



# Quantifying trace metal contamination from marine cathodic protection using *Saccharina latissima* in laboratory and mesocosm exposure experiments

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## ABSTRACT

The increasing deployment of offshore infrastructure has raised concerns about the environmental impact of corrosion protection systems, particularly galvanic anodes, which release trace metals such as zinc and aluminium into the marine environment. Traditional monitoring methods often fail to capture the bioavailable fraction of these contaminants or provide adequate temporal resolution. Here, we investigate the brown macroalga *Saccharina latissima* as a bioindicator of metal emissions from galvanic anodes. Laboratory and mesocosm experiments demonstrated linear relationships between environmental concentrations and metal accumulation, particularly for zinc. Compared to grab and passive sampling, *S. latissima* provided more consistent and representative exposure estimates. These findings highlight the potential of *S. latissima* as a cost-effective and reliable bioindicator for offshore trace metal contamination monitoring and its integration into environmental assessment frameworks.

## 1. Introduction

Anthropogenic inputs of contaminants such as *per-* and poly-fluoroalkyl substances (PFAS), trace metals and pharmaceuticals, in the marine environment have dramatically increased over the last few decades, impacting the health and resilience of associated ecosystems (Tornero and Hanke, 2016). In Europe, these concerns are framed in the Water Framework directive (WFS, 2000) and the Marine Strategy Framework Directive (MSFD, 2008), which aim to achieve good ecological and environmental status of aquatic ecosystems. The MSFD Descriptor 8 specifically addresses those contaminants, identifying priority substances and providing environmental quality standards to ensure that contaminants in marine environments are maintained at levels that do not cause pollution effects on marine ecosystems (MSFD, 2017). Among these contaminants, trace metals put the most pressure on the environment in the impacted areas (Andersen et al., 2022), especially in seaports and other industrialized coastal areas (Ansari et al., 2004; Jahan and Strezov, 2018; Manzetti, 2020; Özşeker et al., 2022). However, data on the environmental health conditions in relation to

trace metal contamination in offshore marine areas are scarce. Moreover, data from localized sources, such as offshore oil rigs, installations for renewable energy or shipping lanes, are lacking or interpretation is complicated because of the dynamic nature of both the sampling vectors and the surrounding environment (Ebeling et al., 2023). Therefore, the impact of marine industrial activities on the environment in the direct vicinity of these point sources is rarely considered to date. Although case studies are available for a few offshore sites such as gas fields (Gomiero et al., 2015) and wind farms (Ebeling et al., 2023; Wang et al., 2023), historical data and common descriptors that are necessary for larger-scale environmental assessments, are limited. As a result, continuous marine-borne emissions from lesser confined sources, such as galvanic anodes, scrubbers and coatings for corrosion protection and antifouling on offshore structures, mainly releasing the trace metals zinc and aluminium, have remained under the radar, even though their impact arises mainly from the scale at which they are used (Barbarin et al., 2023; Gabelle et al., 2012; Kirchgorg et al., 2018; Michelet et al., 2020; Reese et al., 2020). Under MSFD Descriptor 8, zinc is classified as a priority substance due to its ecological relevance, whereas aluminium,

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although not a priority substance, is considered a relevant substance for monitoring (MSFD, 2017).

Trace metal contamination is typically measured in sediment and seawater, obtained through grab sampling with e.g. core samplers and rosette samplers. Information availability is partly limited by the application of these traditional sampling strategies, as they inherently lack (i) information about the fraction of trace metals that might enter the marine ecosystem and accumulate in higher trophic organisms, and (ii) a coherent temporal representation due to the dynamic nature of marine sediment and water bodies. To determine the extent of environmental contamination caused by lesser confined contamination sources, sampling vectors are needed that are localized, sensitive and robust. Various organisms, such as fish, sponges, bivalves and macroalgae, have been proposed as bioindicators to complement direct seawater and sediment sampling, as they can provide historical information with high geographical precision (Amado-Filho et al., 2008; Genta-Jouve et al., 2012; Ghosn et al., 2020; Goldberg et al., 1983; Rainbow et al., 2002). Macroalgae accumulate trace metals exclusively from the water column, and exhibit a high tolerance and accumulation capacity (Agarwal et al., 2022; Amado Filho et al., 1997; van Ginneken and de Vries, 2018). Additionally, they are sessile, tolerate wide ranges of environmental conditions, are easy to sample and process, and are widely abundant year-round, which makes them ideal bioindicators when species are chosen locally (Al-Homaidan et al., 2021). More specifically, the use of macroalgal transplants offers additional advantages over the use of native populations, such as the elimination of local adaptive behavior, metal tolerance building and a dependency on the availability of native populations. Furthermore, using transplants offers better control over geographical localization and exposure periods. The increasing interest in offshore macroalgae cultivation opens the door to the use of macroalgal transplant biomonitoring near offshore structures such as offshore wind farms (Banach et al., 2020; Furness and Furness, 2025; Macias et al., 2025; Moscicki et al., 2024; O'Shea et al., 2024; Ross et al., 2025).

Nonetheless, macroalgal transplant biomonitoring has, to date, not been directly applied in offshore areas, due to a lack of standardized methodologies and a limited understanding of their metal uptake behavior (García-Seoane et al., 2018a; Vázquez-Arias et al., 2023b). The few macroalgal transplant studies performed in the field have a more comparative character, and typically assess differences in accumulation capacity between species (Amado Filho et al., 1999), strains (Sáez et al., 2015) and populations (García-Seoane et al., 2020). However, knowledge on the key uptake mechanisms and respective relationships between environmental and accumulated concentrations, which are key to a quantitative assessment of environmental contamination, are lacking in field experiments with macroalgal transplants.

Some trace metal concentration factors were determined under laboratory conditions in early studies. For example, concentration factors have been reported for zinc accumulation in the commonly researched macroalgal species *Ulva Lactuca*. Gutknecht (1965) derived a concentration factor of  $0.3 \times 10^3$  L seawater/kg fresh biomass; Amado Filho et al. (1997) derived a value of 6 to  $7 \times 10^3$  L seawater/kg dry biomass and van Ginneken and de Vries (2018) obtained a value of  $4 \times 10^3$  L seawater/L seaweed moisture. Note also that there is no consensus over the units in which the concentration factors are expressed. Nonetheless, these experiments provide a first indication of the relationship between the environmental trace metal concentrations and the accumulated trace metal content in macroalgae.

More recently, efforts have been made to determine such concentration factors in wild populations in natural environments. For example, for zinc accumulation in *Ulva* sp., Akcali and Kucuksezgin (2011) determined a concentration factor of  $36.4 \times 10^3$  L seawater/L fresh biomass, whereas Conti and Cecchetti (2003) obtained a value of  $8.1 \times 10^3$  L seawater/L fresh biomass. Concentration factors differ widely between studies since, among others, the sample species, the sampling time and location, and type and speciation of the investigated

trace metals vary widely (Chernova and Shulkin, 2019; García-Seoane et al., 2018b). To explore the potential of using macroalgae for quantitative monitoring of offshore trace metal contamination, normalization of the uptake behavior in macroalgal species for offshore environments is key.

In this study, we focus on the macroalgal species *Saccharina latissima*. *S. latissima* is a fast-growing kelp species that is able to withstand high wave heights and harsh conditions typically found offshore (Bak et al., 2018; Buck and Buchholz, 2005), and therefore, standalone and integrated offshore cultivation of this species in offshore wind farms is emerging (Bernard et al., 2020; Kotta et al., 2022; Maar et al., 2023; Moscicki et al., 2024; O'Shea et al., 2024). *S. latissima* typically has a high polysaccharide content, which can reach up to 40% of the total biomass and is composed mainly of alginates (Sæther et al., 2024). Consequently, passive uptake mechanisms such as physical adsorption and extracellular retention by adsorption in polysaccharide cell walls are hypothesized to be the main uptake pathways in *S. latissima* (Deniz and Karabulut, 2017; Mazur et al., 2018; Vázquez-Arias et al., 2023a), which could be exploited for the quantitative assessment of trace metal emissions from hidden sources.

Two types of galvanic anodes were used as contamination source in this study: zinc based anodes, commonly used for ships protection (Bird et al., 1996; Rees B. et al., 2017; Vignesh and Sathiy, 2024), and aluminium based anodes, which are widely used to protect marine structures against corrosion (Ebeling et al., 2023; Gabelle et al., 2012; Kirchgorg et al., 2018; Levallois et al., 2023). These zinc- or aluminium-based anodes act as sacrificial alloys to protect immersed structures, potentially releasing significant quantities of trace metals in the environment due to their active dissolution. This study focuses on the relationships between anode release, exposure time, contamination level and trace metal uptake. Additionally, for the first time, metal accumulation was measured in *Saccharina latissima* under direct controlled exposure to galvanic anodes in a mesocosm.

The primary objectives of the present study are (i) to investigate the relationship between environmental and accumulated metal concentrations in contaminated seawater under controlled laboratory conditions, and (ii) to explore the potential application of this relationship for macroalgal transplant biomonitoring, in a macroalgal species adapted to offshore conditions.

## 2. Materials and methods

### 2.1. Acclimatization of *Saccharina latissima* under laboratory conditions

Adult *Saccharina latissima* fronds were sampled from a local population at a pristine site on the north coast of Brittany, France (48°38'3.6" N, 4°31'37.7" W), at the lowest tide. They were transported in a closed cooling box submerged in seawater (ca. 1 h). The fronds were acclimatized and stored vegetatively in a plastic tank (800 L) for at least 7 days before experimental exposure. The tank was insulated, and the open top was covered with a transparent acrylic sheet. There was a constant flow of decanted and filtered seawater from the bay of Brest in the tank at an arbitrarily fixed rate of 1.6 L/min. A water pump connected to an aquarium cooler ensured constant water circulation in the tank at  $11.5 \pm 1.0$  °C. A LED light bar with bright white light at 1000 lx was placed 0.5 m above the water surface to provide the light needed for photosynthesis under a 12 h/12 h light/dark regime. The seawater in the bay of Brest has standard characteristics of the Northeast Atlantic Ocean, with a salinity of approximately 34 PSU, an oxygen content of approximately 6 mg/L, and a pH of approximately 7.8. The pH and salinity of the seawater were obtained via the SOMLIT buoy located near the seawater intake of the facility near beach Ste. Anne de Portzic, a few kilometers seawards from the city Brest.

## 2.2. Laboratory-scale exposure of *S. latissima* to seawater indirectly contaminated by galvanic anodes

A plastic container was filled with 10 L seawater from the bay of Brest, which was passed through several mechanical filters to remove any suspended matter. An industrial anode comprising aluminium (4.87% Zn, 0.16% In, 0.07% Fe, 0.03% Si, 0.001% Cd, rem. % Al) (BAC corrosion control AS, Denmark) or zinc (> 99.8%), (BAC corrosion control AS, Denmark) was connected to a mesh titanium-mixed metal oxide-coated cathode (MAGNETO, China) via a galvanostat programmed to a constant current output of 0.30 A, delivering an electric current corresponding to a total input of 46 mmol/L electrons (Eq. 1), equivalent to 15.2 mg/L Zn or 4.0 mg/L Al released from the anodes. The total input was set arbitrarily but designed to approximate Zn and Al concentrations that were around 1000 times higher than reported environmental levels in contaminated sites.

$$Q_{e-} = \frac{I \cdot t}{F \cdot V} \quad (1)$$

Where  $I$  is the applied current (A),  $t$  the exposure time (s),  $F$  the Faraday constant (96,485 As/mol), and  $V$  the volume of the solution (L).

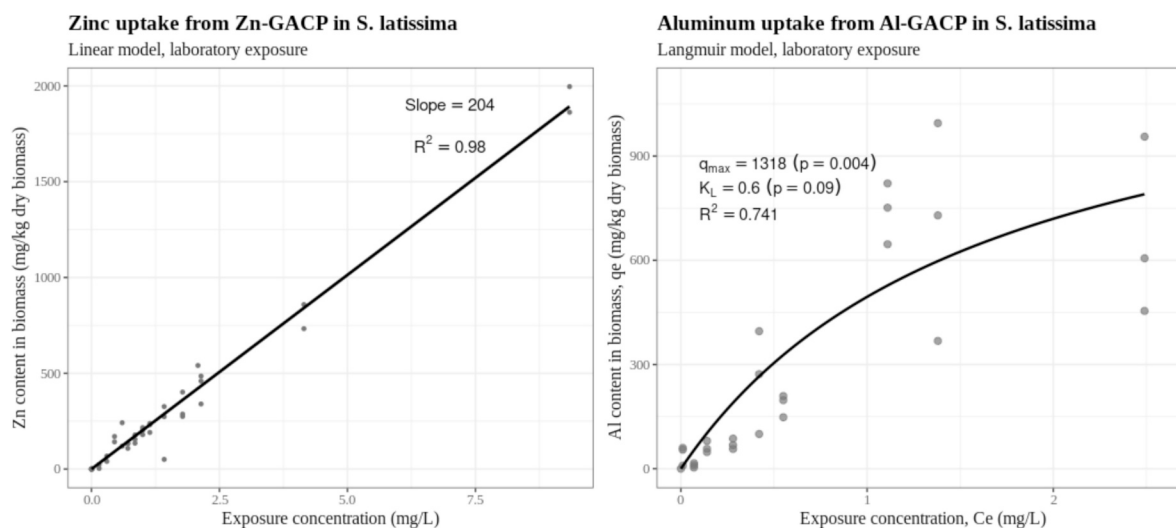
The solution was continuously mixed via a magnetic stirrer. The stock solutions were prepared at room temperature ( $\pm 20$  °C) to ensure reproducibility and stable galvanostat operation. Seawater samples were collected at the end and stored at 12 °C in closed 250 mL plastic bottles. Exposure solutions for laboratory-scale exposure of *S. latissima* were prepared by diluting the contaminated seawater (4.6 mmol/L electrons) to appropriate exposure concentrations (0.15 mg/L, 0.30 mg/L, 0.45 mg/L, 0.60 mg/L, 0.71 mg/L, 0.85 mg/L, 1.00 mg/L, 1.14 mg/L, 1.42 mg/L, 1.78 mg/L, 2.08 mg/L, 2.14 mg/L, 4.15 mg/L and 9.34 mg/L zinc by Zn-GACP and 0.01, 0.07, 0.14, 0.28, 0.42, 0.55, 1.11, 1.38 and 2.49 mg/L aluminium by Al-GACP) and enriching it with 0.1 mL/L Cell-Hi F2P growth medium (Varicon Aqua, United Kingdom) to provide nutrients to the macroalgae. After preparation, the solutions were stored at 12 °C for at least one week prior to use, allowing equilibration to the laboratory exposure temperature. The pH of the solutions was checked after storage and adjusted to 7.8 using 1 M HCl or 1 M NaOH if needed. The exposure concentrations ( $C_e$ ) used in Figs. 1 and 3, were calculated from the dilution factors of the stock solutions and were not analytically measured. Discs ( $\phi$  approx. 8 cm) were cut from the middle part of the

acclimatized *S. latissima* fronds and individually immersed in 100 mL of exposure solution, which was replenished three times per week. The exposed cells were covered with a transparent lid to prevent evaporation, the temperature was kept constant at  $12 \pm 1$  °C, and bright white light was provided in a 12 h/12 h light/dark regime. After exposure periods of up to 28 days, the biomass was recovered and dried at 60 °C, pulverized, and stored in a desiccator until further sample preparation and analysis.

The metal contents were determined for three individuals exposed to seawater contaminated with Zn-GACP or Al-GACP at different contamination levels. Since low amounts of essential metals are naturally present, the background metal contents, obtained from three experimental blanks, were subtracted from the experimentally obtained metal contents. The concentration factor was expressed as L seawater / kg dry *S. latissima* biomass over an exposure period of 28 days. This unit can be converted by correcting for the moisture content of *S. latissima*, which was around 18%.

## 2.3. Monitoring of metal contamination in a mesocosm with galvanic anodes

A mesocosm experiment was performed using three experimental tanks of 80 L. The experimental tanks were insulated and covered with a transparent acrylic sheet. There was a constant flow of decanted and filtered water from the bay of Brest in the tank at a rate of  $0.5 \pm 0.1$  L/min. A water pump connected to an aquarium cooler ensured constant water circulation in the tank at  $11.5 \pm 1.0$  °C. A LED light bar with bright white light at 1000 lx was placed 0.5 m above the water surface to provide the light needed for photosynthesis under a 12 h/12 h light/dark regime. One experimental tank served as a control, and two experimental tanks contained a galvanic corrosion protection (GACP) system consisting of a  $15 \times 30$  cm platinized titanium mesh coupled to aluminium (4.87% Zn, 0.16% In, 0.07% Fe, 0.03% Si, 0.001% Cd, rem. % Al) or a zinc (> 99.8%) anode (BAC Corrosion control AS, Denmark) via a galvanostat. The GACP system was physically separated from the macroalgae by a plastic cage that allowed seawater to flow through. Based on the equation below, the total current delivered electric charge in the tanks, and consequently the trace concentration metals released by the system, are expected to reach a constant level within 11–16 h after initiating the system.



**Fig. 1.** Zinc and aluminium accumulation in *S. latissima* as a function of exposure concentration ( $C_e$ ) after 28 days of exposure to seawater contaminated by GACP on laboratory scale. Points represent measured metal content in the biomass ( $q_e$ ). In the left panel, the solid line shows the fitted linear regression constrained through the origin. The slope of the regression corresponds to the concentration factor for Zn (204 L seawater / kg dry biomass). In the right panel, the solid line shows the fitted Langmuir isotherm, included as a mechanistic comparison to illustrate potential saturation behavior at the highest exposure concentrations. The exposure concentrations ( $C_e$ ) were calculated from the dilution factors of the stock solution and were not analytically measured.

$$c(t) = \frac{V^*I + (c_0 - V^*I)e^{-\frac{Q^*t}{V}}}{1000 \cdot F} \quad (2)$$

$c(t)$  represents the total current input, expressed in mmol/L electrons at time  $t$  in minutes;  $c_0$  represents the initial current input at time  $t = 0$ , which can deviate from zero if the cathodic protection system was previously set at a different current  $I$  in ampere;  $Q$  represents the water inflow in L/min, assuming that this inflow is equal to the outflow, thereby sustaining a constant volume  $V$  in liters in the tank;  $F$  is the Faraday constant ((96,485 As/mol).

For the tank with the Zn-GACP system, the galvanostat was successively set to reach an equilibrium at 0.0041, 0.011, 0.032 or 0.070 mmol/L delivered electrons, equivalent to a concentration of 0.13, 0.36, 1.05 or 2.29 mg/L zinc released from the anode surface, respectively. For the tank with the Al-GACP system, the settings were 0.0041, 0.024, 0.054 or 0.099 mmol/L electrons, equivalent to a concentration of 0.037, 0.22, 0.49 or 0.89 mg/L aluminium released from the anode surface, respectively.

Once steady-state concentrations were reached, fronds from four acclimatized *S. latissima* individuals ( $\approx 600$  g total biomass) were added to each tank. Biomass samples (10 g) were taken at day 7, day 14, day 21 and day 28, and stored at  $-20$  °C until the end of the exposure period, when the remaining biomass was collected, and all biomass was dried at  $60$  °C and stored in a desiccator until further analysis.

Each exposure lasted 28 days, followed by one day of tank and GACP system cleaning and 1 day of equilibration at the new current setting before the next exposure began. The Zn- and Al-GACP tanks ran in parallel from April to September 2024. All *S. latissima* originated from a single batch collected in March 2024 and maintained in an acclimatization tank until use. Simultaneously, 30 mL grab water samples were taken at the same timepoints. From day 14 to day 28 of the exposure period, a diffusive gradient thin film (DGT) LSNM-NP passive sampler with a Chelex binding layer (DGT Research, United Kingdom) was immersed in the tank with the Zn-GACP system, and a DGT LSNT-NP passive sampler with a titanium oxide/Metsorb binding layer (DGT Research, United Kingdom) was immersed in the tank with the Al-GACP system. After retrieval, the samplers were rinsed with demineralized water and transported to the laboratory for elution and analysis.

#### 2.4. Seaweed, seawater and sampler preparation prior to chemical analysis

To determine the metal content in seaweed, a total of 0.5 g of oven-dried *S. latissima* was digested with 5 mL of 65% HNO<sub>3</sub>, Suprapur grade, (Merck/Supelco, Germany) in a microwave destructor for 30 min (20 min heating ramp to 200 °C, 10 min at 200 °C), following the provided Anton Paar Plant Materials 20SVT50 method. The digested fraction was diluted four times using Milli-Q water (18.2 M $\Omega$ -cm, Millipore system). All samples were stored at 4 °C until analysis. Labile fractions were obtained by determining the total metal content in DGT passive samplers. To determine the metal content in the passive samplers, receiving gels (chelex-100 mixed with titanium oxide in the tank with the Al-GACP system, or chelex-100 in the tank with the Zn-GACP system) were extracted from the DGT passive samplers. The gels were incubated in 1 mL of 1 M HNO<sub>3</sub> solution prepared using 65% nitric acid, for at least 24 h at room temperature. The binding gels were removed from the solution; the sample was diluted five times with Milli-Q water. Afterwards, all samples were stored at 4 °C until further analysis. Seawater grab samples were separated into fractions to differentiate between dissolved, acid-soluble and non-reversible bound metals. For the total metal content in the seawater, seawater samples were digested in a microwave destructor (Multiwave 5001 with 20SVT rotor, Anton Paar, Belgium) following the EPA3015A procedure, (U.S. EPA, 2007). For the dissolved fraction, seawater samples were filtered through a 0.45  $\mu$ m PTFE syringe filter and subsequently acidified to 0.8% HNO<sub>3</sub> using 65% nitric acid. For the acid-soluble fraction, seawater samples were

acidified to 0.8% HNO<sub>3</sub> using 65% nitric acid and subsequently filtered via a 0.45  $\mu$ m PTFE syringe filter. All samples were prepared in triplicates and stored at 4 °C until analysis.

#### 2.5. Chemical analysis

The concentrations of the main components of the galvanic anodes in the prepared samples, i.e., zinc for the zinc galvanic anode and aluminium and zinc for the aluminium galvanic anode, were measured via inductively coupled plasma optical emission spectroscopy (ICP-OES; Spectro Arcos FHX, model 76,004,553, SPECTRO Analytical Instruments, Germany). A 0.1–10 mg/L metal concentration range prepared from the multi-element ICP standard solution CR-61 (Carl Roth) was used for calibration purposes. Analytical yields determined from spiked samples were 106% for Zn and 102% for Al, with a slight upwards drift of the instrument during the run, and measured concentrations were corrected accordingly. Limits of detection (LoD) and limits of quantification (LoQ) were derived from the ICP-OES calibration data. LoD values were obtained directly from the instrument software and correspond to the  $3\sigma$  criterion. LoQ values were calculated from the calibration curve using the following equation:

$$LoQ = \frac{10 \cdot StErr}{slope} \quad (3)$$

LoD values for the seawater samples were 0.04 mg/L for Zn and 0.06 mg/L for Al, and LoQ values for the seaweed samples were 2.08 mg/kg for Zn and 7.05 mg/kg for Al. For the seawater samples, background concentrations of Al and Zn were measured in triplicate. Then, fractions were calculated as follows: (i) the total metal content was obtained directly from the seawater after microwave digestion, (ii) the soluble fraction was obtained from the metal content in the samples filtered at 0.45  $\mu$ m before acidification, (iii) the acid-soluble fraction was calculated by subtracting the soluble fraction from the metal content in the acidified samples, and (iv) the non-reversible fraction, which contains the remaining metals that are unavailable in the water column due to strong complexation or ligation with biological matter, was calculated by subtracting the soluble and acid-soluble fractions from the total metal content in the digested samples.

For the seaweed samples, relationships between the metal content in the seaweed, exposure time and exposure concentration were evaluated using linear and Langmuir regression models. The regression models were selected based on the assumption that metal uptake in *S. latissima* is governed by passive uptake pathways. The metal content was expressed in function of the exposure concentration. The datasets were evaluated to ensure that model assumptions were met. For the linear model, independence of residuals was assessed using the Durbin-Watson test, normality of residuals using the Shapiro-Wilk test, and homogeneity of variances using the Harrison-McCabe test. For the Langmuir model, independence and normality of the residuals was assessed via a residuals plot and using the Shapiro-Wilk test. Furthermore, the Akaike Information Criterion (AIC), and  $R^2$  were used for model selection. For the seaweed samples in the mesocosm experiment, the metal content in the biomass was plotted against the exposure time at each exposure concentration. If the slope was positive, statistically supported ( $p < 0.10$ ), and represented  $\geq 10\%$  relative change over the observed period, the metal content at 28 days was derived from a linear model. Otherwise, it was taken as the mean of the observed values with a 95% confidence interval reflecting the temporal variability, assuming steady state. Because only four exposure concentration levels were available, classical regression diagnostics lack statistical power. Thus, model selection for relating the metal content in the biomass with the exposure concentration relied on  $R^2$  and the Akaike Information Criterion (AIC).

For the DGT extracts, the average metal concentration in the seawater was calculated using the following equation (Zhang and Davison, 1995):

$$c_{DGT} = \frac{(M\Delta g)}{D_m t A} \quad (4)$$

where  $c_{DGT}$  represents the metal concentration in the seawater,  $M$  is the mass of the metal accumulated in the resin,  $\Delta g$  is the diffusive layer thickness,  $D_m$  is the diffusion coefficient of the metal,  $t$  is the exposure time of the DGT passive sampler in the mesocosm, and  $A$  is the exposure area. Diffusion coefficients for the average water temperatures over the deployment period were used as provided by DGT Research.

Comparisons between the released concentrations and those obtained from the three sampling approaches (grab samples, seaweed, and DGT passive samplers) were performed within each charge level using two-sided Wilcoxon rank-sum tests and permutation tests based on 10,000 random label permutations (Good, 2000). Effect sizes were reported as rank-based  $r$  statistics and Hodges–Lehmann location shifts (median differences) with 95% confidence intervals. To assess overall agreement across charge levels, stratified permutation tests were conducted in which labels were permuted within levels, combining level-specific median differences using weighted means. Zero values were retained as valid observations and were not replaced. Agreement between the tested methods and the released metal concentrations was further evaluated using Bland–Altman plots (Bland and Altman, 1986), with the released mean per level as the reference. Limits of agreement were calculated as follows:  $LOA = \text{bias} \pm 1.96 \cdot SD$ .

All statistical analyses were performed in R (version 4.5.2), and all statistical outputs are provided in the supplementary information.

### 3. Results

#### 3.1. Metal release and chemical speciation

Triplicate seawater samples were taken after seawater contamination, and the metal concentrations in the different water fractions are summarized in Table 1. In seawater contaminated with Zn-GACP, released zinc remained mostly in dissolved form. However, in seawater contaminated with Al-GACP, released zinc and aluminium were both mostly present in the acid-soluble fraction, indicating coprecipitation and sorption of zinc onto aluminium particles, which strongly reduces the dissolved, bioavailable fraction (Tokoro et al., 2015). During seawater contamination with Al-GACP, the pH of the seawater lowered from 8.1 to 7.9.

#### 3.2. Metal uptake in *Saccharina latissima* exposed to seawater indirectly contaminated by galvanic anodes

A clear increase in the accumulated metal content was recorded with increasing contamination levels in seawater. (Fig. 1). For the uptake of zinc in seaweed exposed to seawater contaminated by Zn-GACP, a linear regression model corresponded well to the data in the experimental range ( $R^2 = 0.98$ ,  $p < 0.001$ ). Residuals showed no evidence of autocorrelation (Durbin-Watson  $p$ -value 0.90) or heteroscedasticity (Harrison-McCabe  $p$ -value 0.998). The Shapiro-Wilk test indicated a deviation from perfect normality ( $p$ -value 0.0002), which was attributed to a few extreme observations. Because of the robustness of linear regression to

**Table 1**  
Metal speciation in seawater contaminated with Zn-GACP or Al-GACP,  $n = 3$ .

	Zn-GACP	Al-GACP	
	Zinc content (mg/L)	Aluminium content (mg/L)	Zinc content (mg/L)
Total	15.2 ± 0.1	4.0 ± 0.04	0.3 ± 0.01
Soluble	15.0 ± 0.2	0.44 ± 0.06	0.05 ± 0.01
Acid-soluble	0.2 ± 0.2	3.0 ± 0.56	0.26 ± 0.01
Non-reversible	< LoD (0.04)	0.5 ± 0.6	< LoD (0.06)

moderate non-normality and the absence of structural violations, the linear model was retained. The concentration factor for zinc from Zn-GACP is given as the slope of the regression model,  $204 \pm 4.5$  L seawater / kg dry biomass.

For the uptake of aluminium in seaweed exposed to seawater contaminated by Al-GACP, a linear regression model did not fit well, showing significant heteroscedasticity and autocorrelation. The Langmuir model was also fitted to explore potential saturation behavior at the highest exposure concentrations (pseudo- $R^2 \approx 0.74$ ). The maximum adsorption capacity  $q_{\max}$  was  $1318 \pm 415$  mg/kg ( $p = 0.0038$ ), and the affinity constant  $K_L$  was  $0.60 \pm 0.34$  L/mg ( $p$ -value 0.092). The Akaike Information Criterion (AIC) and the residual standard error for the Langmuir model were lower than for the linear model. Residuals show no obvious autocorrelation and pass normality (Shapiro-Wilk  $p$ -value 0.607), but a larger variation at high exposure concentrations hints at heteroscedasticity.

Compared to the linear model, the Langmuir model captured better the overall trend, but due to the large spread at higher exposure concentrations, the Langmuir model only serves as a qualitative indication of potential saturation.

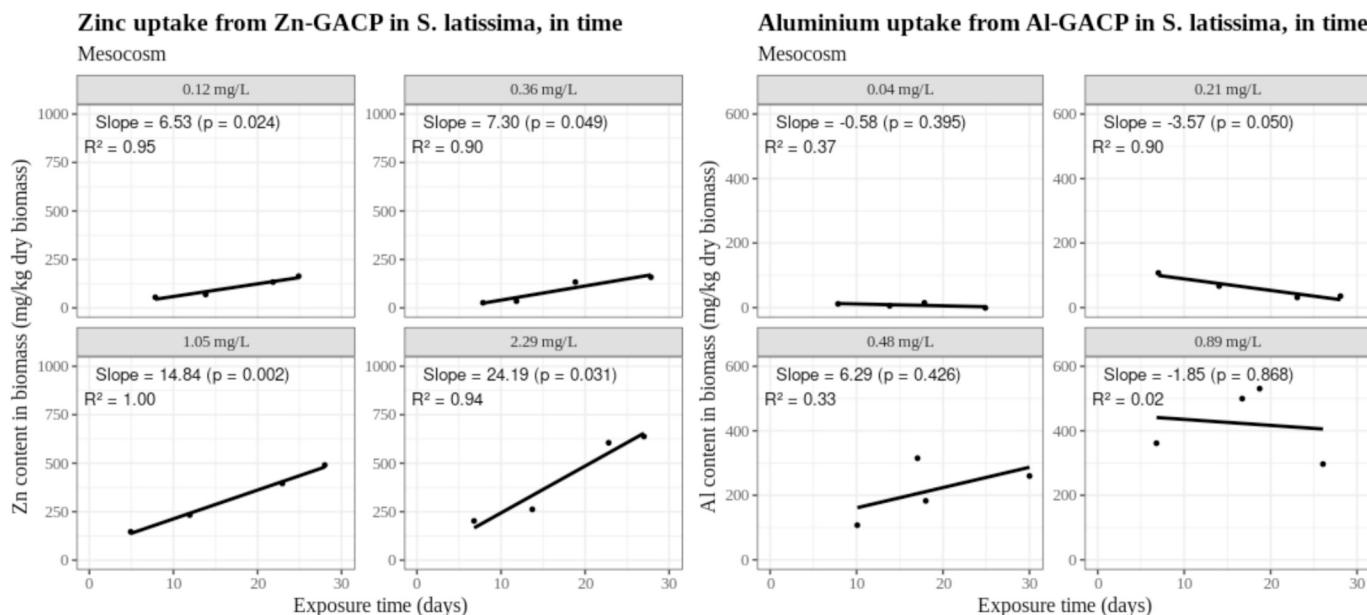
Although the Al-GACP alloy contained 4.87% Zn, the measured Zn content in *S. latissima* exposed to Al-GACP did not differ from the control across the tested exposure range (Fig. S1). This indicates that under the experimental conditions used here, zinc released from the Al-GACP was not accumulated by the seaweed.

#### 3.3. Metal uptake in *Saccharina latissima* under direct exposure to galvanic anodes in a mesocosm

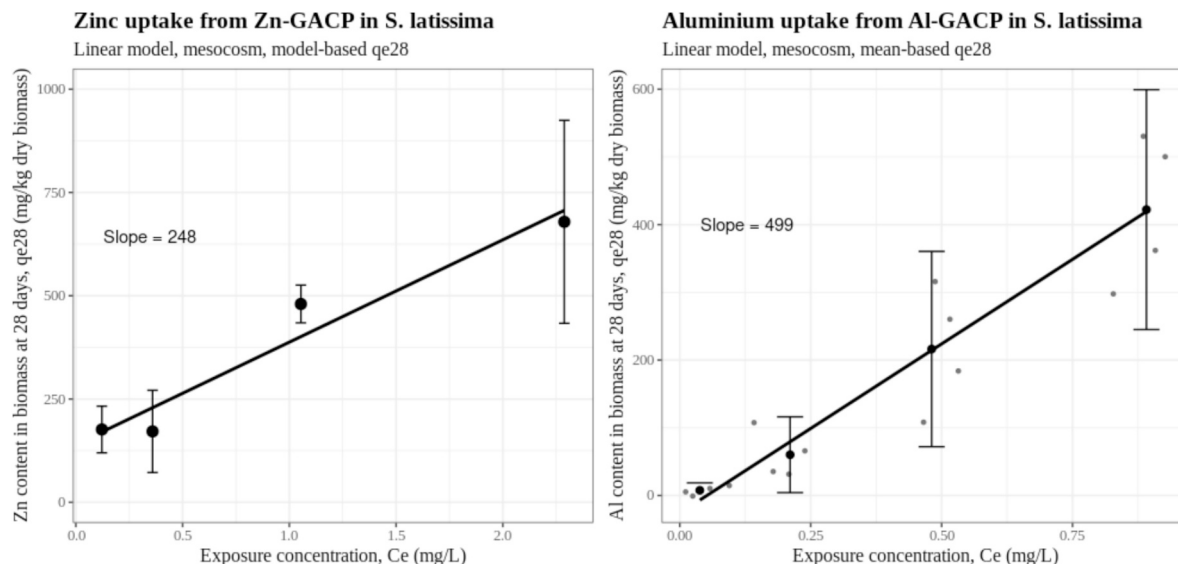
As can be seen in Fig. 2, time-series regressions showed consisted positive slopes for the zinc content in *S. latissima* exposed to Zn-GACP (relative change >10% with  $p$ -value <0.10), consistent with its stable inorganic speciation and the absence of strong complexation effects. Thus,  $q_e$  at 28 days (Fig. 3a) was estimated from the linear models. For the aluminium content in *S. latissima* exposed to Al-GACP, time series patterns varied. No clear increase was observed over time, and the decreasing  $R^2$  showed an increase in temporal variability with the exposure concentration. As time is not a meaningful predictor,  $q_e$  at 28 days (Fig. 3b) was estimated from the mean of observed values with a 95% confidence interval. (See Fig. 4.)

The derived zinc content in *S. latissima* exposed to Zn-GACP at day 28 increased with exposure concentration, from 176 mg/kg dry biomass (95% CI 120–233) at 0.12 mg/L to 679 mg/kg dry biomass (95% CI 433–925) at 2.29 mg/L. In a similar manner, the derived aluminium content in *S. latissima* exposed to Al-GACP at day 28 increased with exposure concentration, from 7.7 mg/kg dry biomass (95% CI –3.3 - 18.7) at 0.04 mg/L to 422 mg/kg dry biomass (95% CI 245–599) at 0.89 mg/L. A linear model was used to describe the resulting relationships between the metal content in the biomass and the exposure concentrations after 28 days, yielding a slope of approximately 248 L seawater / kg dry biomass for Zn from Zn-GACP (Fig. 3a), and a slope of approximately 499 L seawater / kg dry biomass for Al from Al-GACP (Fig. 3b). As the regressions were based on only four concentration levels, formal statistical inference is not meaningful. Nevertheless, the zinc content in the biomass after 28 days showed narrow confidence intervals at each exposure concentration, and no evidence suggested deviation from proportional uptake ( $R^2 = 0.94$ , AIC = 48.8) within the tested time and exposure concentration range. As a result of the extrapolation method, the confidence intervals increased with the exposure concentration, reflecting the time-related variability between the measurements for aluminium from Al-GACP (see also Fig. 2b). Although the linear model fitted extremely well to the derived Al content in the biomass at 28 days ( $R^2 = 0.99$ , AIC = 37.3), the model cannot be validated due to the large CI and lack of statistical sampling depth.

At the end of each experiment, the tank and the GACP system were inspected. In the tank with the Al-GACP system, a white corrosion



**Fig. 2.** Zinc and aluminium contents in *S. latissima* under direct exposure to a Zn- or Al-GACP system regulated by a constant delivered electric charge as a function of exposure time. Linear regressions (black lines) illustrate the direction and strength of temporal trends, which inform the decision rule for deriving  $q_{e28}$ . Zn shows consistently increasing and uniform uptake over time, whereas Al shows no clear upward trend and increasing temporal variability at higher concentrations. The exposure concentrations ( $C_e$ ) were calculated from the measured electrical current over the galvanostat unit, using eq. 2.



**Fig. 3.** Zinc and aluminium contents in *S. latissima* under direct exposure to a Zn- or Al-GACP system regulated by a constant delivered electric charge as a function of exposure concentration in a mesocosm. The Zn content was corrected for the exposure time. The exposure concentrations ( $C_e$ ) were calculated from the measured electrical current over the galvanostat unit, using eq. 2. In contrast to zinc, where the confidence intervals represent the uncertainty of the model-based prediction of  $q_e$  at day 28, the aluminium confidence intervals primarily reflect time-related variability. Say something about model and mean.

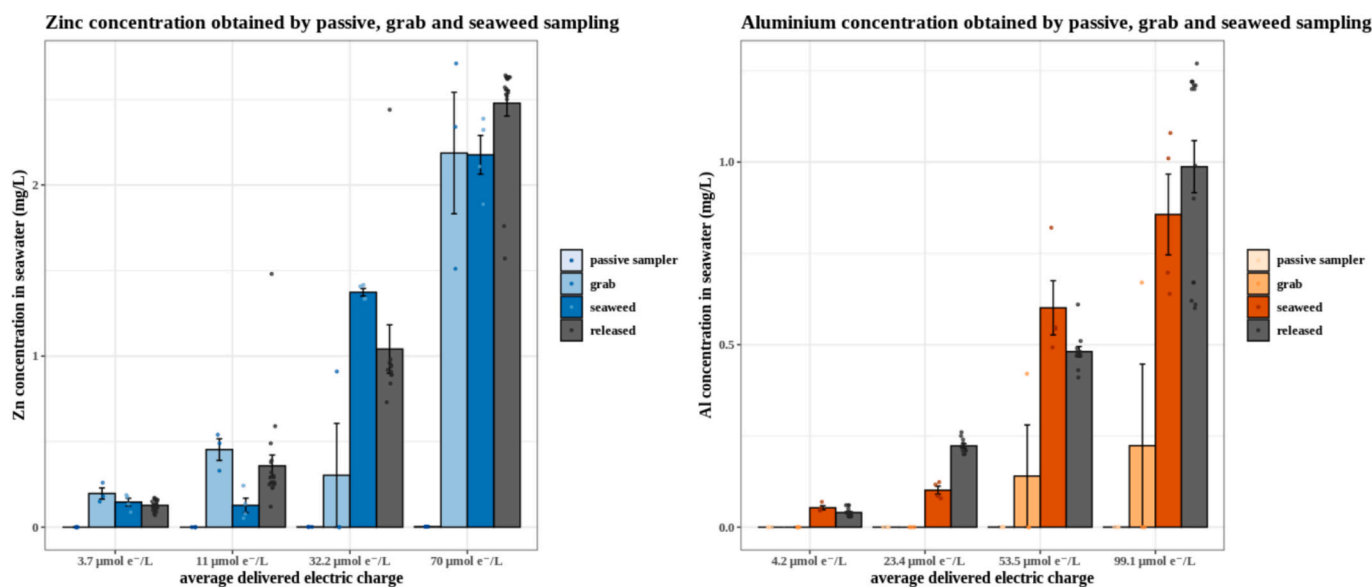
product layer had formed on anode surface, and solids were also found on the cage surrounding the anode, as well as on the bottom of the tank. The pH of the seawater varied between 7.8 and 8.0. Although some aluminium was detected in the seawater, the observed aluminium concentrations in the seawater of the exposure tanks were mostly below the quantification limit (0.2 mg/L).

### 3.4. Comparison of sampling methods in a mesocosm

Across all contamination levels, seaweed sampling followed the expected increase in metal concentration in the mesocosm most closely. Grab sampling performed better in the mesocosm with Zn-GACP than in

the mesocosm with Al-GACP. Passive sampling strongly underestimated metal concentrations in both cases.

Per-level Wilcoxon rank-sum and permutation tests could not be evaluated due to heavy censoring, mostly in grab sampling and passive sampling ( $p$ -value NA). We therefore report the overall stratified permutation comparisons of the sampling methods against the concentration of released metals. The concentration of Zn in the seawater contaminated with a Zn-GACP system and its variation, obtained via seaweed sampling (LoQ 2.08 mg/kg dry biomass) and direct sampling (LoQ 0.05 mg/L), corresponded to the calculated concentration in the tank (Benjamini-Hochberg (BH)-adjusted  $p$ -value >0.3). Limited amounts of Zn were found in the Chelex binding layer of the passive



**Fig. 4.** Concentrations of zinc and aluminium in experimental tanks with a Zn- or Al-GACP system — measured via passive DGT sampling, grab sampling and macroalgae sampling—versus the calculated concentrations of released zinc and aluminium in the tanks.

samplers (LoQ 0.003 μg/L), resulting in a strong underestimation of the calculated concentration of released zinc in the tank ( $\chi^2 = 23.55$ , BH-adjusted p-value <0.0001). For Al in seawater contaminated with Al-GACP, the measured concentration by grab sampling (LoQ 0.18 mg/L) and passive sampling (LoQ 0.02 μg/L) was significantly underestimated (grab sampling:  $\chi^2 = 14.67$ , BH-adjusted p-value <0.001; passive sampling:  $\chi^2 = 24.44$ , BH-adjusted p-value <0.0001). Seaweed sampling did not differ significantly from the calculated concentration of released aluminium in the tank ( $\chi^2 = 0.35$ , p-value 0.55).

Bland-Altman analyses (Fig. S4) confirmed these findings. Passive and grab sampling showed systematic negative biases for both metals (Zn: -1.00 and -0.22; Al: -0.43 and -0.34), with wide limits of agreement. Seaweed measurements showed minimal bias (Zn: -0.05; Al: -0.03) and the narrowest limits of agreements.

#### 4. Discussion

##### 4.1. Chemical speciation partially determines the bioavailability of released trace metals

The seawater used was sampled from the bay of Brest (beach Ste. Anne de Portzic, France), a few kilometers seaward from the city Brest. Although there are a few minor sources of industrial inputs of trace metals in the area, they are not expected to significantly increase the trace metal content of the seawater in the bay (Lemière and Clozel, 2000). The typical trace metal concentrations reported for the Northeast Atlantic open ocean are in the pico- to nanomolar scale and increase in coastal environments, especially near river outlets and in enclosed areas with little current (conform (Bruland and Franks, 1983; Ellwood and Van den Berg, 2000; Reese et al., 2020, 2020; Saager et al., 1997) and references therein).

For both aluminium and zinc, the background levels were inferior to the detection limits/values reported for the open ocean or well below the studied contamination range and were therefore not expected to interfere with the outcome of these experiments.

In Zn-GACP-contaminated seawater, zinc was predominantly present in dissolved form, consistent with earlier observations (Baars and Croot, 2011). This suggests that under the tested conditions, nearly all the released zinc remained bioavailable. At physiological pH, zinc is present as a mixture of  $Zn^{2+}$  or  $Zn(OH)^+$ , in roughly equal amounts (Reichle et al., 1975). The solubility limit of zinc in seawater,  $13 \times 10^5$  mol/L, is

well above the tested concentration. Based on the electrochemical properties of the anode, the delivered electric charge is expected to be fully used for the dissolution of zinc.

In a marine environment, zinc can adsorb to suspended particles and sediments (Bird et al., 1996; Rousseau et al., 2009), thereby removing dissolved zinc from the water column. However, in this study, no sediment was present since the seawater used was decanted and filtered. The negligible acid-soluble fraction confirms that there was no significant complexation with other dissolved or suspended matter.

In seawater (pH 8.0, 20 °C) contaminated with Al-GACP, both zinc and aluminium were present predominantly in acid-soluble forms. Under physiological seawater conditions,  $Al^{3+}$  quickly reacts to form the insoluble  $Al(OH)_3$ , which strongly controls the dissolved aluminium fraction (Angel et al., 2015; Savenko and Savenko, 2011; Ščančar and Milačić, 2006). According to Savenko and Savenko (2011),  $Al(OH)_2^+$  dominates below pH 8.05, whereas  $Al(OH)_4^-$  becomes the major species above pH 8.10. Teien et al. (2006) suggest that dissolved aluminium in seawater mainly consist of cationic species, while other speciation studies (Angel et al., 2015; Ščančar and Milačić, 2006) consistently report that at typical seawater pH (pH 8.0–8.2),  $Al(OH)_4^-$  is the predominant dissolved form, while at lower pH other polymeric species are present. The pH of the seawater from the bay of Brest was slightly lower (pH 7.8–8.0) than oceanic seawater (8.0–8.2), thus it is possible that cationic aluminium species such as  $Al(OH)_2^+$  dominate, while  $Al(OH)_4^-$  is present to a smaller extent. However, as aluminium speciation in the presence of a precipitate is highly dynamic and sensitive to small pH variations (Angel et al., 2015), the exact speciation of dissolved fractions cannot be assigned.

The solubility limit for aluminium in seawater is governed by the equilibrium of dissolved species with amorphous  $Al(OH)_3$  (s). Reported solubility limits range from ~30–40 μg/L (at pH 8.05–8.10) (Savenko and Savenko, 2011), to ~500 μg/L (Angel et al., 2015), depending on the ageing state and reactivity of the precipitate. In our experiments, the dissolved aluminium concentration stabilized at 0.44 mg/L, which lies within the upper range of values reported for systems containing freshly formed aluminium hydroxides. With soluble aluminium representing only ~10% of the total aluminium content in the solution, our results are in agreement with previous studies, which showed that aluminium anode consumption did not result in a significant increase in aluminium in the water column but rather in the suspended particulate matter (SPM) and sediments (Caplat et al., 2020; Deborde et al., 2015; Gabelle

et al., 2012; Leleyter et al., 2018). Although the pH in seawater is buffered, we observed a slight decrease during anode release. This is likely caused by the formation of aluminium hydroxides and electrolysis at the cathode. The concentration of dissolved aluminium initially exceeded the solubility limit, but the concentration stabilized within two hours of continuous stirring. We observed that most of the released aluminium precipitated almost instantaneously at the anode surface.

Zinc from Al-GACP appeared largely in the acid-reversible fraction, whereas in the absence of aluminium it remained dissolved. We thus conclude that in the presence of suspended aluminium particles, zinc co-precipitates to form mixed metal hydroxide complexes (Tokoro et al., 2015). This co-precipitation may influence bioavailability, reducing the zinc uptake in *S. latissima*.

#### 4.2. Metal uptake from galvanic anodes in *S. latissima* appears to be linear on laboratory scale

After exposing *S. latissima* to seawater contaminated by GACP, elevated metal contents were observed in the biomass. The regression analysis between metal content and environmental concentration was linear, suggesting that the uptake mechanism is governed by passive uptake. The uptake of zinc from Zn-GACP in *S. latissima* followed a robust linear trend, whereas aluminium uptake from Al-GACP showed greater variability, likely reflecting the strong dependence of aluminium speciation on pH and other seawater parameters. The bioavailability of aluminium to seaweed is primarily governed by the fraction that remains dissolved, as particulate aluminium hydroxides are not expected to be taken up. Among the dissolved species, positively charged complexes are generally considered more likely to interact with the negatively charged functional groups in the algal polysaccharide matrix. Under experimental conditions (pH 7.8–8.0), several mononuclear aluminium hydrolysis products may occur, including  $\text{AlOH}^{2+}$ ,  $\text{Al}(\text{OH})_2^+$ , and, at slightly higher pH,  $\text{Al}(\text{OH})_4^-$  (Botté et al., 2022; Savenko and Savenko, 2011). While  $\text{Al}(\text{OH})_4^-$  is negatively charged and therefore less likely to adsorb, positively charged species such as  $\text{AlOH}^{2+}$  and  $\text{Al}(\text{OH})_2^+$  are likely to be taken up, provided they do not aggregate into larger colloidal complexes such as  $[\text{Al}_2(\text{OH})_2(\text{H}_2\text{O})]^{4+}$ . Under physiological conditions, aluminium is present predominantly as the insoluble  $\text{Al}(\text{OH})_3$ , and the bioavailable, dissolved aluminium fraction might be highly variable, as aluminium speciation around physiological conditions is sensitive to small changes in pH, temperature and salinity. At pH < 8.05, dissolved aluminium increasingly shifts towards positively charged species such as  $\text{Al}(\text{OH})_2^+$  (Savenko and Savenko, 2011), and aluminium solubility increases with decreasing pH (Pitre et al., 2014). Consistent with this, we found that the aluminium content in *S. latissima* increased with decreasing pH from 8 to 5.5 (Fig. S2), supporting the enhanced availability of positively charged dissolved aluminium species under more acidic conditions.

The lack of accumulation of zinc from Al-GACP in *S. latissima* likely reflects the chemical conditions created by the experimental setup. Despite a one-week equilibration period of the exposure solutions to the exposure conditions, the rapid release of metals from the Al-Zn-In anode to reach above-realistic concentrations may have promoted rapid local oversaturation and the formation of Al hydroxides, leading to less reversible co-precipitation or sorption of Zn onto these solids. This is supported by the metal speciation results, showing that Zn was largely present in the acid-soluble fraction rather than in the dissolved, bioavailable fraction. Consequently, the laboratory Al-GACP exposure experiment was less suitable for evaluating low-level Zn uptake from Al-based anodes. It potentially underestimated the accumulation of Zn in *S. latissima*, reflecting the chemical speciation of Zn in this setup rather than the sampling capacity. Field-scale or long-term exposures, where Zn remains in the dissolved phase, would be required to evaluate Zn uptake from Al-based anodes under realistic environmental conditions.

The concentration factor for zinc in *S. latissima* exposed to seawater contaminated by Zn-GACP was lower than the concentration factor for

aluminium in *S. latissima* exposed to seawater contaminated by Al-GACP. This may be related to the electric charge of the dissolved metal species, which influences their interaction with the negatively charged extracellular polysaccharide matrix of the seaweed. The hypothesized uptake mechanism involves passive adsorption driven by ionic interactions. Advanced imaging techniques such as synchrotron X-ray fluorescence microscopy or fluorescence microscopy using chemo-selective sensors in future experiments could help testing this hypothesis by visualizing the intracellular and extracellular distribution of metals within the algal tissues (Vázquez-Arias et al., 2023a, 2023b). Such approaches have been successfully applied to brown algae to map metal hotspots, identify binding compartments and infer uptake pathways (Leonardo et al., 2014; Mijovilovich et al., 2023).

At the highest tested exposure concentration, the zinc content in *S. latissima* did not reach saturation. Therefore, the maximum uptake capacity of *S. latissima* for zinc exceeds 1900 mg / kg dry biomass. For aluminium, the maximum uptake capacity could not be determined as the uptake was limited, potentially by the reduced bioavailability of aluminium in the seawater at higher concentrations. The accumulation capacity of *S. latissima* for zinc did not appear to be limited to the given range under an exposure time of 28 days, which opens doors to potential other applications, such as seawater decontamination via biological remediation strategies (Arecio et al., 2021; Deniz and Karabulut, 2017; Luo et al., 2020). Note that concentration factors cannot be directly compared due to differences in experimental approaches, and the extrapolability of the linear model in this study to lower concentrations should be confirmed by a transplant study.

#### 4.3. *Saccharina latissima* as a sentinel for trace metal contamination assessment in a mesocosm

For zinc from Zn-GACP, the regression analysis in the mesocosm experiment was linear, similar to previous findings at the laboratory scale (Fig. 1), but the calculated concentration factor of 248 L seawater/kg dry biomass was considerably greater, potentially because of improved mixing and a greater seawater-to-seaweed volume ratio, resulting in more efficient uptake. Based on the hypothesis that metal uptake is governed by a passive uptake process, it is expected that the linear relationship extends to concentrations below the experimental range in this study. A field study on the Irish coast on the complementary brown kelp species *Alaria esculenta* confirms this hypothesis, as a similar correlation was found, with environmental zinc concentrations as low as 0.5 µg/L (Reis et al., 2016). The present data suggest that *S. latissima* has a greater affinity for zinc, which could make it a favorable sampling vector for offshore monitoring of trace metals from “hidden” sources such as galvanic anodes, where metals are constantly emitted in relatively low amounts and remain dissolved over longer time scales. There are, to our knowledge, no reported concentration factors for zinc or aluminium in *S. latissima*, but reported concentration factors in other seaweeds were several times higher than the experimental concentration factors. Along the Aegean coast, Akcali and Kucuksezgin (2011) reported zinc concentration factors from approximately  $1.5 \times 10^4$  to  $5.5 \times 10^4$  across several macroalgae species. Experimental work by Amado Filho et al. (1997) showed similarly high zinc accumulation capacities, with concentration factors on the order of  $10^3$  to  $10^4$ , depending on species and exposure level. Reis et al. (2016) reported zinc concentration factors in *Alaria esculenta* between  $2 \times 10^4$  and  $7 \times 10^4$ , with seasonal variations. Taken together, these studies indicate that zinc concentration factors commonly fall within the  $10^3$ – $10^4$  range or higher, which is several times above the concentration factors observed in our experimental system. This is likely due to the reduced exposure time in comparison to field studies, where exposure times may extend to the full lifetime of the sampled biomass. Owing to the gradual uptake of metals over a prolonged period, the seaweed provided a time-weighted rolling average of the environmental concentration, in which fluctuations in environmental concentrations were not detected.

The aluminium uptake of *S. latissima* directly exposed to Al-GACP in the mesocosm showed a more distinct linear trend than that in the laboratory exposed to seawater contaminated in a controlled manner. The stronger correlation could be a result of more consequent metal speciation. Under physiological conditions, aluminium speciation in seawater and the agglomeration kinetics of  $\text{Al}(\text{OH})_3$  are highly sensitive to small changes in concentration, pH and temperature (Lydersen, 1990; Savenko and Savenko, 2011). In the laboratory, colloidal aluminium hydroxides might have settled, resuspended or redissolved during preparation and handling of the exposure solutions, causing variation in the exposure solutions. In the mesocosms, the working volumes are much greater, and the seaweed was directly exposed to the Al-GACP system without interference related to seawater manipulation. The linear increase in the metal content of *S. latissima* due to increasing exposure concentration and the absence of an increase in the metal content of *S. latissima* due to increasing exposure time suggest that after a short initial period, the aluminium released by the GACP system remained mainly present in the form of non-bioavailable aluminium hydroxides, such as  $\text{Al}(\text{OH})_3$  and  $\text{Al}(\text{OH})_4^-$ , which are not taken up by the seaweed, although this has been suggested by previous studies (Barrat et al., 2024). These findings are in agreement with previous data from Deborde et al. (2015), who not only observed the formation of a loose, poorly adherent white layer on the surface of galvanic anodes with similar compositions but also reported that the dissolved aluminium concentrations in discrete seawater samples remained close to background levels (Deborde et al., 2015). Moreover, metal uptake in polysaccharide-rich brown seaweed, like *S. latissima*, is most likely driven by the adsorption of metal ions and small, positively charged inorganic complexes into polysaccharide cell walls rather than via the adsorption of metal particles onto the seaweed surface, as suggested by other authors (Barrat et al., 2024).

Note that (a) the maximum exposure time was one month, which is much lower than the realistic exposure times of annually cultivated *S. latissima* (six to eight months) and perennial *S. latissima* populations (up to several years), except in the case of distinct, short contamination events and (b) the maximum contamination level of zinc was 9.4 mg/L, which is approximately fifteen times higher than the highest reported Zn contamination level from previous studies from different European estuaries ((Beiras et al., 2003; Bird et al., 1996; Chiffolleau et al., 1999; Fernández et al., 2008; Law et al., 1994; Matthiessen et al., 1999; Pérez-López et al., 2003; Prego et al., 2006)). Note that previously determined trace metal are average bulk concentrations, and, in the near vicinity of a contamination source, trace metal concentrations might be higher than those reported. However, as bioaccumulation appears to be linear with respect to exposure time and exposure concentration in the tested concentration range, we expect that it is possible to extrapolate these findings to seaweed transplants exposed to realistic exposure conditions (García-Seoane et al., 2020). To the best of our knowledge, there is no data available on aluminium and zinc accumulation in *S. latissima* from areas with quantified contamination levels.

While our study provides the first quantitative evidence for linear metal uptake in *S. latissima*, future transplant experiments under field conditions are essential to confirm the extrapolation of these results to longer exposure times and lower, more environmentally relevant concentrations. Furthermore, the metal desorption from macroalgae has not been investigated but could interfere with field experiments in dynamic environments with short-lived contamination events or decreasing contamination levels over time. Proceeding from our results, we hypothesized that trace metal accumulation in *S. latissima* is based on the adsorption of metallic ions in the extracellular polysaccharide matrix. Consequently, other polysaccharide-rich seaweed species could also be suitable sampling vectors. It must be noted that the composition of the polysaccharide matrix, and therefore the affinity for trace metals widely varies between macroalgae. It was already shown that different macroalgae species exhibit different accumulation behaviors (García-Seoane et al., 2018b; Rainbow and Phillips, 1993). Therefore, the role of the

composition and mass of the extracellular matrix on metal uptake should be investigated in follow-up studies to test this new hypothesis. Additionally, expressing the metal content in function of the polysaccharide composition and total polysaccharide content of the biomass, instead of the total biomass, could reveal a relationship allowing quantitative trace metal monitoring with multiple species. For aluminium contamination, more research is needed to elucidate the relationships among environmental conditions, metal speciation, and uptake by any sampling vector. However, the present study shows that the suitability of *S. latissima* for biomonitoring purposes of trace metal contamination is not limited by the composition of the galvanic anodes under assessment.

#### 4.4. Comparison of sampling techniques

Although passive samplers are expected to reflect the fraction of dissolved aluminium, previous experiments have suggested that in a semi-closed environment with Al-GACP, the aluminium concentration measured by passive samplers increases, whereas the expected dissolved content obtained by grab sampling does not (Deborde et al., 2015). Here, we observed a different trend: the aluminium concentration obtained via grab sampling increased more strongly than the aluminium concentration obtained via passive sampling. The differences between our results and those reported by Deborde et al. (2015) may be related to hydrodynamic conditions. In our setup, seawater was continuously renewed ( $0.5 \pm 0.1$  L/min) and actively circulated using a pump that generated a jet stream, ensuring vigorous mixing throughout the tank. Deborde et al. (2015) also used continuously renewed seawater (2 L/min), but their system operated under low-circulation conditions, with no active mixing devices visible and a largely still water surface. These less energetic hydrodynamic conditions may have favored the settling of aluminium hydroxide precipitates, whereas our more turbulent conditions likely kept particles in suspension for longer. Grab samples contain not only dissolved but also colloidal aluminium, while the passive samplers exclusively take up dissolved aluminium.

The absolute zinc and aluminium concentrations in seawater contaminated with Zn-GACP or Al-GACP as measured by seaweed were derived via concentration factors based on the same data and therefore cannot be evaluated in an objective manner. However, concentrations measured by all the sampling methods were normalized to the average delivered electric charge. Seaweed sampling was the best indicator for protection strength, followed by grab sampling and passive sampling. Both seaweed sampling and grab sampling reflected the average delivered electric charge better at higher contamination levels than at lower contamination levels. Passive sampling is a promising method for environmental trace metal monitoring, as they were successfully used to measure lower Zn concentrations, among other elements (Zhang and Davison, 1995). However, in this mesocosm-based study, DGT passive samplers did not give good results.

In the present study, the electrical current was modulated according to variations in the water flow to keep the total delivered current in the tank relatively constant. However, the environmental trace metal concentration is expected to fluctuate much more in realistic field scenarios, as it is highly susceptible to weather changes and tidal currents. Since macroalgae transplants accumulate metal over a given exposure period, the resulting metal content is the averaged concentration over this period. Therefore, macroalgae sampling is expected to outperform grab sampling in the field to a larger extent than in the present mesocosm experiment, reflecting the average environmental concentration with much greater precision.

## 5. Conclusion

Our results demonstrate that *Saccharina latissima* accumulated zinc and aluminium from galvanic anode emissions in a concentration-dependent manner. Mesocosm experiments confirmed that uptake is proportional to exposure, without saturation under the tested

conditions. Since laboratory exposure did not exceed the concentration range or exposure time in the mesocosm experiment, the nonlinear uptake behavior observed for Al is likely a result of variable aluminium speciation in the exposure solution. Compared with grab and passive sampling, *S. latissima* provided more stable and representative estimates of exposure in the mesocosm. We conclude that *S. latissima* can be a reliable and cost-effective bioindicator for quantitative offshore monitoring of trace metal contamination. Its use in transplant-based bio-monitoring could significantly improve current assessment frameworks, especially in areas impacted by hidden and continuous emission sources such as galvanic anodes.

Although the methodology in this study was original and not yet confirmed by experimental reproduction, our results are the first indication that macroalgae are suitable for quantitative evaluation in mesocosm with high seawater renewal. Additional experiments are needed to unravel the mechanisms of trace metal accumulation in macroalgae, including desorption kinetics, the impact of environmental parameters and biomass composition. Microscopic localization techniques can provide insights into the mechanisms of uptake. Further research using field transplants is recommended to investigate the bio-monitoring potential of *S. latissima* for trace metal contamination from galvanic anodes in realistic environments.

### CRedit authorship contribution statement

**Caya de Leeuw van Weenen:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicolas Larché:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Barbara Rossi:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Emiliano Pinori:** Writing – review & editing, Validation. **Lise Appels:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Conceptualization.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Microsoft 365 Copilot in order to summarize the content of the paper to ensure that the abstract was complete and concise. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2026.119362>.

### Data availability

The research data and supplementary information are made available through KU Leuven's Research Data Repository (RDR) via the following DOI: [10.48804/KD2NDX](https://doi.org/10.48804/KD2NDX)

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