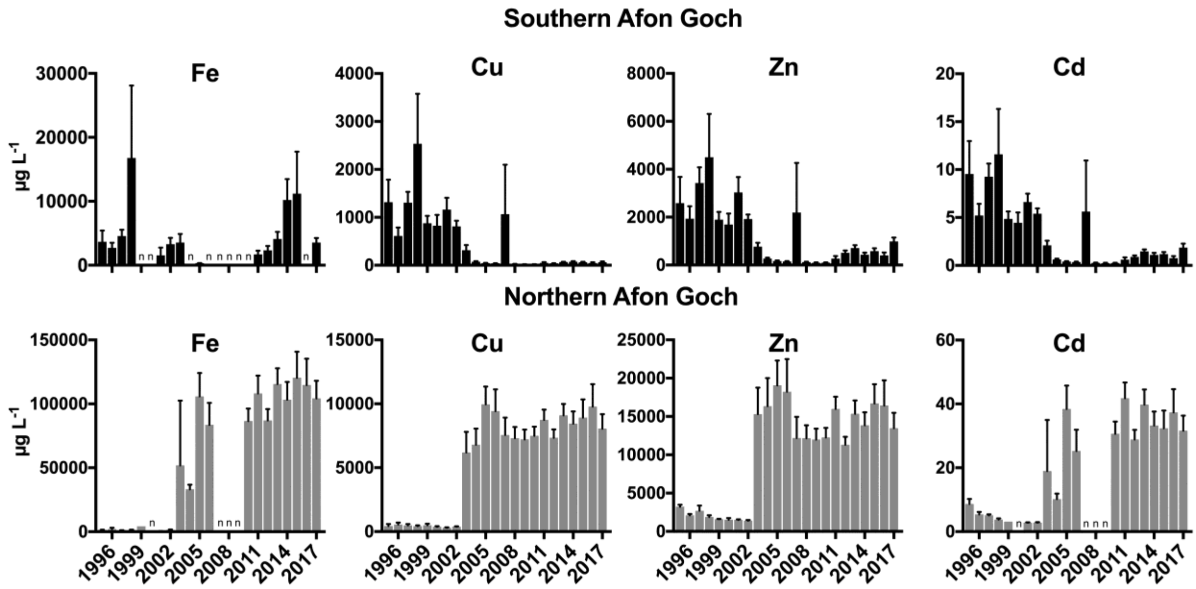

Figure 2. Historical metal concentration data (1995 – 2017) for water samples taken from the Southern Afon Goch before entering Dulas Estuary and the Northern Afon Goch before entering the Irish Sea at Amlwch. Data are mean (± SEM) values of total (dissolved and particulate) Fe, Cu, Zn and Cd concentrations for each year where data was available. Data was collected by Natural Resources Wales with 1 – 27 readings collected per year. For some years, individual metal datasets were not available (n: not determined).

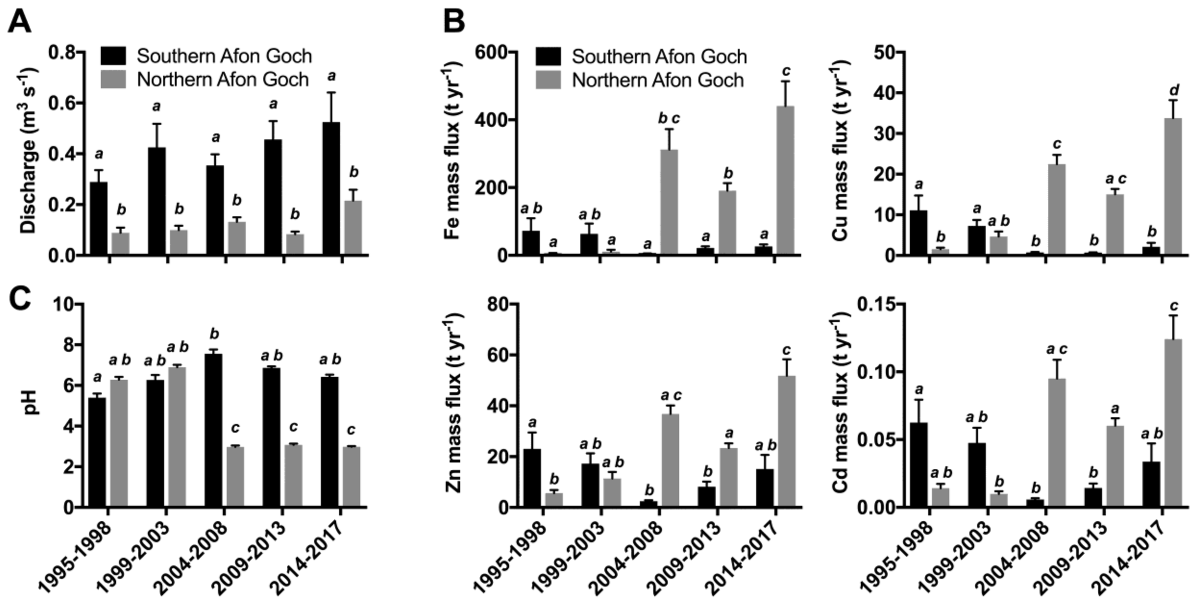

Figure 3. Historical (1995 – 2017) discharge (A), mass flux data for total (dissolved and particulate) Fe, Cu, Zn and Cd (B), and pH data (C) for water samples entering Dulas Estuary and Dulas Bay via the Southern Afon Goch and entering the Amlwch coastal water via the Northern Afon Goch. Data are mean (± SEM) values for the indicated time periods. Values that do not share lowercase letters are significantly different ($P < 0.05$). Data was collected by Natural Resources Wales with 1 – 27 readings collected per year.

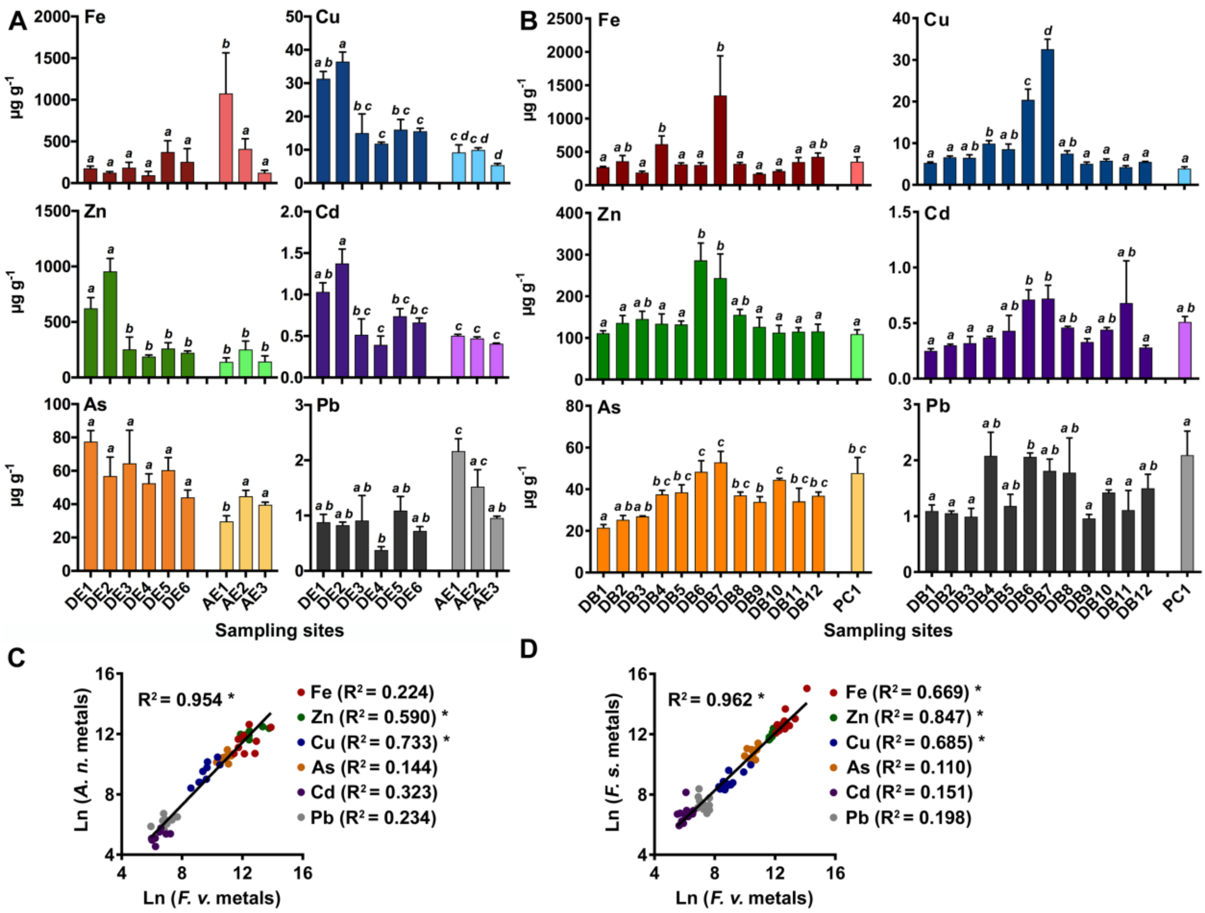

Figure 4. (A and B) Metal concentrations associated with *Fucus vesiculosus* collected from sampling sites at Dulas Estuary (DE1 – DE6) and Afon Alaw Estuary (AE1 – AE3) (A), and from Dulas Bay (DB1 – DB12) and Porth Cwyfan (PC1) (B). All samples were collected in 2017. Data are mean (± SEM) of three *Fucus* samples from each site. Values that do not share lowercase letters are significantly different ($P < 0.05$). (C and D) Correlations between metal concentrations associated with *F. vesiculosus* (*F. v.*) and *Ascophyllum nodosum* (*A. n.*) collected from Dulas Estuary and Afon Alaw Estuary (C), and between metal concentrations associated with *F. vesiculosus* and *Fucus serratus* (*F. s.*) collected from Dulas Estuary and Afon Alaw Estuary (D). Correlations were performed for all metals grouped together and each metal individually. All metal concentration data (as µg per mg) was natural log transformed. An asterisk indicates a significant correlation ($P < 0.05$). Metal concentration data for *A. nodosum* and *F. serratus* are shown in Fig. S2 and Fig. S3, respectively.

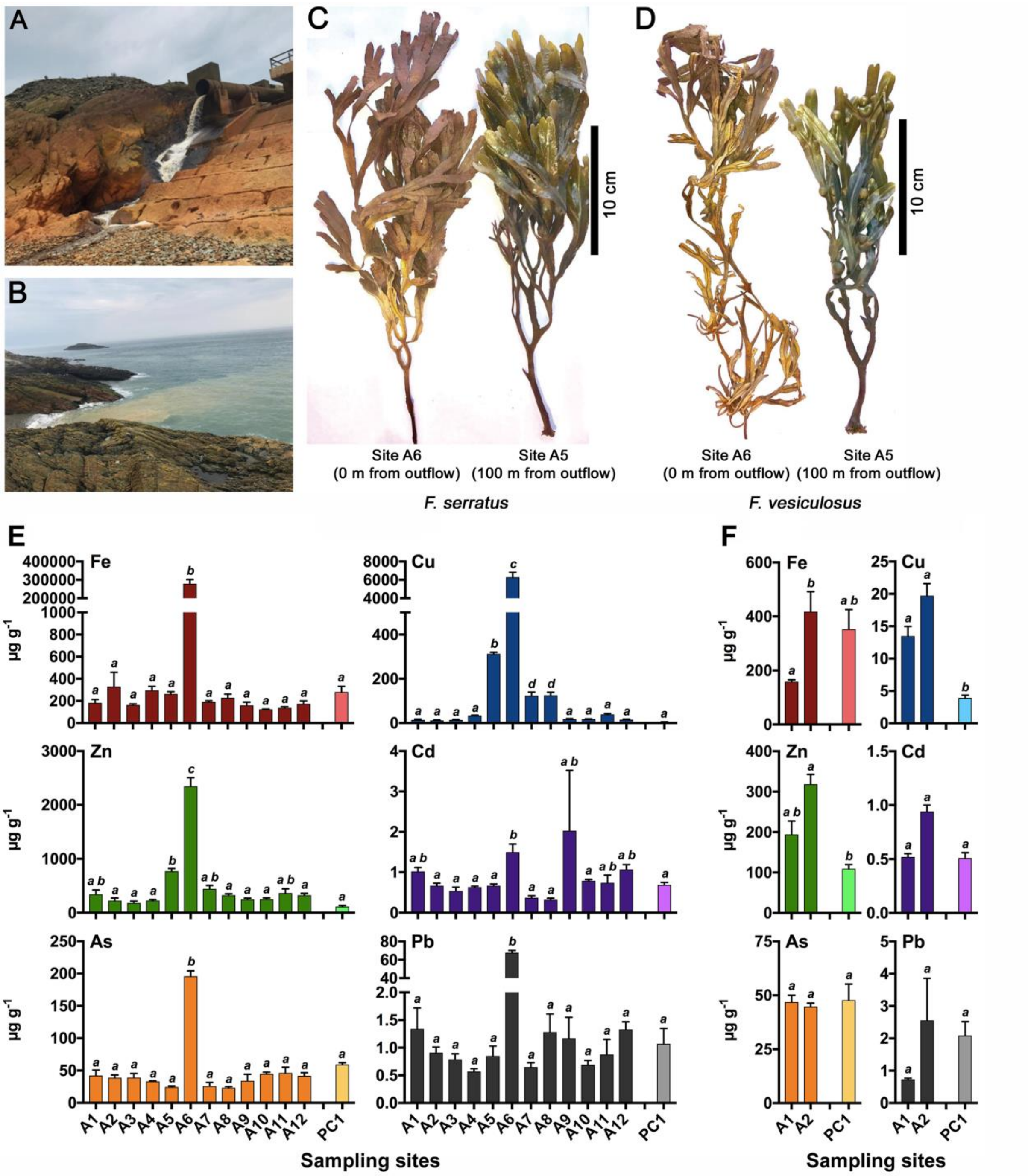

Figure 5. AMD pollution at Amlwch from the Northern Afon Goch outflow. (A) Water from the Northern Afon Goch as it enters the Amlwch coast via a dredge pipe at site A6. (B) The effluent plume moving easterly after entering the Irish Sea at site A6. (C and D) Representative *Fucus serratus* (C) and *Fucus vesiculosus* (D) samples collected from site A6 at the source of AMD effluent outflow and from site A5, which is 100 m from the outflow. (E and F) Metal concentrations associated with *Fucus serratus* (E) and *Fucus vesiculosus* (F) collected from sampling sites at Amlwch (A1 – A12) and Porth Cwyfan (PC1) collected in 2017. *F. vesiculosus* was only present at high abundance along the Amlwch coast at sites A1 and A2. Data are mean (± SEM) of three *Fucus* samples from each site. Values that do not share lowercase letters are significantly different ($P < 0.05$).

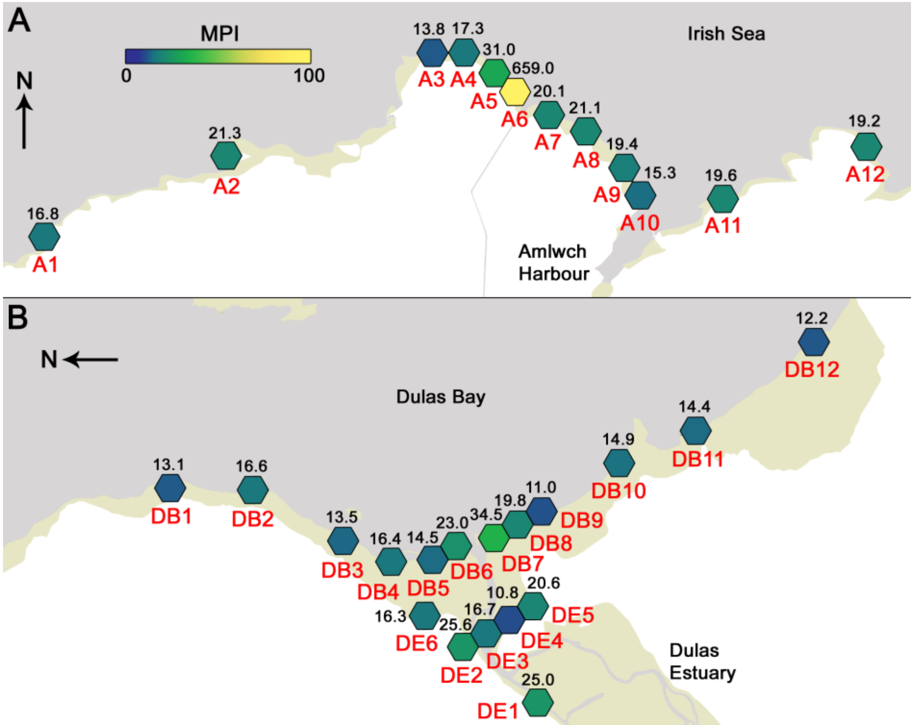

Figure 6. Geographical distribution of metal contamination using Metal Pollution Index (MPI) values in Anglesey coastal waters in 2017. MPI values were determined from cumulative Fe, Cu, Zn, Cd, As and Pb concentrations associated with *Fucus* sp. at each sample site.

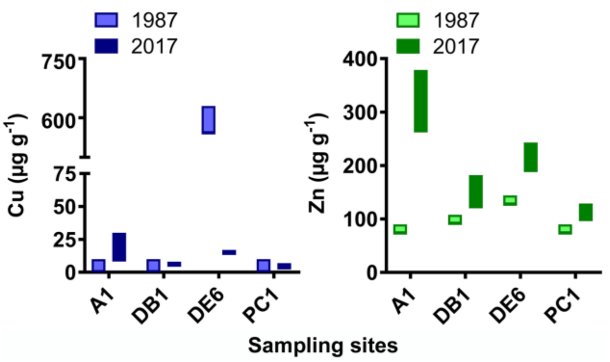

Figure 7. Historical comparison between *Fucus* sp. samples collected in 1987 and 2017 from Amwlch (site A1), Dulas Bay (site DB1), Dulas Estuary (site DE6) and Porth Cwyfan (site PC1) for Cu and Zn concentration. Boxes show minimum and maximum concentration ranges.