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First quantitative biomonitoring study of two ports (marina, commerce) in French littoral area: Evaluation of metals released into the marine environment and resulting from galvanic anodes

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1 **Research paper**

2 **Title**

3 First quantitative biomonitoring study of two ports (marina, commerce) in French littoral
4 area: Evaluation of metals released into the marine environment and resulting from galvanic
5 anodes

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Abstract

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Coastal areas are important interface environments between marine and terrestrial ecosystems and are also areas of high economic activity related, among others, to trade, fishing and tourism. The port areas of La Rochelle (France) are part of these areas with strong anthropic activities and are therefore subject to potential contamination, particularly with trace metals. Among the various sources of discharges, galvanic (or sacrificial) cathodic protection can be questioned. Indeed, few studies have been carried out on the potential impact of the degradation of sacrificial anodes in ports on the health of bivalves. A study was therefore carried out on the black scallop *Mimachlamys varia* in the commercial port and the marina of La Rochelle using the caging method. Several biomarkers of the health status of individuals were observed in the laboratory (SOD, GST, MDA, AChE, and LAC). Among the different results obtained over two exposure periods, it appears that the potential effects of

46 sacrificial anodes, whether new or present on site for several years, are masked by meteorological
47 conditions, as well as by harbour activities.

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49 **Keywords:** biomarkers, environmental quality, trace metals, port area, cathodic protection

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52 **1. Introduction**

53 Coastal areas are important interface environments between marine and terrestrial ecosystems
54 and are populated by abundant living communities. They are generally areas of high economic activity
55 related to trade, fishing and tourism, among others. Port areas, which are essential to the maritime
56 economy, concentrate these activities in commercial, fishing and pleasure ports. Their activities, very
57 different from each other, can vary according to the years, the seasons and the commercial interests of
58 each region and/or country.

59 Indeed, looking at the commercial port of La Rochelle (France), the activity depends on the
60 harvest season with the transport of cereals, or the oil activity for example. They require the use of
61 cargo ships, which can reach a size of more than 200 meters long, for maritime transport, but also
62 trucks for road transport and freight trains for rail transport. In 2020, traffic has been estimated at
63 8,925,015 tons for the commercial port of La Rochelle (PALR website). In comparison, the marina of
64 La Rochelle (first marina of the Atlantic coast with more than 5000 places for boaters) has a different
65 activity, punctuated by the tourist seasons (April to October). The vessels encountered are much
66 smaller (between 6 and 20 meters long on average) and their uses are dedicated to pleasure boating,
67 fishing and nautical activities such as sailing.

68 These two port areas, as well as all those of the Atlantic coast, have developed marine
69 ecosystems within their basins, which may be subject to various contaminations from these port
70 activities. Trace metal elements are particularly at issue. Indeed, beyond their presence in the natural
71 environment (geological background), over concentrations of anthropic origin can be generated by
72 port activities. They can come from roadways, river discharges, careening stations (present both in the
73 commercial port and in the marina), but also from cathodic protection equipment. Too much of these
74 metals can lead to multiple risks, both for marine organisms and for consumers (Fasulo et al., 2012 ;
75 Kumar et al., 2022 ; Prato et al., 2019 ; Sureda et al., 2011).

76 Cathodic protection has existed since the 1820's, but has been mainly used since the Second
77 World War for the protection of underground oil and gas pipelines. Today, it is used in many fields

78 where structures can be subjected to corrosion, and in particular in the maritime field. Sacrificial
79 anodes are mainly made of zinc or aluminum. They must be made of a specific alloy that meets the NF
80 EN 12496 standards for anode conformity. In port areas, the alloy most often used is aluminum-based.
81 A large part of the infrastructures (quays, piles, sheet piles, etc.) present in port areas are made of steel
82 and are therefore subject to the process of corrosion with sea water, thus reducing their life span. The
83 hulls of ships are also affected. The basic principle of galvanic cathodic protection is to slow down the
84 corrosion of the steel structure thanks to a galvanic anode connected with the steel. A corrosion cell is
85 then created, which accelerates the corrosion of the anode and decreases significantly that of the steel
86 (Refait et al., 2015; Refait et al., 2013). The anodes are corroding instead of the steel structure and are
87 thus commonly called sacrificial anodes. In marine applications, the galvanic anodes are generally
88 made of an alloy based on aluminum (Al > 90 %), zinc (Zn, 3 - 4 %) and indium (In < 0.1 %) and their
89 life span is estimated at 15 years. This requires regular renewal of all the protections established on the
90 structures. Large quantities of anodes are therefore necessary to protect the submerged infrastructure,
91 and consequently the quantity of corrosion products resulting from the degradation of the anodes
92 seems considerable. However, the duration of dissolution being important and the concerned water
93 masses being always renewed, this quantity of corrosion products represents a negligible metal mass
94 flow. The cathodic protection is essential for the ports, without being compulsory: it allows to reduce
95 the financial costs for the maintenance of the structures and to limit the safety problems related to the
96 degradation of these structures. The question of the impact of cathodic protection on the marine
97 environment is nevertheless raised (Deborde et al., 2015). However, to date, few biomonitoring
98 studies in harbor environments have addressed the potential impact of cathodic protection (Jupp et al.,
99 2017). On the other hand, several studies have been conducted in laboratories, notably on mussels
100 (Mao et al., 2011) or oysters (Mottin et al., 2012).

101 In view of the various studies already carried out in the laboratory, it seemed interesting to
102 look at the potential impact of sacrificial anodes through an environmental approach in port areas. The
103 interest is twofold since it should allow to observe the effect or not of sacrificial anodes on a bivalve
104 mollusk directly in the marine environment, but also to compare the environments by choosing the

105 study sites on two port areas: the commercial port and the marina of La Rochelle. The choice of
106 species was made for the black scallop *Mimachlamys varia*. Described in the literature as a sentinel
107 species of the marine environment, this filter feeder has a sedentary lifestyle, allowing it to
108 bioaccumulate and tolerate large quantities of contaminants in its body (Bustamante and Miramand,
109 2005 ; Lacroix et al., 2015 ; Luna-Acosta et al., 2010a ; Luna-Acosta et al., 2010b ; Sureda et al.,
110 2011). It is also a species prized by consumers (Prato et al., 2019). A comparative study conducted in
111 2017 within the La Rochelle marina identified the scallop as a more sensitive bioindicator than the
112 oyster and mussel for conducting multi-biomarker monitoring in harbor spaces (Breitwieser et al.,
113 2017). Therefore, it seemed appropriate to use this species for the study presented here.

114 Thus, the two-year study first examined whether or not the presence of sacrificial anodes
115 influenced the health status of black scallops *M. varia* in the two port areas using biomarkers.
116 Secondly, it is important to distinguish between recent galvanic cathodic protection systems and older
117 ones. The dissolution rate of a galvanic anode is the highest at the beginning of the process, when the
118 anode is connected to a previously unprotected steel structure. As time passes, a mineral layer
119 (calcareous deposit) progressively covers the steel surface so that the electrical current, i.e. the anode
120 dissolution rate, required for cathodic protection is significantly decreased (Humble, 1948). Therefore,
121 this study compares sites where the cathodic protection was recently implemented with sites where the
122 cathodic protection system operates for years. Finally, the last objective was to see if port activities
123 had an impact or not on the bioaccumulation of trace metals by bivalves.

124 **2. Material and methods**

125 ***2.1 Sampling sites and caging transplantation procedure***

126 Two study sites are located in the marina and two study sites are located in the commercial
127 port (La Rochelle, Nouvelle-Aquitaine Region). They are exposed to potential diffuse chronic
128 contamination of sacrificial anodes with trace metals. The study sites are presented as follows: the
129 service port (POS, Commercial port) and the Lazaret 1 basin (LAZ1, Marina) are exposed to
130 sacrificial anodes installed in the vicinity; pontoon 519 (P519, Commercial port) and the Lazaret 2

131 basin (LAZ2, Marina) are not exposed to sacrificial anodes (Figure 1). The reference site, Marsh, is
132 located north of La Rochelle (L'Houmeau, CNRS site). It consists of former oyster beds not subject to
133 harbor influences. This site has already been used as a reference site in previous studies (Barbarin et
134 al. 2022 ; Breitwieser et al., 2020). The same sites were used in 2020 and 2021 with a change in site
135 configuration in 2021: P519 and LAZ2 were equipped in December 2020 with new sacrificial anodes.
136 The characteristics of the sites are specified in Table 1.

137 Between 2020 and 2021, the same method of collecting individuals was used: individuals
138 (Black scallop, *Mimachlamys varia*) were collected from the Loix site (Ile de Ré, less subject to port
139 influences, (Barbarin et al., 2022 ; Breitwieser et al., 2018a ; Breitwieser et al., 2017) in November
140 and caged in the reference site (Marsh) for two months to be acclimated. The scallops were then
141 transplanted to the study sites in the harbor spaces. On each day of experimentation (D0, D07, D21,
142 D30), individuals were counted in order to evaluate mortality, then seven individuals per site were
143 collected (the collection at D0 took place only at the reference site) and were placed in liquid nitrogen
144 in order to preserve enzymatic activities. Low mortality (less than 10 % for each site) was observed
145 during the study. Individuals were then placed at -80°C before being prepared for biochemical analysis
146 and quantification of trace metals. In addition, at D0 on the five study sites, water and sediment
147 samples were taken in order to establish an initial state of the environment at the time of the bivalves'
148 installation.

149 **2.2 Water physicochemical assessments**

150 For each day of sampling, the physicochemical parameters of the water (Temperature (°C) and
151 Salinity (PSU)) were measured using a HANNA probe (HI9829 multiparameter). The calibration of
152 the probe was performed before each sampling period.

153 **2.3 Trace element assessments**

154 Al, Zn, In, Cu, and Fe analyses were performed with a Varian Vista-Pro ICP-OES and a
155 Thermofisher XSeries II ICP-MS. For bivalves, sample preparation and analysis were performed using
156 the method used in previous studies (Breitwieser et al., 2017 ; Milinkovitch et al., 2015). Average

157 recoveries for bivalves were (in %): Al (99), Zn (98), Cu (96), Fe (87). Average trace element
158 concentrations are expressed as $\mu\text{g.g}^{-1}$ dry weight. For seawater, the limits of quantification (in $\mu\text{g.l}^{-1}$)
159 were: Al (200), Zn (2500 (in 2020) and 550 (in 2021)), Cu (10 (in 2020) and 2 (in 2021)), Fe (2500 (in
160 2020) and 550 (in 2021)). For sediments, the average recoveries were (in %): Al (106), Zn (96), Cu
161 (91), Fe (90). and the limits of quantification (in $\mu\text{g.g}^{-1}$) for sediment were: Al (20), Zn (4), Cu (1)
162 and Fe (4).

163 **2.4 Biomarker assessments**

164 All biomarker assays (SuperOxide Dismutase (SOD), Glutathione-S Transferase (GST),
165 MalonDiAldehyde (MDA), Acetylcholinesterase (AChE), Laccase (LAC)) were performed using the
166 same instrument: SpectroStar Nano, with specific absorbance for each type of biomarker. Assays were
167 performed on scallop (*M. varia*) digestive glands from all study sites at D0, D07, D21 and D30 (n=7
168 per site and date). Data are obtained as IU.mg protein for SOD, GST, AChE, and LAC. Data obtained
169 for MDA are in $\mu\text{M.mg}$ protein. All assays are described in previous publications (Barbarin et al.,
170 2022 ; Breitwieser et al., 2017).

171 **2.5 Statistical analysis**

172 All statistical analyses were performed using R software (RStudio, version 1.4.1103).
173 Homogeneity of variances was assessed using Fligner-Killeen tests and normality of variables was
174 also tested using a Shapiro-Wilk test. Quantification analysis of biomarkers (SOD, GST, MDA, AChE
175 and LAC) was performed using the reference value method of Barbarin et al. 2022. For this purpose,
176 linear regression equations were created from the digestive gland weight, temperature and salinity
177 measurements from the Marsh reference site. The r^2 values obtained for each of the equations and their
178 precisions are shown:

$$179 \text{SOD (U.mg protein}^{-1}\text{)} = - 5703.0 - 46.5 (\text{weight d.glands}) + 33.6 (\text{weight d.glands})^2 + 23.7 (\text{temperature})$$
$$180 \quad - 1.2 (\text{temperature})^2 + 444.4 (\text{salinity}) - 8.7 (\text{salinity})^2$$

$$181 R^2 = 0.66 \text{ and Precision} = 54 \%$$

182 **GST (U. mg protein⁻¹)** = - 953.8 - 2.4 (weight d.glands) + 3.9 (weight d.glands)² - 1.5 (weight d.glands)³
183 + 0.9 (temperature) - 0.1 (temperature)² + 0.001 (temperature)³ + 108.7 (salinity)
184 - 4.1 (salinity)² + 0.1 (salinity)³

185 R² = 0.66 and Precision = 58 %

186 **MDA (μM.mg protein⁻¹)** = 1047.4 - 36.7 (weight d.glands) + 43.7 (weight d.glands)² - 15.8 (weight d.glands)³
187 + 9.3 (temperature) - 1.3 (temperature)² + 0.1 (temperature)³ - 122.2 (salinity)
188 + 4.7 (salinity)² - 0.1 (salinity)³

189 R² = 0.62 and Precision = 60 %

190 **AChE (U. mg protein⁻¹)** = 12026.0 - 217.2 (weight d.glands) + 294.8 (weight d.glands)²
191 - 116.2 (weight d.glands)³ - 120.9 (temperature) + 13.4 (temperature)²
192 - 0.5 (temperature)³ - 1418.5 (salinity) + 57.4 (salinity)² - 0.8 (salinity)³

193 R² = 0.34 and Precision = 58 %

194 **LAC (U. mg protein⁻¹)** = - 867.8 - 7.9 (weight d.glands) + 9.0 (weight d.glands)² - 3.0 (weight d.glands)³
195 - 8.2 (temperature) + 1.0 (temperature)² - 0.04 (temperature)³ + 103.8 (salinity)
196 - 4.0 (salinity)² + 0.1 (salinity)³

197 R² = 0.54 and Precision = 54 %

198 Using these equations, it was possible to calculate, for each study site, the predicted values, as
199 well as the residuals (difference between the measured and predicted values). The residuals are then
200 used for the k-means clustering method to establish the indicators. The latter specify the health status
201 of the individuals studied and are established using the k-means clustering method (Barbarin et al.,
202 2022 ; Barrick et al., 2016 ; Devin et al., 2014). They are presented in Table S1. After the initial
203 analyses, a correlation study within each variable group is performed (biomarkers, metals). Then, a
204 second correlation study is performed this time with the most relevant variables to set up a principal
205 component analysis (PCA). The PCA identifies the main linear combinations of variables (Press et al.,

206 2013 ; Saporta 2006 ; Venables and Ripley, 2013) and extreme values. Data for metal quantification in
207 water and sediment are analyzed in parallel.

208 **3. Results**

209 ***3.1 Biomarkers responses***

210 The biomarker quantification results are shown in Figure 2. For some data, the values
211 predicted by the statistical models (linear regressions) are not displayed due to the lack of precision of
212 the statistical models. Each of the graphs presents the biomarker results obtained at D0, D07, D21 and
213 D30 sampling in winter 2020 and winter 2021. As a reminder, the study sites are divided according to
214 the characteristics presented in Table 1. Each graph is created in the same way: data symbolized by
215 circles correspond to measured values in the laboratory and data symbolized by triangles correspond to
216 predicted values. The black line represents the data measured at the Marsh reference site.

217 For SOD (Figure 2A), we see that the predicted and measured data are similar, reflecting a
218 good prediction of the model at these dates, with some exceptions. In 2020 at D0, the predicted values
219 are significantly lower than the measured ones and conversely, in 2021 at D30 they are significantly
220 higher. In the latter case, the values predicted contamination of individuals that was not measured.
221 Concerning the study sites, several of them present expected lethal effects (light red zone), in
222 particular the site P519 at the following dates: D07 and D21 (2020), D07 (2021). The LAZ1 and POS
223 sites are also affected. At D07 (2020), significant differences ($p < 0.05$) are obtained between all the
224 study sites, however, as previously indicated, only site P519 appears to be contaminated. This site was
225 not equipped with an anode at this time but is located near the ship repair facility of the commercial
226 port. This contamination is again visible at D21 (2020) for the P519 site, but also for the POS site and,
227 to a lesser extent, the LAZ1 site. Significant differences in activity were also found between these
228 three sites at this date (D21 (2020)). The suspected contamination did not only concern the
229 commercial port but also the marina. Heavy rainfall was recorded at this time, thus increasing the
230 discharge from the road. No difference can be made for this biomarker between the sites with and

231 without anode in 2020, nor when 2021. On the other hand, it can be seen that SOD activity has
232 significantly decreased between 2020 and 2021.

233 Regarding GST (Figure 2B), almost all predicted values were higher than measured values,
234 except at D21 and D30 (2020). However, few assumed contamination were found: LAZ1 (D07) and
235 POS (D30) in 2020; POS (D07) and P519 (D30) in 2021. A significant difference was found between
236 LAZ1 (with anode) and LAZ2 (without anode) at the marina at D07 in 2020. The drawbacks of this
237 result are that they are not repeated at subsequent dates, and the opposite result is observed at the
238 commercial port sites. Defining whether there is therefore an influence of anodes on GST activity
239 becomes complicated.

240 For MDA (Figure 2C), the predicted values are globally close to the measured values, which is
241 consistent with the predictive capacity of the model evaluated at 60 %. Concerning the measured
242 values, some assumed contaminations are observable: P519 and LAZ1 (D07, 2020); LAZ1 (D21,
243 2020); POS (D30, 2020); POS, P519 and LAZ2 (D07, 2021); LAZ2 (D21, 2021). Of these values,
244 some have expected sublethal effects and some have expected lethal effects. This reflects an increase
245 in cell membrane peroxidation.

246 Then, AChE (Figure 2D) naturally shows a high activity. In case of contamination, the activity
247 of acetylcholinesterase is degraded, explaining the inversion of the graph with respect to the other
248 biomarkers. Several differences are observable between the predicted and measured data. Potential
249 contaminations are visible on: POS (D07, 2020) ; POS and P519 (D30, 2021). These alterations in
250 AChE activity remain punctual and insufficient to define an implication of anode activity on the health
251 status of bivalves.

252 Finally, for the LAC (Figure 2E), almost all data (predicted and measured) show potential
253 alterations in the bivalve immune system. Again, it is not possible with the graphs to determine
254 whether or not sacrificial anodes have an influence on biomarkers. Port activities, as well as weather
255 conditions, influence the biomarker results too significantly to distinguish the potential effect of
256 anodes.

257 **3.2 Levels of trace elements**

258 Trace metal concentrations obtained in black scallop digestive glands, seawater and sediments
259 are presented in Table 3, Table S2 and Table S3 respectively. As In concentrations are generally below
260 the limits of quantification for all three compartments, results for this trace element will not be
261 presented in this document.

262 Based on the results presented in Table 3, trace elements present in bivalves are in the
263 majority at the study sites in harbor areas compared to the Marsh reference site. This shows that,
264 despite naturally occurring trace elements in the environment, anthropogenic activities influence the
265 bioaccumulation of these metals in scallops. Then some differences were obtained in 2020 between
266 sites with and without anodes: POS / P519 for Al, Zn and Fe. Site P519, without sacrificial anodes in
267 2020, still has the highest concentrations. This can be explained by the proximity of the site to the ship
268 repair facility, and prevents any distinction from being made on the influence or not of anodes. No
269 significant difference was obtained between sites LAZ1 and LAZ2 for the marina in that year. In 2021,
270 other comparisons could be made: POS / P519 for Al, Zn, Cu and Fe. No one site appears to
271 predominate over the other although P519 has the highest trace element concentrations on several
272 occasions. For marina sites: LAZ1 is higher than LAZ2 (with some significant differences) for Zn and
273 Cu. Site LAZ1 has the highest concentrations for these two metals (except for D30 where the same
274 concentration is observed at both sites). As for the commercial port, distinguishing between the two
275 sites is complex since neither of them stands out.

276 The results for seawater (Table S2) are not very usable since most of the results are below the
277 limits of quantification. In contrast, for sediment (Table S3), better results were obtained. The
278 commercial port sites, POS and P519, show almost all of the highest concentrations, mainly in 2020
279 for the POS site for Al and Zn and in 2021 for the P519 site with Zn, Cu and Fe. The results for site
280 P519 can again be explained by the presence of the ship repair facility nearby. Except in 2020 with the
281 POS site, none of the results allow to observe a potential effect of the anodes on the seawater and
282 sediment compartments.

283 **3.3 Factor analysis to underline relationships between biomarker responses and trace**
284 **element assessments**

285 To complete this study, a principal component analysis is performed with the data collected
286 from the bivalve *M. varia*. Axes 1 and 2 of the PCA, with 56.1 % of the data explained were chosen
287 (Figure 3). From the figure, no demarcation is visible between sites. However, it can be noted that site
288 LAZ2 (marina) is more driven by trace elements, while site LAZ1 (marina) is more driven by
289 biomarkers, as well as site P519 (commercial port). In terms of correlations, the biomarkers are
290 globally positively correlated with each other and no correlation is measured with metals. Only Fe and
291 Al are positively correlated with each other.

292 **4. Discussion**

293 The Pertuis Charentais are marine areas protected by the OSPAR convention since 2012,
294 constituting the marine natural park with Natura 2000 protection and conservation areas for birds and
295 habitats. These coastal areas, interface and exchange environment between marine and terrestrial
296 ecosystems, are nevertheless subject to a significant anthropic activity with the development of port
297 activities for trade, fishing or yachting. For several years, these different port areas have implemented
298 an environmental policy aimed at monitoring and protecting marine biodiversity by avoiding
299 excessive impact of port activities, in particular through the ISO 14001 standard (control of
300 environmental impacts, continuous improvement of environmental performance). In addition, several
301 environmental monitoring studies have been carried out, in partnership with the University, within
302 these port areas: (Breitwieser et al., 2020 ; Breitwieser et al., 2018b ; Breitwieser et al., 2017 ; Huguet
303 et al., 2020).

304 Nevertheless, the anthropic pressure is always present and new questions are raised, in
305 particular on the potential effect of galvanic cathodic protection systems installed in port areas in order
306 to preserve the structures against corrosion caused by sea water. This protection against corrosion is
307 necessary to increase the life span of structures, and thus reduce maintenance costs. Sacrificial anodes,
308 which have been present for many years in ports and harbors and are constantly being renewed (the

309 life span of an anode is about 15 years), have been the subject of several studies with the objective of
310 understanding the functioning of sacrificial anodes and their interactions with the marine environment
311 (Caplat et al., 2020 ; Deborde et al., 2015). Other laboratory studies have looked at the impact of
312 anodes on different bivalve species: the hollow oyster *Crassostrea gigas* (Levallois et al., 2022 ;
313 Mottin et al., 2012), or the blue mussel *Mytilus edulis* (Mao et al., 2011). However, no studies have
314 been conducted in the harbor environment on this topic to date, and few studies focus on the black
315 scallop *Mimachlamys varia*. Thus, the study presented in this article is an innovative approach with an
316 active biomonitoring approach in a port environment. Moreover, this study was set up over two years
317 in order to observe whether or not sacrificial anodes have an impact on the health status of the black
318 scallop *Mimachlamys varia* in a port environment (commercial and marina).

319 The different results obtained in winter 2020 and winter 2021 allowed, thanks to the different
320 analyses (Figures. 2 and 3) to show that the port sites had a different activity compared to the
321 reference site Marsh. Moreover, the biomarker analysis approach, by modeling, makes it a little easier
322 to identify possible contamination. On the other hand, it is essential to compare the results with the
323 quantification of trace metals. However, differentiating the potential effect of anodes in relation to port
324 activities, or in relation to the age of the anodes is not possible following an environmental approach.
325 This must necessarily be accompanied by a laboratory study in order to avoid the influence of
326 anthropic activities, as well as the influence of meteorological conditions. Indeed, in winter period,
327 they favor the resuspension of sediments in the sea water, and increase the discharges from the road.
328 At the end of January 2020, the cumulative rainfall was more important with an increase of 22 %, or
329 89.5 mm of average precipitation compared to the normal value established on the measuring station.

330 The same phenomenon was observed in January 2021 with a 33% increase in precipitation, i.e.
331 98.0 mm of average precipitation (data extracted from the La Rochelle Aerodrome meteorological
332 station). Concerning the anodes, a theoretical estimate of the mass of anode consumed per year within
333 a port area was made in a parallel study. Taking into account the lifespan of an anode (15 years), an
334 estimate of the quantity of anodes present in a port area (commercial or marina), as well as the volume
335 of seawater present in this same area considering (for the purposes of the calculation) that it is a closed

336 enclosure with no water renewal, the mass of anode consumed and dissolved in the seawater would be
337 1.38 mg.l⁻¹ per year. This value may seem important, but taking into account the permanent renewal of
338 water in the port areas (current and tide), this concentration decreases considerably and could be
339 considered without risk for marine ecosystems. Thus, the results showed that the potential effects of
340 these anodes were masked by port activities such as careening, but also by meteorological conditions
341 with heavy rainfall. This observation is in line with the conclusion of other scientists that the
342 contribution of anodes to the inputs of trace metals seemed negligible compared to continental inputs
343 on a regional scale (Caplat et al., 2010 ; Deborde et al., 2015 ; Mao et al., 2011 ; Gabelle et al., 2012).

344 However, this environmental approach should not be put aside, and other studies have been
345 carried out on the subject of cathodic protection, in particular on the development of oysters with the
346 replacement of plastic by steel (Hunsucker et al., 2021), or for the protection of offshore wind turbines
347 (Erdogan and Swain, 2021). Other studies, using an experimental approach, have been conducted on
348 oysters or mussels for example (Mao et al., 2011; Mottin et al., 2012), but none on black scallops. It
349 would therefore be interesting to complete the environmental approach presented here with an
350 approach that could be carried out over a longer period of time and in other port areas such as a fishing
351 port for example. Moreover, renewing this experimentation according to an experimental approach in
352 a controlled environment and without the influence of port activities would make it possible to answer
353 the questions left unanswered following this study.

354 **5. Conclusion**

355 This study allowed us to observe that, through an environmental approach in a port
356 environment, it is not possible to observe the potential effect of sacrificial anodes on the health of the
357 black scallop *Mimachlamys varia*. Therefore, it would be interesting to carry out a similar study in a
358 controlled environment in order to be free of the influence of port activities. It would also be possible
359 and interesting to observe if the different components of an anode (Al, In, Cu, Zn, Fe) have an impact
360 on the health status of the black scallop.

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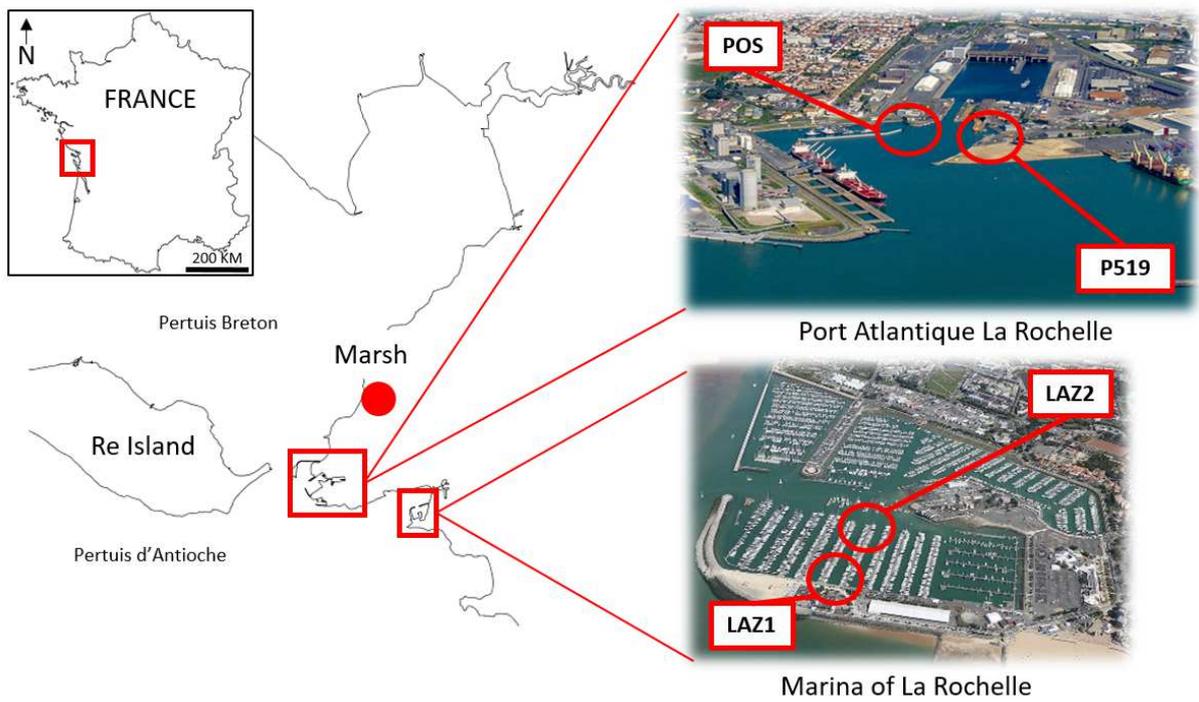
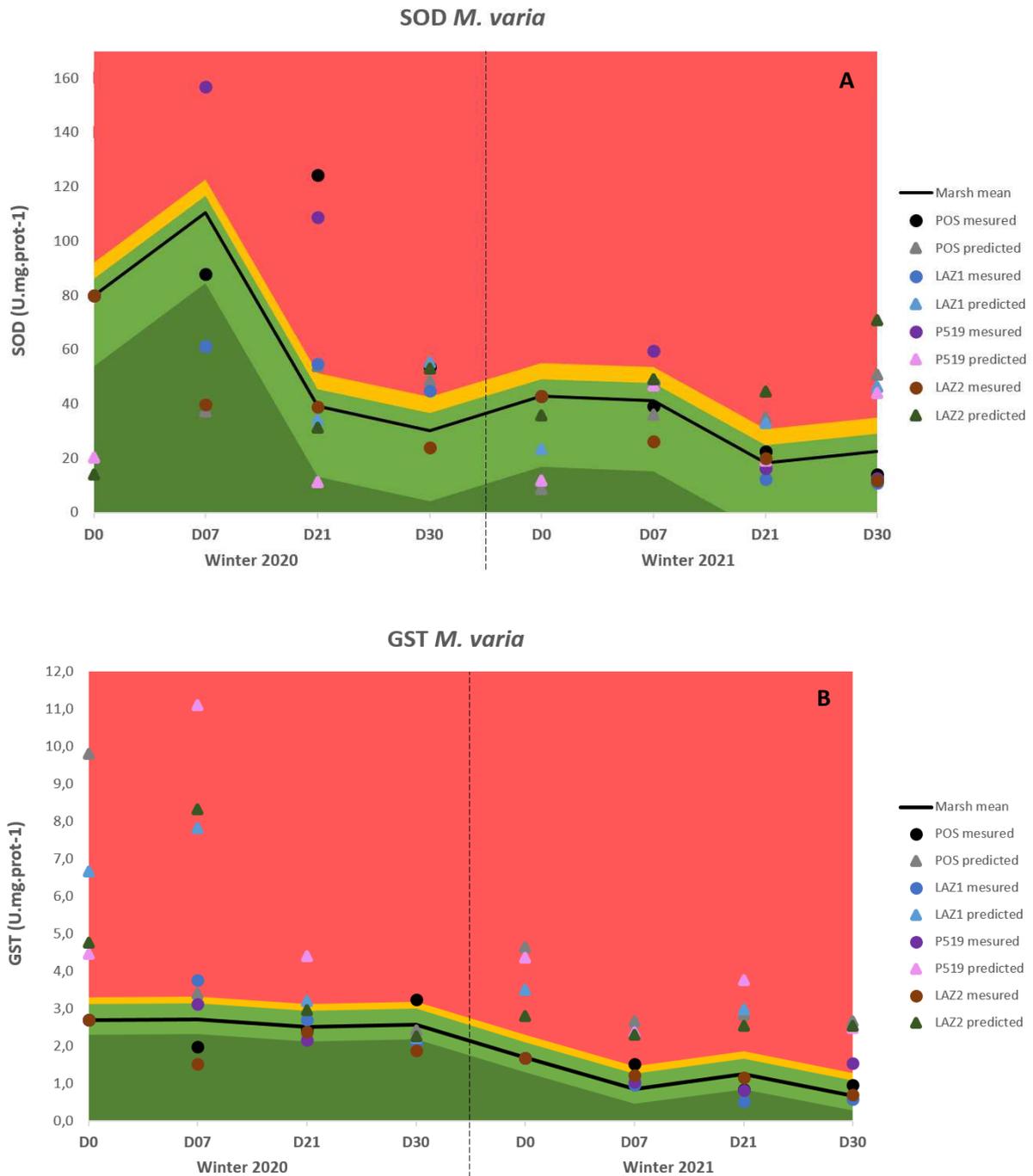
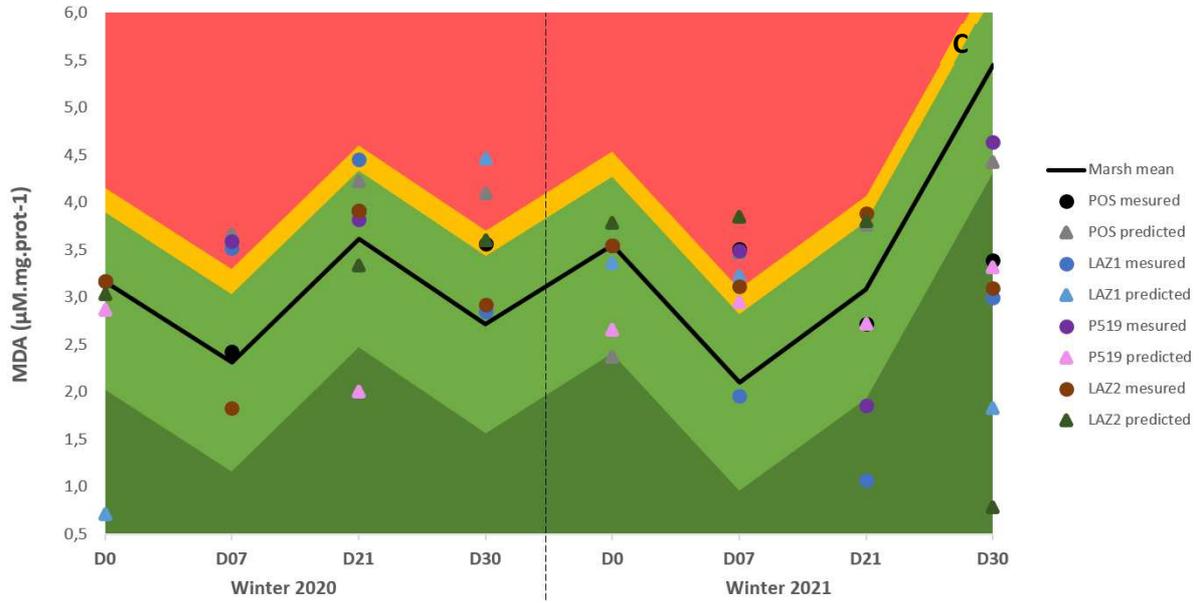


Figure 1: Map showing the location of the study sites. Marsh: reference site (L'Houmeau, CNRS site). Port Atlantique La Rochelle is the name of the commercial port of La Rochelle.

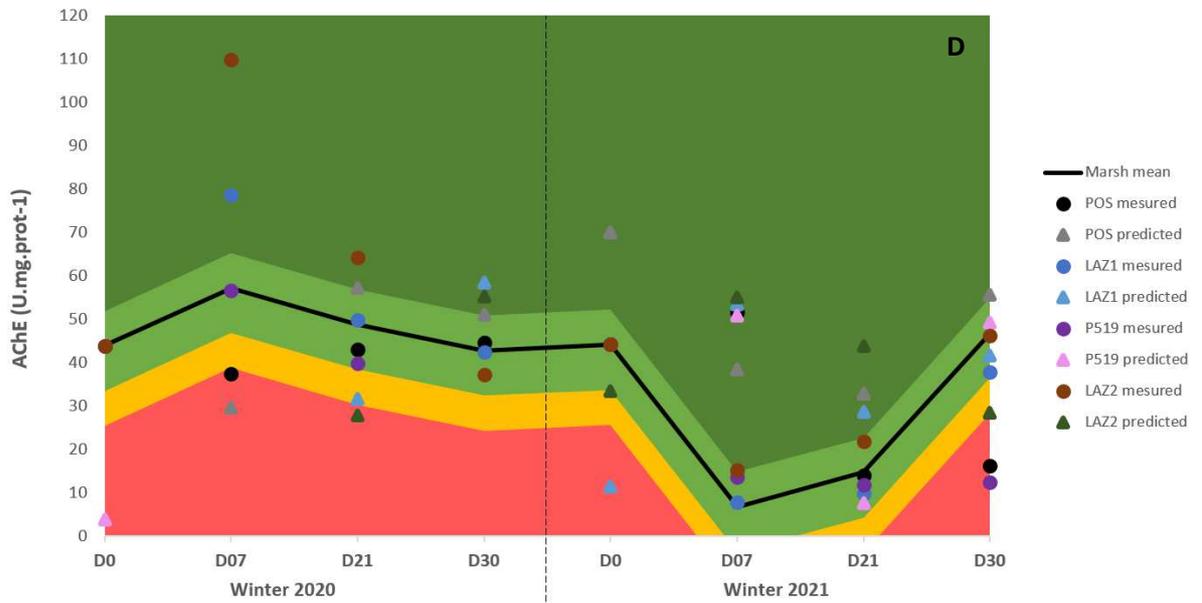
Figure 2: Graphical representations of health indicators for SOD (A), GST (B), MDA (C), AChE (D) and LAC (E). For each graph, the measured and model-predicted values for each study site are represented by circles and triangles respectively (calculations of the predicted values from the equations in the article). Environmental variations (k-means method) are defined by dark green and light green bands (healthy individuals), yellow bands (expected sublethal effects) and light red bands (expected lethal effects). The measured values for the reference site Marsh are represented on each graph by a black line.



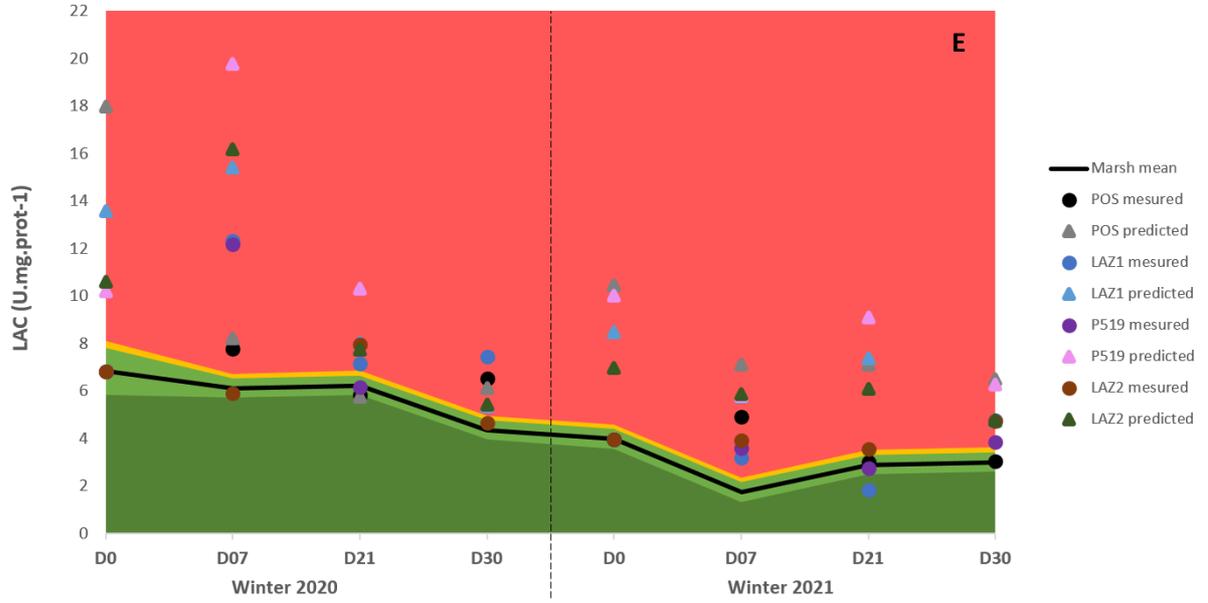
MDA *M. varia*



AChE *M. varia*



LAC *M. varia*



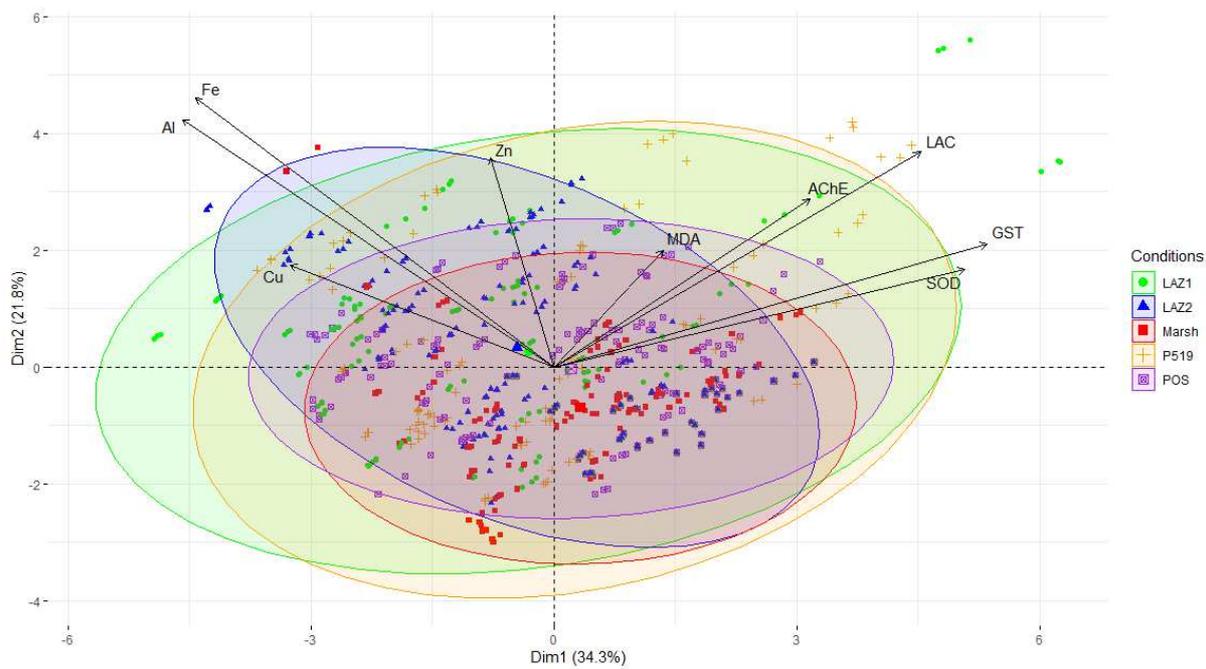


Figure 3: Principal component analysis of biomarker (SOD, GST, MDA, AChE, LAC) and trace element (Al, Zn, Cu, Fe) data obtained from the black scallop *Mimachlamys varia* in 2020 and 2021.

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Table 1 : Characteristics of the study sites.

| Sites | Location | 2020 | 2021 |
|--------------|-----------------|----------------|----------------|
| POS | Commercial port | Old anodes | Old anodes |
| P519 | Commercial port | Without anodes | New anodes |
| LAZ1 | Marina | Old anodes | Old anodes |
| LAZ2 | Marina | Without anodes | New anodes |
| Marsh | Reference | Without anodes | Without anodes |

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Table 2: Average digestive gland weights (\pm SEM) of collected *Mimachlamys varia* black scallops, as well as temperature ($^{\circ}$ C) and salinity (PSU) parameters observed at each sampling day at the Marsh reference site.

| Sampling period | | Digestive gland weight \pm SEM (g) | Temperature ($^{\circ}$ C) | Salinity (PSU) |
|-----------------|-----|--------------------------------------|-----------------------------|----------------|
| 2020 | D0 | 1.02 \pm 0.04 | 5.28 | 24.17 |
| | D07 | 1.04 \pm 0.06 | 8.81 | 24.73 |
| | D21 | 0.95 \pm 0.07 | 10.68 | 27.98 |
| | D30 | 0.98 \pm 0.04 | 10.21 | 23.99 |
| 2021 | D0 | 0.81 \pm 0.03 | 5.80 | 25.3 |
| | D07 | 0.56 \pm 0.03 | 11.48 | 23.5 |
| | D21 | 0.93 \pm 0.05 | 7.28 | 23.23 |
| | D30 | 0.54 \pm 0.05 | 11.70 | 22.87 |

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46 Table 3: Concentration ($\mu\text{g/g}$) of trace metals (Al, Zn, Cu, Fe) in the digestive glands of black scallops *Mimachlamys varia* according to study sites in 2020 and 2021. POS (commercial port): old
 47 anodes; P519 (commercial port): without anode (2020) and new anodes (2021); LAZ1 (marina): old anodes; LAZ2 (marina): without anode (2020) and new anodes (2021). In bold are the highest
 48 concentrations recorded for each trace element in 2020 and 2021. **a** ($p < 0.05$) = significant difference with the reference site; **b** ($p < 0.05$) = significant difference with the site without anode
 49 (2020) or the site with new anode (2021) in each port area.

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| | Year | POS | | | | P519 | | | | LAZ1 | | | | LAZ2 | | | | Marsh | | | |
|----|------|-----|------------------------|------------------------|-----------------------|------|------------------------------|------------------------------|--------------------------------|------|------------------------|------------------------|----------------------------|------|------------------------|-------------------------|--------------------------------|--------------|--------------|--------------|---------------|
| | | D0 | D07 | D21 | D30 | D0 | D07 | D21 | D30 | D0 | D07 | D21 | D30 | D0 | D07 | D21 | D30 | D0 | D07 | D21 | D30 |
| Al | 2020 | - | 128.3 ±27 | 194.3 ±51 | 318.2 ±43 a | - | 345.4 ±37 a, b | 347.3 ±71 | - | - | 341.0 ±92 a | 302.3 ±47 | 482.0 ±75 a | - | 404.7 ±103 a | 410.3 ±80 a | 483.3 ±73 a | 38.7 ±11 | 109.2 ±22 | 254.5 ±46 | 188.9 ±37 |
| | 2021 | - | 455.5 ±123 a | 360.0 ±73 | 369.1 ±87 | - | 241.0 ±60 b | 301.2 ±69 | 628.7 ±106 b | - | 343.1 ±98 | 550.6 ±106 a | 512.5 ±33 | - | 282.9 ±59 | 587.0 ±170 a | 675.1 ±42 | 166.2 ±59 | 203.7 ±53 | 176.6 ±61 | 477.9 ±181 |
| Zn | 2020 | - | 97.3 ±18 | 64.4 ±25 a | 58.4 ±13 a | - | 146.0 ±38 a | 114.9 ±12 a, b | - | - | 105.6 ±24 | 95.6 ±9 | 114.7 ±10 a | - | 92.9 ±6 | 118.4 ±13 a | 96.0 ±8 a | 65.7 ±9 | 97.3 ±13 | 64.4 ±4 | 58.4 ±4 |
| | 2021 | - | 68.1 ±5 a | 110.2 ±9 a | 80.0 ±5 | - | 61.5 ±4 a | 114.2 ±18 a | 96.0 ±7 b | - | 90.6 ±14 | 108.6 ±5 a | 109.5 ±5 a | - | 79.1 ±4 | 76.9 ±4 b | 106.9 ±3 a | 62.1 ±4 | 96.5 ±21 | 85.6 ±5 | 90.6 ±9 |
| Cu | 2020 | - | 20.1 ±3 | 36.3 ±4 a | 17.5 ±2 | - | 19.2 ±4 | 39.3 ±3 a | - | - | 23.6 ±2 | 35.8 ±7 a | 43.8 ±8 a | - | 24.7 ±3 a | 28.5 ±3 a | 35.9 ±5 a | 19.0 ±4 | 18.2 ±3 | 13.2 ±1 | 12.8 ±2 |
| | 2021 | - | 21.4 ±3 | 45.2 ±13 a | 50.1 ±17 a | - | 19.3 ±2 | 24.1 ±2 b | 28.8 ±3 b | - | 28.7 ±6 | 50.5 ±11 a | 72.5 ±6 a | - | 20.5 ±3 | 29.2 ±2 b | 38.0 ±2 a, b | 13.9 ±1 | 25.1 ±8 | 24.7 ±7 | 16.5 ±1 |
| Fe | 2020 | - | 332.0 ±54 | 524.2 ±119 | 581.4 ±82 | - | 801.1 ±91 a, b | 717.9 ±175 | - | - | 765.6 ±165 a | 709.0 ±128 | 979.2 ±145 a | - | 879.3 ±195 a | 945.3 ±140 a | 1088.5 ±131 a | 212.3 ±20 | 261.6 ±35 | 499.9 ±67 | 441.0 ±78 |
| | 2021 | - | 717.5 ±153 | 901.8 ±152 a | 794.4 ±152 | - | 512.8 ±105 | 692.5 ±108 | 1275.2 ±188 b | - | 658.0 ±164 | 866.0 ±142 a | 884.9 ±45 | - | 617.2 ±90 | 1144.1 ±266 a | 1193.5 ±53 | 357.2 ±79 | 500.3 ±92 | 446.8 ±90 | 955.9 ±285 |

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Graphical abstract

