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A reassessment of the use of *Posidonia oceanica* and *Mytilus galloprovincialis* to biomonitor the coastal pollution of trace elements: New tools and tips

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ABSTRACT

The present study gives a summary using state-of-the-art technology to monitor *Posidonia oceanica* and *Mytilus galloprovincialis* as bioindicators of the pollution of the Mediterranean littoral with trace elements (TEs), and discusses their complementarity and specificities in terms of TE bioaccumulation. Furthermore, this study presents two complementary indices, the Trace Element Spatial Variation Index (TESVI) and the Trace Element Pollution Index (TEPI): these indices were shown to be relevant monitoring tools since they led to the ordering of TEs according to the overall spatial variability of their environmental levels (TESVI) and to the relevant comparison of the global TE pollution between monitored sites (TEPI). In addition, this study also discusses some underestimated aspects of *P. oceanica* and *M. galloprovincialis* bioaccumulation behaviour, with regard to their life style and ecophysiology. It finally points out the necessity of developing consensual protocols between monitoring surveys in order to publish reliable and comparable results.

1. Introduction

The pollution of trace elements (TEs) remains a topical subject: on the one hand, because some of these elements, which had been up to now little monitored (*e.g.* Bi, Sb, Mo, etc.) can be considered as pollutants of environmental "emerging concern" (Daughton, 2004, 2005); whereas on the other hand because their world production and use, after having suffered a slight slowdown at the end of the 1990s, is now experiencing new growth (US Geological Survey, http://www.usgs.gov/) as a result of the economic growth of a series of nations (*e.g.* the People's Republic of China, India, etc.; Sievers et al., 2010; Tiess, 2010). Consequently, the threats that represent TE anthropogenic loadings in the environment require to be continuously monitored, from their emission sources down to their ultimate repository, *i.e.* the oceans.

The monitoring of the marine pollution relying upon the use of bioindicator species (*i.e.* biomonitoring; Blandin, 1986) exhibits obvious predominance when compared with the conventional analysis of chemicals in environmental matrices (*i.e.* water and sediments). Biomonitoring reveals biological changes of organisms affected by exogenous chemicals as well as synergistic and integrated effects of pollutants on organisms. It has high sensitivity due to rapid responses induced in organisms exposed to pollutants and allows the relevant monitoring of pollutants found at low environmental levels. It further allows wide sampling even in remote areas and avoids limits of conventional chemical analyses such as continuous sampling or needs of expensive instruments (Zhou et al., 2008). The use of specific bioindicator organisms (*e.g.* seagrasses) has led to the development of different classification tools for assessing the global quality of water bodies and the health status of coastal ecosystems (Montefalcone, 2009; Lopez y Royo et al., 2011). In contrast, there currently exists no satisfactory biomonitoring index to specifically assess the TE pollution status of aquatic bodies. For example, the international Metal Pollution Index (MPI) proposed by Usero et al. (1996), in its current form, only allows to compare the global TE pollution between the different monitored sites of a specific survey, for a given species (Chaudhuri et al., 2007; Lafabrie et al., 2008; Lopez y Royo et al., 2009). We believe that this index could be adapted in order to make the calculated index values comparable between studies, regardless the bioindicator species considered or the list of TEs monitored. In addition, there currently exists no monitoring index providing the ability to order and to compare TEs according to the overall spatial variability of their environmental levels throughout the whole of a studied area. However, we believe that such an index could be useful to efficiently highlight TEs of main environmental concern regarding their discharge to coastal waters.

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A wide variety of species have been shown to be relevant indicators to assess the contamination status of marine ecosystems (Burger, 2006; Zhou et al., 2008). In the Mediterranean, the magnoliophyte *Posidonia oceanica* (L.) Delile and the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 are part of the key bioindicator species integrated for a long time in environmental monitoring surveys (Montefalcone, 2009; Andral et al., 2011). *P. oceanica* and *M. galloprovincialis* respond appreciably and quantitatively to the pollution of TEs (Casas et al., 2008; Benedicto et al., 2011; Luy et al., 2012; Richir et al., 2013) and could complement one another. The two species bioaccumulate TEs dissolved in the water column (Lafabrie et al., 2007; Casas et al., 2008). *P. oceanica* is strongly rooted in sediments and reflects the contamination of this compartment (Lafabrie et al., 2007) whereas *M. galloprovincialis*, as filter feeder, bioaccumulates TEs from their particulate phase (Casas et al., 2008). Together, they could give an overview of the pollution status (water, sediments, suspended matter) of the coastal Mediterranean. However, of the few surveys that have concomitantly used these two bioindicators (Kantin and Pergent-Martini, 2007; Lafabrie et al., 2007; Joksimović and Stanković, 2012), none has so far either discussed *P. oceanica* and *M. galloprovincialis* monitoring complementarity and specificity according to their life style or compared their bioaccumulation behaviour according to their respective ecophysiology. We nevertheless believe that such knowledge is required in order to improve their use as main bioindicators of the coastal pollution of the Mediterranean. Furthermore, the use of specific body compartments of both *P. oceanica* and *M. galloprovincialis* instead of entire organisms has been suggested in some studies (Adami et al., 2002; Roméo et al., 2005; Romero et al., 2007a, 2007b; Salivas-Decaux et al., 2010). Therefore, we believe that a harmonization of monitoring practices is necessary, both in terms of sampling strategy and sample processing, in order to make biomonitoring studies comparable between themselves.

Based on these considerations, the main goal of the present work was to propose new tools and tips regarding to the use of *P. oceanica* and *M. galloprovincialis* to biomonitor the coastal pollution of the Mediterranean with 19 TEs (Al, V, Fe, Cr, Mn, Co, Ni, Cu, Zn, Se, Ag, Cd, Sn, Sb, As, Mo, Be, Pb and Bi). More precisely, detailed objectives were: firstly, to propose a new index, the Trace Element Spatial Variation Index (TESVI) allowing to order and to compare TEs according to the overall spatial variability of their environmental levels throughout the whole of a studied area; secondly, to study and compare the spatial resolution (the response sensitivity) of *P. oceanica* and *M. galloprovincialis* in the biomonitoring of environmental TE pollution loads, and to develop a weighted version of the Metal Pollution Index (Usero et al., 1996) allowing the reliable comparison of global TE pollution levels whatever the bioindicator used; thirdly, to study and compare TE kinetics in *P. oceanica* and *M. galloprovincialis*, with regard to their ecophysiology; and fourthly, to discuss the reliability of using specific compartments of both bioindicator species instead of entire organisms in monitoring surveys.

2. Material and methods

2.1. *P. oceanica* and *M. galloprovincialis* sampling

The 1st objective of the present study aimed to develop a new Trace Element Spatial Variation Index (TESVI), proposed to order and to compare TEs according to the overall spatial variability of their environmental levels throughout the whole of a studied area. To this end, *P. oceanica* (n = 15) were sampled at 15 m depth in April 2007 in 18 sites (diamonds in Figs. 1a and 1c) remote from a few km to hundreds of km and located along Provence-Alpes-Côte d'Azur (PACA) and Corsican Mediterranean coasts of France (TE concentrations detailed in Luy et al., 2012; St Raphaël and Cap Roux sites were properly reordered according to their west-to-east geographic localization; maps of both manuscripts cover the same 41–44° south-to-north (wrong scaling in Luy et al., 2012) and 5–10° west-to-east geographic area).

The 2nd objective aimed to study and compare the response sensitivity of *M. galloprovincialis* and *P. oceanica* in the biomonitoring of coastal TE pollution loads. To this end, rope-grown *M. galloprovincialis* were purchased from the pristine shellfish farm SARL Etang de Diane, eastern Corsica (bold-thick right cross in Fig. 1a), in March 2010 (Richir and Gobert, 2014). Mussels were carefully detached from ropes with a ceramic scalpel and individuals with sizes ranging from 60 to 70 mm were stored in 8 conchylicultural pouches (n = 103 mussels in each pouch) mounted on PVC tubing (Andral et al., 2004). Two man-made mussel biointegrator stations were immersed for 3 months at 7–10 m depth, from March to June 2010 (*i.e.* during mussel sexual dormancy), in 4 stations remote from 1 to 3 km in Calvi Bay area, northwestern Corsica. The 4 stations were respectively in front of the oceanographic station STARESO, beside an aquaculture farm, in the vicinity of the pipe discharging the treated domestic wastewater of Calvi city and at the Punta Bianca, just outside of the Bay influence (right-crosses in Figs. 1b and 1c). At haul-out time (June 2010), pouches were recovered by divers, while *P. oceanica* shoots (n = 15) were concomitantly sampled between 13 and 22 m depth (circles in Figs. 1b and 1c), depending on site configuration. To study TE bioaccumulation in *P. oceanica* along a gradient of disturbance, additional seagrasses (n = 9–10) were further sampled at 8–9 m depth in May 2010 in 9 stations spaced approximately 300 m along a radial following the coastline at the back of the Ajaccio Bay, western Corsica (squares in Fig. 1d), with increasing distance from the port and urban centre of Ajaccio city. To discuss *P. oceanica* response sensitivity at two very different spatial scales, observations from monitoring surveys along the Ajaccio Bay radial (present study) or along the French Mediterranean littoral (TE concentrations detailed in Luy et al., 2012) are compared in the results and discussion section.

The 3rd objective aimed to study and compare TE kinetics in *M. galloprovincialis* and *P. oceanica*, with regard to their ecophysiology. To this end, rope-grown *M. galloprovincialis* were purchased from the shellfish farm SARL Etang de Diane in February 2011 (Richir and Gobert, 2014). Once detached from ropes, mussels with sizes ranging from 50 to 80 mm were stored in 2 conchylicultural pouches, each subdivided into 9 individual sub-pouches for time-scaled sampling (n = 25 in each sub-pouches), and mounted on PVC tubing. The 2 man-made mussel biointegrator stations were immersed for 4.5 months, from February to June 2011, just under the sea surface near the oceanographic station STARESO (leaning cross in Fig. 1c), and regularly sampled during that time interval. Regarding *P. oceanica*, samples (n = 15) were collected at 10 m depth in March, June and November of years 2008–2010 in the pristine seagrass bed in front of the oceanographic station STARESO (Luy et al., 2012; Richir et al., 2013), in the Calvi Bay (triangle in Fig. 1c), and in the Plateau des Chèvres (diamond 4 in Fig. 1a) near Cortiou sewage outfall discharging wastewater from Marseille city (Oursel et al., 2013). Results from this mussel-caging experiment and results from the seasonal monitoring of TE levels in *P. oceanica* are discussed in the results and discussion section concomitantly with complementary observations from previous published studies.

P. oceanica sampled to meet objectives 1–3 were always collected randomly by divers within areas of minimum 25 m^2. Finally, the 4th objective aiming to discuss the reliability of using specific mussel or seagrass compartments instead of entire individuals in biomonitoring surveys was met through the compilation of observations from previous published studies.

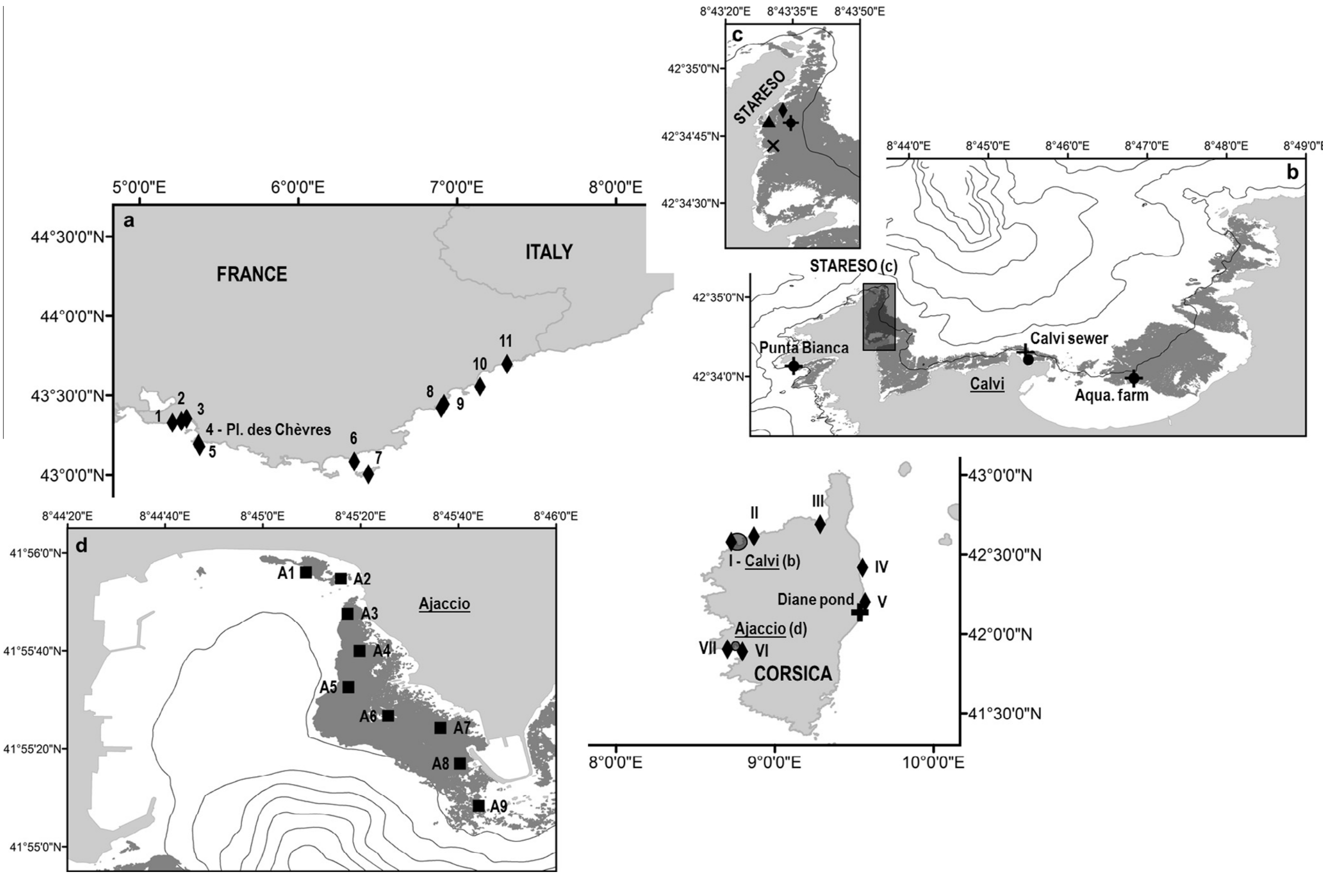

Fig. 1. (a) Map of the northwestern Mediterranean. Diamonds ♦ – 18 sites located along coasts of the French Mediterranean littoral, remote from one another of a few to hundreds of km and sampled for *P. oceanica* ($n = 15$) at 15 m depth in April 2007 (Luy et al., 2012). Arabic and Roman numbers represent sites along continental Provence-Alpes-Côte d'Azur (1–11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Bold-thick right cross ✚ – The shellfish farm SARL Etang de Diane on the eastern Corsican coast where rope-grown *M. galloprovincialis* were purchased in March 2010 and February 2011 (Richir and Gobert, 2014). Zoomed Calvi and Ajaccio areas of maps (b) and (d) are dark grey circled. (b) Zoom in Calvi Bay area. Right crosses + – Caged *M. galloprovincialis* ($n = 103$ in each pouch) immerged in duplicate at 7–10 m depth from March to June 2010 in 4 stations in Calvi Bay area, remote from one another by a distance of 1 to 3 km: STARESO (in STARESO (c) zoomed area), aquaculture farm, Calvi city sewer and Punta Bianca. Circles ● – *P. oceanica* ($n = 15$) sampled between 13 and 22 m depth in June 2010, concomitantly when caged mussels were retrieved from water. (c) Zoom in STARESO area. Triangle ▲ – *P. oceanica* ($n = 15$) seasonally sampled at 10 m depth in March, June and November of years 2008–2010; an identical sampling effort was performed at 10 m depth in Plateau des Chèvres site (map (a), diamond 4). Leaning cross × – Caged *M. galloprovincialis* ($n = 25$ in each of several sub-pouches) immerged just below the sea surface from February to June 2011 and regularly sampled during that time interval. ♦, + and ● symbols are the same than on maps (a) and (b). (d) Zoom in Ajaccio area. Squares ■ – *P. oceanica* ($n = 9–10$) sampled at 8–9 m depth in May 2010 in 9 stations remote from one another of around 300 m along a radial located at the back of the Ajaccio Bay, with increasing distance from the port and urban centre of Ajaccio city (from station A1 to station A9). Medium grey areas on maps (b), (c) and (d) are *P. oceanica* biocoenoses, and lines are 25 m depth equidistant isobaths (Abadie, 2012; ANDROMEDE OCEANOLOGIE et STARESO, 2012).

2.2. *P. oceanica* and *M. galloprovincialis* sample processing

All collected *P. oceanica* (to meet 1st–3rd objectives) were treated according to the biometric method proposed by Giraud (1979). Epiphytes were scraped from leaves with the aid of a ceramic scalpel blade (Dauby and Poulicek, 1995). Leaves were then freeze dried (BenchTop 3L, VirTis Company Inc.) and weighed.

Of the 103 mussels placed in conchylicultural pouches for the spatial biomonitoring of TEs in Calvi Bay area (to meet the 2nd objective), 24–25 individuals were randomly considered for analysis. The 24–25 mussels from the duplicated cages immerged in each of the 4 monitored stations were afterwards regarded as originating from one single cage (*i.e.* one mussel cage by station; Andral et al., 2004; Benedicto et al., 2011) since mean concentrations in TEs between duplicated cages did not differ (results from additional statistics not shown). Of the 25 mussels placed in sub-pouches for the monitoring of TE kinetics in *M. galloprovincialis* with regard to their ecophysiology (to meet the 3rd objective), 12 individuals were randomly considered for analysis. *M. galloprovincialis* were measured with an electronic calliper (0.01 mm). Soft tissues (byssus excluded) were carefully removed from shells with a ceramic scalpel blade. Mussels soft tissues were freeze dried (BenchTop 3L, VirTis Company Inc.) and weighed. Their shells were oven dried (48 h at 60 °C) and weighed to calculate individual condition indices (CI; Andral et al., 2004).

P. oceanica and *M. galloprovincialis* dried samples were cryogenically ground with liquid nitrogen in an agate mortar and re-lyophilized to eliminate condensed ambient water vapour. Dried powders were mineralized in Teflon bombs in a closed microwave digestion lab station (Ethos D, Milestone Inc.). The digestion procedure performed was a nitric acid-hydrogen peroxide mineralization (HNO_3/H_2O_2; suprapur grade, Merck). Digestates were diluted to an appropriate volume of 50 ml prior to being analysed.

2.3. Trace element analysis

TE levels were determined by inductively coupled plasma mass spectrometry (ICP-MS) using dynamic reaction cell (DRC) technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). Analytical accuracy was checked by analysing Certified Reference Materials (CRMs):

BCR 60 (*Lagarosiphon major*; $n = 8–20$; mean recovery = 87 ± 21%), BCR 61 (*Platyhypnidium riparioides*; $n = 8$; mean recovery = 87 ± 23%), BCR 62 (*Olea europaea*; $n = 8–20$; mean recovery = 94 ± 6%) and BCR 278 (mussel tissue; $n = 3$; mean recovery = 95 ± 9%) from the JCR's Institute for Reference Materials and Measurements; GBW 07603 (bush branches and leaves; $n = 10–23$; mean recovery = 97 ± 19%, Se excluded) from the Chinese Institute of Geophysical and Geochemical Exploration; NIST 1577c (bovine liver; $n = 10$; mean recovery = 103 ± 8%), NIST 1566b (oyster tissue; $n = 10$; mean recovery = 95 ± 8%) and NIST 2976 (mussel tissue; $n = 10$; mean recovery = 106 ± 7%) from the American National Institute of Standards and Technology. The global mean recovery of certified values, all CRMs and TEs together, was 96 ± 14% (Se excluded for GBW 07603; no certified value for Sn).

For each TE, detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated according to Currie (1999) or Grinzaid et al. (1977), depending on their specific blank distribution (normal or not). Data were analysed as TE concentrations on a dry weight basis and are expressed in $\mu g\ g_{DW}^{-1}$. The number of decimals, for each TE, is retained throughout the entire manuscript (Tables 2–5) in order to facilitate the comparison of concentrations between species and/or studied areas.

2.4. Data analysis

2.4.1. Index calculation

To order and to compare TEs according to the overall spatial variability of their environmental levels along French Mediterranean coasts, using *P. oceanica* as bioindicator species, Trace Element Spatial Variation Index (TESVI) values were calculated, for each TE, as follows:

$$\mathrm{TESVI} = \left[(x_{\max}/x_{\min})/\left(\sum(x_{\max}/x_i)/n\right)\right] * \mathrm{SD},$$

where $x_{\max}$ and $x_{\min}$ are the maximum and minimum mean concentrations recorded among the n sites, x_i are the mean concentrations recorded in each of the n sites, and SD is the standard deviation of the weighted sum $\sum(x_{\max}/x_i)/n$. The higher the index value for a given TE, the more its environmental levels globally vary throughout the whole of the studied area the index is applied to (punctual contaminations and overall coastal spatial heterogeneity of TE levels taken into account). The overall spatial variability of TE levels, summarized in the form of single TESVI values, can further be graphically compared by using a proportional ordinate (concentration) scaling between TEs. The proportional ordinate scaling is obtained by multiplying the minimum mean concentration of each TE recorded among the n sites by the highest $x_{\max}/x_{\min}$ mean concentration ratio calculated among all the studied TEs.

To compare global TE pollution levels between monitored sites using either *M. galloprovincialis* or *P. oceanica* as bioindicator species, a weighted version of the Metal Pollution Index (MPI) proposed by Usero et al. (1996) was used. This index was renamed Trace Element Pollution Index (TEPI), to include non-metallic chemical elements, and a data pre-treatment was performed prior calculating TEPI values, *i.e.* the mean normalization. This pre-treatment standardizes the data, by converting all the variable mean values to unity and the rest close to unity. This is useful when data of very different magnitude, such as concentrations of various TEs, are present (Moreda-Pineiro et al., 2001). TEPI values were calculated, for each site or station, as follows:

$$\mathrm{TEPI} = (\mathrm{Cf}_1 * \mathrm{Cf}_2 \ldots \mathrm{Cf}_n)^{1/n},$$

where Cf_n is the mean normalized concentration of the TE n in a given monitored site or station. The higher the index value, the more polluted is the monitored site or station. The TEPI allows to differentiate little contaminated sites or stations from highly polluted ones with an efficiency equivalent to that of the MPI, while minimizing the variability related to the number and the sorting of TEs used for its calculation (tested on random lists of TEs; results from additional statistics not shown). This weighted index further allows, contrary to the MPI, the reliable comparison of global TE pollution levels between monitoring surveys, and that even if the list of monitored TEs and/or bioindicator species used differ.

Table 1
Trace Element Spatial Variation Index (TESVI) values calculated from trace element (TE) mean concentrations in *P. oceanica* ($n = 15$) sampled at 15 m depth in April 2007 in 18 sites located along the French Mediterranean littoral and remote from one another of a few to hundreds of km. Analysed TEs have been either little or broadly monitored in that species. For each TE, $x_{\max}/x_{\min}$ is the ratio between the maximum ($x_{\max}$) and minimum ($x_{\min}$) mean concentrations recorded among the 18 sites, and $\sum(x_{\max}/x_i)/18$ (mean ± SD) is the mean ratio between the maximum mean concentration ($x_{\max}$) and each of the 18 site mean concentrations (x_i). TESVI = $[(x_{\max}/x_{\min})/(\sum(x_{\max}/x_i)/18)] * \mathrm{SD}$. The higher the TESVI value for a given TE, the more its levels globally varied throughout the whole of the French Mediterranean littoral. Data used are from Luy et al. (2012). The site with the highest mean concentration reported by these authors is also given for each TE. BAL = blades of *P. oceanica* adult leaves.

	$x_{\max}/x_{\min}$	$\sum(x_{\max}/x_i)/18 \pm$ SD	TESVI	Site $x_{\max}$
TEs little monitored in *P. oceanica*				
Be	3.1	1.6 ± 0.6	1.0	Ajaccio Nord
Al	7.5	2.2 ± 1.8	6.1	Ajaccio Nord
V	14.5	5.9 ± 5.0	12.3	Antibes
Mn	2.2	1.6 ± 0.4	0.5	St Raphaël
Co	2.9	1.8 ± 0.5	0.7	St Raphaël
As	10.6	5.9 ± 2.7	4.9	Pl. des Chèvres
Se	1.7	1.3 ± 0.2	0.3	Calvi
Mo	22.8	13.6 ± 6.2	10.5	Aregno
Ag	3.1	1.9 ± 0.6	0.9	La Vesse
Sn (BAL)	6.9	3.5 ± 1.9	3.8	Corbière
Sb	4.4	3.6 ± 0.7	0.9	Bravone
Bi	13.6	6.1 ± 3.5	7.9	Pl. des Chèvres
TEs broadly monitored in *P. oceanica*				
Cr	6.0	3.6 ± 1.3	2.2	St Florent
Fe	4.4	2.0 ± 0.9	1.9	Bravone
Ni	2.4	1.6 ± 0.3	0.5	St Raphaël
Cu	3.4	1.9 ± 0.7	1.2	Villefranche
Zn	19.6	13.3 ± 4.4	6.5	Bravone
Cd	3.9	1.9 ± 0.7	1.4	St Raphaël
Pb	4.4	2.7 ± 1.2	2.0	Ajaccio Nord

2.4.2. Statistical analyses

Statistical analyses were performed with STATISTICA (Statsoft, Inc.). Significant differences between mean TE concentrations measured in caged *M. galloprovincialis* ($n = 48–49$, except for Be: $n = 24–25$) immersed from March to June 2010 in the 4 stations of Calvi Bay area and significant differences between mean TE concentrations measured in *P. oceanica* ($n = 15$) concomitantly sampled when mussels were retrieved from water were highlighted through one-way analysis of variance (one-way ANOVA), followed by Tukey HSD pairwise comparison test of means with equal (*P. oceanica*) or unequal (*M. galloprovincialis*) n's ($p < 0.05$), after testing for normality and homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric analysis of variance (Kruskal-Wallis test) was performed when assumptions prior to ANOVAs (normality and/or homoscedasticity) were not achieved, followed by Dunn pairwise comparison test of means ($p < 0.05$). One-way ANOVA or Kruskal-Wallis test were further performed on raw or log-transformed TE concentrations measured in *P. oceanica* ($n = 9–10$) sampled in May 2010 in the 9 stations located along the Ajaccio Bay radial, to study the significance ($p < 0.05$) of their spatial evolution with increasing distance from the port and urban centre of Ajaccio city.

Table 2

Trace element (TE) concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 48–49, except for Be: n = 24–25) purchased from the Diane salty pond (eastern Corsica, France) and immerged between 7 and 10 m depth from March to June 2010 in 4 stations in Calvi Bay area (northwestern Corsica, France), and in *P. oceanica* (n = 15) sampled between 13 and 22 m depth in June 2010 concomitantly when mussels were retrieved from water. Trace Element Pollution Index (TEPI) values were calculated for each station and bioindicator species from mean normalized concentrations of the 19 TEs. The 4 sampled stations STARESO, aquaculture farm, Calvi city sewer and Punta Bianca were remote from one another by a distance of 1 to 3 km. Letters represent significant differences between stations for each bioindicator species. *, ** and struck-through values represent concentrations $<L_Q$, $<L_D$ or $<L_C$, respectively. For each bioindicator species, the largest difference in concentrations (in %) between stations for each of the 19 TEs and TEPI values is also given.

Species-Station	Al	V	Fe	Cr	Mn	Co	Ni	Cu	Zn	Se
Mytilus galloprovincialis										
STARESO	36.7 ± 21.3^{a}	1.52 ± 0.45^{a}	81 ± 21^{ab}	0.310 ± 0.111^{a}	2.3 ± 0.4^{a}	0.47 ± 0.15^{ab}	1.01 ± 0.35^{a}	3.29 ± 0.54^{a}	121 ± 38	3.36 ± 0.48^{a}
Aquaculture farm	25.8 ± 17.2^{ab}	1.29 ± 0.41^{b}	74 ± 19^{a}	0.284 ± 0.066^{b}	2.0 ± 0.3^{b}	0.43 ± 0.17^{a}	0.85 ± 0.23^{b}	2.80 ± 0.52^{b}	117 ± 56	2.97 ± 0.47^{b}
Calvi sewer	55.0 ± 29.7^{c}	1.37 ± 0.37^{bc}	101 ± 35^{b}	0.324 ± 0.094^{ab}	2.5 ± 0.6^{a}	0.52 ± 0.20^{b}	1.02 ± 0.30^{ab}	3.35 ± 0.79^{ab}	121 ± 69	3.37 ± 0.63^{ab}
Punta Bianca	25.0 ± 13.2^{b}	1.55 ± 0.44^{ac}	77 ± 19^{a}	0.290 ± 0.076^{ab}	2.3 ± 0.5^{ab}	0.47 ± 0.17^{ab}	1.01 ± 0.31^{a}	3.22 ± 0.52^{b}	113 ± 57	3.28 ± 0.47^{ab}
largest difference	120%	20%	36%	14%	25%	22%	20%	20%	7%	14%
Posidonia oceanica										
STARESO	19.2 ± 3.5^{a}	10.42 ± 1.78^{a}	40 ± 3^{a}	0.211 ± 0.015^{ab}	37.2 ± 3.0^{a}	1.72 ± 0.12^{a}	29.21 ± 2.68^{a}	5.69 ± 0.87^{a}	76 ± 6^{a}	0.35 ± 0.03^{a}
Aquaculture farm	85.6 ± 15.0^{b}	6.46 ± 1.62^{b}	83 ± 10^{b}	0.232 ± 0.028^{a}	36.3 ± 3.7^{a}	1.84 ± 0.21^{a}	23.21 ± 1.60^{b}	8.07 ± 1.19^{b}	70 ± 7^{a}	0.38 ± 0.04^{b}
Calvi sewer	35.3 ± 7.6^{c}	2.43 ± 0.46^{c}	89 ± 13^{b}	0.184 ± 0.024^{b}	37.2 ± 3.5^{a}	1.83 ± 0.18^{a}	18.79 ± 1.04^{b}	8.50 ± 0.96^{b}	107 ± 11^{b}	0.33 ± 0.03^{a*}
Punta Bianca	8.1 ± 1.6^{d}	1.29 ± 0.37^{d}	35 ± 3^{a}	0.139 ± 0.011^{c}	53.6 ± 5.8^{b}	2.35 ± 0.26^{b}	28.54 ± 1.54^{a}	7.91 ± 1.11^{b}	106 ± 7^{b}	0.36 ± 0.03^{ab}
largest difference	954%	708%	155%	68%	47%	36%	55%	49%	52%	16%

Species-Station	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi	TEPI
Mytilus galloprovincialis										
STARESO	0.018 ± 0.021^{a}	0.74 ± 0.21	0.014 ± 0.017*	0.014 ± 0.010^{a}	13.73 ± 1.50^{ab}	5.45 ± 1.95^{a}	0.0067 ± 0.0054**	0.84 ± 0.33	0.0120 ± 0.0047	1.01
Aquaculture farm	0.010 ± 0.004^{b}	0.74 ± 0.18	0.015 ± 0.021*	0.010 ± 0.006^{b}	12.81 ± 1.35^{a}	5.21 ± 1.62^{b}	0.0110 ± 0.0055*	0.85 ± 0.28	0.0105 ± 0.0040	0.91
Calvi sewer	0.015 ± 0.019^{ab}	0.80 ± 0.29	0.017 ± 0.014*	0.012 ± 0.003^{a}	13.37 ± 1.16^{ab}	4.86 ± 1.80^{b}	0.0138 ± 0.0061	0.92 ± 0.33	0.0127 ± 0.0043	1.09
Punta Bianca	0.014 ± 0.007^{a}	0.71 ± 0.21	0.012 ± 0.009*	0.013 ± 0.003^{a}	14.29 ± 1.31^{b}	5.44 ± 2.07^{a}	0.0064 ± 0.0036**	0.99 ± 0.37	0.0122 ± 0.0043	0.96
largest difference	79%	12%	42%	35%	12%	12%	114%	18%	21%	20%
Posidonia oceanica										
STARESO	0.452 ± 0.071^{a}	2.15 ± 0.19^{a}	0.022 ± 0.005^{a*}	0.255 ± 0.028	1.40 ± 0.33^{a}	2.52 ± 0.69^{ab}	0.0047 ± 0.0009^{a**}	0.66 ± 0.08^{a}	0.0043 ± 0.0004^{a}	0.91
Aquaculture farm	0.551 ± 0.084^{b}	1.95 ± 0.19^{b}	0.035 ± 0.005^{b*}	0.268 ± 0.024	1.02 ± 0.17^{bc}	1.74 ± 0.30^{a}	0.0134 ± 0.0030^{b}	0.82 ± 0.12^{b}	0.0046 ± 0.0008^{a}	1.08
Calvi sewer	0.559 ± 0.080^{b}	2.28 ± 0.18^{a}	0.033 ± 0.006^{b*}	0.271 ± 0.031	0.92 ± 0.13^{c}	3.53 ± 1.14^{b}	0.0053 ± 0.0011^{a**}	0.97 ± 0.08^{c}	0.0076 ± 0.0022^{b}	1.00
Punta Bianca	0.660 ± 0.083^{c}	3.07 ± 0.21^{c}	0.014 ± 0.002^{a**}	0.254 ± 0.029	1.29 ± 0.42^{ab}	3.61 ± 1.80^{b}	~~0.0030~~ ± 0.0006^{c}	0.53 ± 0.05^{d}	0.0025 ± 0.0001^{c}	0.78
largest difference	46%	58%	162%	7%	52%	108%	350%	84%	205%	38%

Table 3

Trace element (TE) concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 9–10) sampled at 8–9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France), and in the two supplementary Ajaccio Sud and Ajaccio Nord sites sampled at 15 m depth for *P. oceanica* (n = 15) in April 2007 by Luy et al. (2012). Trace Element Pollution Index (TEPI) values were calculated by site and station from mean normalized concentrations of the 19 TEs. The 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial. The significance of the overall spatial evolution of TE concentrations along the radial from station A1 to station A9 (*i.e.* with increasing distance from the port and urban centre of Ajaccio city) is given for each TE (s.d. or s.i. = significant decrease or increase, respectively, p < 0.05; n.s. = no significant evolution, p > 0.05). * and ** represent TE concentrations $<L_Q$ or $<L_D$, respectively. Analytical limits for Ajaccio Sud and Ajaccio Nord sites are from Luy et al. (2012).

Study-Station/Site	Al	V	Fe	Cr	Mn	Co	Ni	Cu	Zn	Se
Present study	(s.d.)	(s.d.)	(s.d.)	(s.d.)	(n.s.)	(s.d.)	(s.d.)	(s.d.)	(n.s.)	(s.d.)
A1	430.9 ± 167.8	7.51 ± 1.27	347 ± 120	0.743 ± 0.481	163.6 ± 17.3	2.65 ± 0.23	19.28 ± 1.89	15.96 ± 1.46	106 ± 11	1.02 ± 0.24
A2	274.4 ± 78.7	5.67 ± 0.68	279 ± 69	0.454 ± 0.098	140.0 ± 16.5	2.46 ± 0.26	18.04 ± 1.61	14.22 ± 1.47	115 ± 7	0.77 ± 0.13
A3	139.0 ± 38.6	3.50 ± 0.80	152 ± 30	0.327 ± 0.069	146.6 ± 35.7	4.56 ± 0.46	16.05 ± 1.65	18.20 ± 2.47	91 ± 7	0.71 ± 0.10
A4	149.0 ± 36.7	3.39 ± 1.67	163 ± 25	0.382 ± 0.030	94.8 ± 8.1	1.62 ± 0.11	15.46 ± 1.91	10.11 ± 0.73	78 ± 3	0.62 ± 0.05
A5	71.5 ± 27.3	3.00 ± 0.91	75 ± 14	0.207 ± 0.034	143.7 ± 59.7	1.55 ± 0.17	14.51 ± 1.67	9.60 ± 1.32	98 ± 13	0.61 ± 0.09
A6	78.1 ± 42.1	2.86 ± 0.48	77 ± 17	0.172 ± 0.030	149.5 ± 32.1	1.53 ± 0.34	14.20 ± 1.83	7.37 ± 0.97	92 ± 20	0.56 ± 0.09
A7	92.4 ± 31.0	1.54 ± 0.32	80 ± 11	0.187 ± 0.036	78.7 ± 10.8	1.81 ± 0.24	17.09 ± 2.95	6.99 ± 2.23	103 ± 12	0.48 ± 0.06
A8	103.5 ± 29.8	2.82 ± 0.38	128 ± 33	0.220 ± 0.035	69.3 ± 9.7	1.54 ± 0.28	14.52 ± 1.20	6.28 ± 1.13	81 ± 11	0.59 ± 0.05
A9	63.1 ± 25.2	2.91 ± 1.73	86 ± 52	0.265 ± 0.064	122.8 ± 18.9	1.22 ± 0.21	11.99 ± 2.36	6.39 ± 1.37	62 ± 7	0.56 ± 0.10
Luy et al. (2012)										
Ajaccio Sud	111.8 ± 23.9	3.50 ± 0.86	80 ± 9	0.206 ± 0.026	55.9 ± 2.3	2.65 ± 0.09	34.04 ± 1.38	8.37 ± 0.26	89 ± 6	0.28 ± 0.03*
Ajaccio Nord	151.0 ± 28.6	6.81 ± 2.79	143 ± 7	0.242 ± 0.017	55.2 ± 2.1	1.56 ± 0.06	21.79 ± 0.64	7.51 ± 0.38	72 ± 7	0.20 ± 0.02**

Study-Station/Site	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi	TEPI
Present study	(n.s.)	(s.i.)	(s.d.)	(n.s.)	(n.s.)	(n.s.)	(s.d.)	(s.d.)	(s.d.)	
A1	0.706 ± 0.075	0.92 ± 0.06	0.234 ± 0.073	0.352 ± 0.032	1.55 ± 0.29	2.57 ± 0.58	0.0319 ± 0.0061	6.01 ± 0.93	0.0612 ± 0.0121	1.55
A2	0.475 ± 0.055	1.19 ± 0.10	0.173 ± 0.036	0.277 ± 0.025	1.40 ± 0.15	1.73 ± 0.13	0.0203 ± 0.0026	5.12 ± 0.69	0.0483 ± 0.0101	1.25
A3	0.550 ± 0.088	0.76 ± 0.10	0.101 ± 0.018	0.336 ± 0.042	1.26 ± 0.17	2.83 ± 0.69	0.0174 ± 0.0043	5.81 ± 0.72	0.0230 ± 0.0022	1.09
A4	0.569 ± 0.098	0.87 ± 0.10	0.103 ± 0.019	0.314 ± 0.019	1.57 ± 0.08	2.41 ± 0.22	0.0173 ± 0.0029	4.41 ± 0.31	0.0285 ± 0.0026	0.98
A5	0.604 ± 0.104	1.53 ± 0.15	0.048 ± 0.015	0.296 ± 0.039	1.55 ± 0.21	3.40 ± 1.06	0.0114 ± 0.0039	3.00 ± 0.99	0.0169 ± 0.0033	0.84
A6	0.417 ± 0.083	1.47 ± 0.21	0.045 ± 0.016	0.311 ± 0.068	1.23 ± 0.12	3.45 ± 0.45	0.0088 ± 0.0052*	3.07 ± 0.64	0.0112 ± 0.0032	0.76
A7	0.533 ± 0.155	1.80 ± 0.18	0.047 ± 0.016	0.294 ± 0.042	1.62 ± 0.15	2.25 ± 0.56	0.0085 ± 0.0018*	1.67 ± 0.22	0.0103 ± 0.0011	0.72
A8	0.499 ± 0.095	1.61 ± 0.21	0.072 ± 0.015	0.306 ± 0.041	1.16 ± 0.10	2.98 ± 0.23	0.0105 ± 0.0039*	1.52 ± 0.17	0.0103 ± 0.0017	0.76
A9	0.354 ± 0.076	1.37 ± 0.14	0.110 ± 0.077	0.303 ± 0.029	2.73 ± 0.41	5.79 ± 4.66	0.0081 ± 0.0027*	2.96 ± 0.79	0.0093 ± 0.0039	0.80
Luy et al. (2012)										
Ajaccio Sud	1.133 ± 0.123	2.45 ± 0.14	0.045 ± 0.026	0.201 ± 0.021	2.21 ± 0.16	3.37 ± 0.30	0.0077 ± 0.0012*	2.32 ± 0.49	0.0105 ± 0.0013	0.83
Ajaccio Nord	0.523 ± 0.021	1.56 ± 0.08	0.050 ± 0.013	0.188 ± 0.015	1.70 ± 0.09	2.38 ± 0.21	0.0113 ± 0.0014	4.85 ± 0.92	0.0134 ± 0.0012	0.82

Table 4

Seasonal and pluriannual kinetics of trace element (TE) concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of Stareso (Calvi Bay, northwestern Corsica, France) and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France). The sampling of June 2009 is missing for Plateau des Chèvres site, and Sn concentrations are only available for samplings of November 2010 in both *P. oceanica* beds. *, ** and struck-through values represent TE concentrations <L_Q, <L_D or <L_C, respectively. *P. oceanica* intermediate and adult leaf foliar surfaces (FS; mean ± SD, in cm^2) and leaf numbers (mean ± SD) are also given. As *P. oceanica* leaf and shoot foliar surfaces and leaf numbers did not significantly ($p < 0.05$) differ between June 2008 and June 2010 in Plateau des Chèvres site, they were averaged to calculate the missing mean foliar surfaces and leaf numbers of June 2009.

Seagrass bed-Date	Al	V	Fe	Cr	Mn	Co	Ni	Cu	Zn	Se	Ag	Cd
STARESO												
03/17/08	51.7 ± 6.1	3.75 ± 0.70	45 ± 5	0.131 ± 0.005	33.9 ± 2.7	1.72 ± 0.16	24.38 ± 3.10	6.34 ± 0.45	67 ± 6	0.38 ± 0.04	0.503 ± 0.056	2.00 ± 0.11
06/01/08	38.6 ± 5.6	3.82 ± 0.31	45 ± 3	0.206 ± 0.008	45.5 ± 2.9	1.69 ± 0.09	22.11 ± 1.33	5.92 ± 0.31	63 ± 6	0.48 ± 0.02	0.470 ± 0.036	1.97 ± 0.11
11/10/08	29.7 ± 3.7	2.41 ± 0.66	45 ± 2	0.238 ± 0.053	43.4 ± 5.7	1.21 ± 0.21	16.87 ± 1.68	8.69 ± 0.86	48 ± 6	0.26 ± 0.04*	0.625 ± 0.072	1.55 ± 0.15
03/04/09	30.1 ± 3.9	2.15 ± 0.32	42 ± 2	0.103 ± 0.005	39.1 ± 2.5	1.95 ± 0.07	25.84 ± 2.03	6.45 ± 0.42	82 ± 3	0.31 ± 0.03*	0.570 ± 0.035	2.42 ± 0.11
06/01/09	36.1 ± 5.5	5.40 ± 1.15	52 ± 6	0.219 ± 0.010	45.8 ± 3.5	1.93 ± 0.11	23.90 ± 0.88	5.17 ± 0.31	81 ± 4	0.37 ± 0.03	0.477 ± 0.043	2.30 ± 0.08
11/14/09	42.2 ± 10.3	1.74 ± 0.51	45 ± 3	0.217 ± 0.030	51.8 ± 3.5	1.10 ± 0.10	17.02 ± 1.50	8.42 ± 0.46	52 ± 3	0.30 ± 0.02*	0.863 ± 0.081	2.04 ± 0.11
03/11/10	36.7 ± 6.5	1.32 ± 0.43	48 ± 3	0.115 ± 0.009	41.3 ± 2.8	1.77 ± 0.13	30.20 ± 1.64	7.50 ± 0.41	89 ± 4	0.35 ± 0.02	0.703 ± 0.048	2.96 ± 0.08
05/31/10	70.4 ± 16.8	3.94 ± 0.65	52 ± 4	0.188 ± 0.032	35.3 ± 4.1	1.76 ± 0.20	24.84 ± 1.73	5.48 ± 0.94	85 ± 7	0.43 ± 0.05	0.472 ± 0.094	2.25 ± 0.23
11/01/10	17.8 ± 4.3	1.50 ± 0.59	39 ± 6	0.201 ± 0.038	37.1 ± 3.7	1.23 ± 0.14	18.55 ± 2.80	8.52 ± 1.82	62 ± 7	0.18 ± 0.03*	0.756 ± 0.245	1.86 ± 0.20
Pl. des Chèvres												
03/20/08	30.9 ± 5.8	6.30 ± 2.04	53 ± 6	0.163 ± 0.035	27.2 ± 2.1	1.21 ± 0.16	22.39 ± 1.14	11.57 ± 1.13	73 ± 7	0.27 ± 0.03*	1.201 ± 0.295	1.81 ± 0.06
06/25/08	24.0 ± 2.5	4.27 ± 0.67	56 ± 4	0.195 ± 0.020	35.3 ± 1.4	1.62 ± 0.11	16.98 ± 1.06	7.57 ± 0.68	82 ± 5	0.32 ± 0.02*	1.018 ± 0.404	1.46 ± 0.07
11/28/08	27.2 ± 5.9	2.36 ± 0.60	49 ± 3	0.141 ± 0.013	29.2 ± 3.8	1.15 ± 0.22	17.43 ± 2.69	10.02 ± 0.61	71 ± 10	0.26 ± 0.02*	1.007 ± 0.102	1.65 ± 0.14
03/18/09	62.4 ± 11.0	4.16 ± 1.95	79 ± 12	0.270 ± 0.043	23.0 ± 1.8	1.28 ± 0.09	19.68 ± 1.10	11.00 ± 0.57	78 ± 3	0.32 ± 0.03*	0.924 ± 0.076	1.54 ± 0.06
06/14/09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11/19/09	41.4 ± 6.0	4.57 ± 1.66	71 ± 3	0.236 ± 0.025	45.3 ± 2.6	1.20 ± 0.18	17.13 ± 2.44	11.05 ± 0.61	63 ± 3	0.30 ± 0.02*	1.013 ± 0.096	1.38 ± 0.09
03/17/10	38.8 ± 4.3	2.33 ± 0.60	63 ± 4	0.187 ± 0.014	26.0 ± 2.7	1.60 ± 0.16	19.87 ± 1.29	13.13 ± 0.97	94 ± 8	0.31 ± 0.03*	1.066 ± 0.092	1.98 ± 0.16
06/03/10	55.3 ± 6.8	4.10 ± 1.30	71 ± 4	0.265 ± 0.016	38.4 ± 4.0	1.62 ± 0.12	18.29 ± 1.36	9.32 ± 0.62	85 ± 6	0.36 ± 0.01	0.643 ± 0.055	1.58 ± 0.13
11/10/10	24.3 ± 4.0	2.84 ± 1.14	56 ± 11	0.207 ± 0.016	61.9 ± 7.0	1.89 ± 0.21	21.61 ± 1.91	8.63 ± 0.97	88 ± 8	0.31 ± 0.03*	0.919 ± 0.169	1.71 ± 0.16

Seagrass bed-Date	Sn	Sb	As	Mo	Be	Pb	Bi	FS inter. leaves	FS adult leaves	Nb inter. leaves	Nb adult leaves
STARESO											
03/17/08	nd	0.213 ± 0.013	1.91 ± 0.14	1.56 ± 0.15	0.0061 ± 0.0015**	0.69 ± 0.06	0.0048 ± 0.0004	107 ± 48	83 ± 37	3.9 ± 0.8	3.0 ± 0.9
06/01/08	nd	0.233 ± 0.008	2.32 ± 0.46	3.75 ± 0.51	0.0058 ± 0.0014**	0.90 ± 0.04	0.0058 ± 0.0007	158 ± 33	231 ± 76	3.6 ± 1.1	4.5 ± 1.2
11/10/08	nd	0.204 ± 0.043	0.89 ± 0.13	1.76 ± 0.28	0.0071 ± 0.0014*	1.05 ± 0.13	0.0080 ± 0.0010	46 ± 11	100 ± 22	3.5 ± 0.5	4.3 ± 0.6
03/04/09	nd	0.206 ± 0.015	1.35 ± 0.15	1.81 ± 0.44	0.0047 ± 0.0008**	0.64 ± 0.05	0.0044 ± 0.0005	114 ± 32	88 ± 40	4.3 ± 0.7	3.1 ± 0.8
06/01/09	nd	0.272 ± 0.013	1.99 ± 0.19	1.70 ± 0.32	0.0051 ± 0.0009**	0.95 ± 0.07	0.0077 ± 0.0007	167 ± 32	228 ± 62	2.5 ± 0.5	3.7 ± 0.9
11/14/09	nd	0.207 ± 0.020	1.01 ± 0.06	2.81 ± 0.40	0.0056 ± 0.0005**	1.11 ± 0.12	0.0082 ± 0.0006	47 ± 12	81 ± 26	3.3 ± 0.5	3.7 ± 0.7
03/11/10	nd	0.187 ± 0.011	1.20 ± 0.12	2.29 ± 0.61	0.0058 ± 0.0007**	0.61 ± 0.03	0.0057 ± 0.0003	113 ± 49	96 ± 36	3.5 ± 0.7	3.3 ± 0.9
05/31/10	nd	0.241 ± 0.030	1.95 ± 0.36	1.56 ± 0.24	0.0091 ± 0.0011*	0.73 ± 0.09	0.0064 ± 0.0012	145 ± 32	144 ± 52	2.7 ± 0.6	2.9 ± 0.8
11/01/10	0.028 ± 0.007*	0.212 ± 0.026	0.62 ± 0.13	1.34 ± 0.17	0.0052 ± 0.0017**	0.80 ± 0.13	0.0072 ± 0.0011	35 ± 9	109 ± 31	2.7 ± 0.5	3.8 ± 0.8
Pl.des Chèvres											
03/20/08	nd	0.136 ± 0.012	2.35 ± 0.54	1.23 ± 0.11	~~0.0030~~ ± 0.0007	0.87 ± 0.14	0.0214 ± 0.0041	92 ± 26	61 ± 20	3.6 ± 0.9	2.8 ± 0.7
06/25/08	nd	0.236 ± 0.019	1.85 ± 0.19	2.03 ± 0.20	~~0.0021~~ ± 0.0005	0.82 ± 0.06	0.0163 ± 0.0014	90 ± 17	101 ± 27	2.5 ± 0.5	3.6 ± 0.7
11/28/08	nd	0.167 ± 0.028	1.11 ± 0.16	1.31 ± 0.21	~~0.0023~~ ± 0.0005	1.15 ± 0.18	0.0238 ± 0.0037	35 ± 14	78 ± 27	2.4 ± 0.6	3.6 ± 0.9
03/18/09	nd	0.173 ± 0.009	2.56 ± 0.50	1.54 ± 0.08	0.0043 ± 0.0013**	1.02 ± 0.10	0.0280 ± 0.0022	64 ± 20	48 ± 15	3.5 ± 0.7	2.9 ± 0.6
06/14/09	nd	n.d	n.d	n.d	n.d	n.d	n.d	88 ± 22	94 ± 29	2.7 ± 0.5	3.7 ± 0.8
11/19/09	nd	0.203 ± 0.024	2.89 ± 0.56	2.48 ± 0.67	0.0044 ± 0.0011**	2.43 ± 0.17	0.0408 ± 0.0024	25 ± 13	82 ± 31	2.2 ± 0.7	3.9 ± 0.6
03/17/10	nd	0.169 ± 0.013	1.34 ± 0.26	1.95 ± 0.23	0.0034 ± 0.0006**	1.06 ± 0.05	0.0450 ± 0.0026	72 ± 33	43 ± 23	3.7 ± 0.8	2.9 ± 0.9
06/03/10	nd	0.242 ± 0.008	2.57 ± 0.70	2.87 ± 0.33	0.0035 ± 0.0006**	1.05 ± 0.06	0.0229 ± 0.0015	86 ± 26	87 ± 30	2.9 ± 0.5	3.8 ± 0.9
11/10/10	0.049 ± 0.005	0.227 ± 0.044	1.15 ± 0.27	2.02 ± 0.51	0.0040 ± 0.0006**	1.69 ± 0.38	0.0334 ± 0.0023	19 ± 15	115 ± 38	1.6 ± 0.7	4.1 ± 0.7

Table 5
Temporal kinetics of trace element (TE) concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 – n = 30 – and 02/12/2011 – n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). * represent TE concentrations <L_Q. Mussel Condition Index values (CI ± SD; Andral et al., 2004) are also given.

Date	Al	V	Fe	Cr	Mn	Co	Ni	Cu	Zn	Se
02/11/11	155.1 ± 51.1	6.19 ± 1.72	151 ± 39	0.465 ± 0.125	14.4 ± 3.8	0.58 ± 0.12	1.23 ± 0.33	4.96 ± 1.51	65 ± 21	2.58 ± 0.78
02/12/11	230.3 ± 184.6	5.20 ± 2.29	195 ± 119	0.615 ± 0.392	10.0 ± 4.6	0.67 ± 0.24	1.54 ± 0.62	4.73 ± 1.50	78 ± 39	2.79 ± 0.77
02/14/11	30.2 ± 11.5	6.30 ± 2.32	91 ± 24	0.324 ± 0.072	5.4 ± 1.0	0.85 ± 0.31	1.65 ± 0.76	4.38 ± 0.80	104 ± 46	3.12 ± 0.38
02/16/11	29.6 ± 13.0	2.52 ± 1.28	64 ± 16	0.244 ± 0.075	4.6 ± 1.6	0.53 ± 0.19	0.74 ± 0.53	3.97 ± 0.99	69 ± 22	3.63 ± 0.95
02/19/11	96.9 ± 38.7	1.87 ± 0.77	106 ± 24	0.335 ± 0.109	6.4 ± 2.4	0.60 ± 0.19	0.72 ± 0.24	4.39 ± 1.01	88 ± 29	3.19 ± 0.63
02/22/11	36.8 ± 18.1	1.54 ± 0.67	76 ± 20	0.278 ± 0.095	4.7 ± 1.8	0.57 ± 0.23	0.62 ± 0.29	4.02 ± 1.06	96 ± 39	4.29 ± 1.01
02/25/11	78.5 ± 62.2	1.03 ± 0.35	93 ± 38	0.304 ± 0.131	5.7 ± 2.7	0.48 ± 0.23	0.53 ± 0.18	4.62 ± 1.26	89 ± 38	3.56 ± 0.90
02/28/11	69.5 ± 42.5	1.06 ± 0.25	86 ± 27	0.254 ± 0.093	5.5 ± 2.4	0.48 ± 0.16	0.55 ± 0.19	4.41 ± 1.22	84 ± 37	3.47 ± 0.91
04/04/11	159.2 ± 100.1	1.84 ± 1.23	150 ± 59	0.424 ± 0.117	4.8 ± 1.3	0.60 ± 0.19	0.90 ± 0.24	3.85 ± 0.61	77 ± 25	4.42 ± 0.88
03/07/11	37.2 ± 27.4	2.38 ± 0.71	77 ± 20	0.283 ± 0.057	4.3 ± 0.9	0.67 ± 0.22	0.87 ± 0.25	3.57 ± 0.56	86 ± 44	4.44 ± 0.95
03/11/11	38.7 ± 29.8	1.97 ± 0.41	76 ± 15	0.409 ± 0.327	4.4 ± 1.3	0.65 ± 0.24	0.80 ± 0.23	3.88 ± 0.80	90 ± 43	5.16 ± 0.88
03/14/11	62.4 ± 24.6	1.83 ± 0.37	91 ± 16	0.411 ± 0.146	4.7 ± 1.3	0.80 ± 0.26	0.88 ± 0.31	3.85 ± 0.64	107 ± 31	4.84 ± 0.88
03/28/11	64.1 ± 88.6	1.84 ± 0.46	98 ± 56	0.473 ± 0.264	4.5 ± 2.7	0.72 ± 0.28	0.86 ± 0.24	3.79 ± 0.75	143 ± 105	3.52 ± 0.62
04/06/11	41.8 ± 17.8	1.42 ± 0.27	88 ± 19	0.496 ± 0.169	4.2 ± 1.3	0.70 ± 0.32	0.79 ± 0.23	4.30 ± 0.76	120 ± 75	3.63 ± 0.42
04/12/11	51.0 ± 22.4	1.53 ± 0.48	100 ± 30	0.380 ± 0.098	4.4 ± 1.6	0.82 ± 0.19	1.02 ± 0.28	4.31 ± 0.55	103 ± 23	3.47 ± 0.65
06/04/11	21.0 ± 10.2	3.10 ± 1.50	78 ± 14	0.420 ± 0.172	2.2 ± 0.3	0.61 ± 0.19	1.00 ± 0.38	3.00 ± 0.43	112 ± 45	3.50 ± 0.41
06/21/11	41.9 ± 28.7	4.37 ± 1.29	105 ± 29	0.419 ± 0.119	2.5 ± 0.3	0.70 ± 0.18	1.02 ± 0.24	3.05 ± 0.36	135 ± 83	3.30 ± 0.40

Date	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi	CI
02/11/11	0.012 ± 0.003	0.36 ± 0.08	0.029 ± 0.013	0.011 ± 0.002	35.50 ± 5.91	22.21 ± 4.73	0.0121 ± 0.0034*	0.24 ± 0.10	0.0082 ± 0.0028	0.243 ± 0.036
02/12/11	0.013 ± 0.006	0.38 ± 0.16	0.034 ± 0.019	0.014 ± 0.005	31.12 ± 6.70	15.29 ± 6.07	0.0144 ± 0.0066	0.40 ± 0.21	0.0090 ± 0.0034	0.214 ± 0.059
02/14/11	0.021 ± 0.007	0.54 ± 0.11	0.030 ± 0.021	0.017 ± 0.003	28.37 ± 3.08	15.74 ± 3.05	0.0092 ± 0.0020*	0.61 ± 0.18	0.0152 ± 0.0127	0.128 ± 0.032
02/16/11	0.027 ± 0.011	0.58 ± 0.21	0.020 ± 0.004*	0.015 ± 0.003	25.52 ± 4.36	9.80 ± 4.73	0.0080 ± 0.0024*	0.53 ± 0.14	0.0114 ± 0.0048	0.152 ± 0.033
02/19/11	0.030 ± 0.008	0.47 ± 0.12	0.029 ± 0.008	0.018 ± 0.004	23.64 ± 3.69	7.40 ± 3.50	0.0130 ± 0.0027	0.98 ± 0.33	0.0141 ± 0.0024	0.156 ± 0.035
02/22/11	0.037 ± 0.022	0.71 ± 0.23	0.026 ± 0.010	0.018 ± 0.007	28.97 ± 2.54	7.43 ± 3.54	0.0090 ± 0.0033*	1.26 ± 0.57	0.0134 ± 0.0040	0.163 ± 0.039
02/25/11	0.030 ± 0.015	0.47 ± 0.12	0.030 ± 0.015	0.017 ± 0.007	26.81 ± 5.23	4.95 ± 1.93	0.0090 ± 0.0053*	0.69 ± 0.29	0.0144 ± 0.0046	0.225 ± 0.074
02/28/11	0.023 ± 0.008	0.46 ± 0.18	0.027 ± 0.012	0.015 ± 0.004	25.62 ± 5.61	4.70 ± 1.74	0.0107 ± 0.0054*	0.55 ± 0.18	0.0133 ± 0.0050	0.201 ± 0.053
04/04/11	0.039 ± 0.015	0.74 ± 0.18	0.046 ± 0.016	0.027 ± 0.007	27.41 ± 4.80	5.92 ± 2.21	0.0197 ± 0.0099	0.96 ± 0.37	0.0227 ± 0.0048	0.125 ± 0.029
03/07/11	0.030 ± 0.013	0.74 ± 0.18	0.017 ± 0.005*	0.018 ± 0.003	25.02 ± 2.67	6.29 ± 2.03	0.0082 ± 0.0029*	0.71 ± 0.17	0.0180 ± 0.0033	0.125 ± 0.031
03/11/11	0.035 ± 0.013	0.85 ± 0.27	0.015 ± 0.005*	0.018 ± 0.004	29.15 ± 4.76	5.09 ± 1.40	0.0085 ± 0.0026*	0.69 ± 0.08	0.0153 ± 0.0034	0.138 ± 0.040
03/14/11	0.047 ± 0.037	0.82 ± 0.20	0.028 ± 0.012	0.017 ± 0.003	25.42 ± 3.90	5.02 ± 2.12	0.0117 ± 0.0045*	0.92 ± 0.30	0.0198 ± 0.0133	0.120 ± 0.027
03/28/11	0.033 ± 0.016	0.67 ± 0.14	0.029 ± 0.014	0.025 ± 0.006	19.16 ± 1.55	3.62 ± 1.29	0.0098 ± 0.0038*	0.90 ± 0.25	0.0143 ± 0.0033	0.092 ± 0.019
04/06/11	0.030 ± 0.011	0.67 ± 0.16	0.029 ± 0.015	0.029 ± 0.014	17.68 ± 1.89	3.66 ± 1.27	0.0087 ± 0.0019*	0.74 ± 0.27	0.0137 ± 0.0029	0.091 ± 0.026
04/12/11	0.029 ± 0.006	0.72 ± 0.16	0.031 ± 0.029	0.036 ± 0.014	17.95 ± 1.72	3.64 ± 1.29	0.0119 ± 0.0064	1.14 ± 0.42	0.0179 ± 0.0041	0.077 ± 0.019
06/04/11	0.015 ± 0.006	0.75 ± 0.17	0.019 ± 0.010*	0.020 ± 0.004	20.23 ± 2.16	3.70 ± 0.86	0.0076 ± 0.0042*	1.03 ± 0.24	0.0115 ± 0.0034	0.077 ± 0.018
06/21/11	0.010 ± 0.002	0.80 ± 0.13	0.053 ± 0.087	0.034 ± 0.020	20.35 ± 2.25	2.42 ± 0.59	0.0120 ± 0.0069	1.17 ± 0.59	0.0154 ± 0.0051	0.066 ± 0.008

3. Results and discussion

For clarity purpose, only some representative examples chosen among the 19 studied TEs are graphically illustrated throughout the manuscript, and discussion mainly revolves around these selected examples. The complete set of data is however available under graphical format in the associated annexes published online alongside the electronic version of the manuscript.

3.1. An index ordering and comparing trace elements according to the overall spatial variability of their environmental levels

The 1st objective of this work aimed to test a Trace Element Spatial Variation Index (TESVI) to order and to compare TEs according to the overall spatial variability of their environmental levels throughout the whole of a studied area. This new index was calculated for the 19 TEs measured in *P. oceanica* (n = 15) sampled at 15 m depth in April 2007 in 18 sites located along the French Mediterranean littoral (diamonds in Fig. 1; Table 1; TE concentrations detailed in Luy et al., 2012). Of these 19 TEs, 12 had not been previously recorded or had little published information for *P. oceanica* (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi), contrary to the 7 remaining ones (Cr, Fe, Ni, Cu, Zn, Cd and Pb; Pergent-Martini and Pergent, 2000; Luy et al., 2012). The range of TESVI values, calculated for TEs little monitored in *P. oceanica*, went from 0.3 for Se to 12.3 for V, a range more important that the one reported for TEs classically monitored in that species (from 0.5 for Ni to 6.5 for Zn). TESVI values were listed in ascending order as: Se, Ni, Mn, Co, Sb, Ag, Be, Cu, Cd, Fe, Pb, Cr, Sn, As, Al, Zn, Bi, Mo, V. The spatial variability of TE environmental levels was then graphically compared by using a proportional ordinate (concentration) scaling between TEs (Annex A). The example given for Ni, Pb, Al and V in Fig. 2 properly demonstrates that the higher the index value (Table 1), the more environmental levels of a TE spatially varied throughout the whole of the French Mediterranean littoral. Ni levels remained similar between sites (Fig. 2a), contrary to Pb contaminations in the vicinity of big city centres such as Marseille, Villefranche or Ajaccio (Fig. 2b). The higher spatial variability of Al levels was likely related to the natural heterogeneity of sedimentary facies and not to any anthropogenic activities (Fig. 2c), contrary to V contaminations resulting from the transport, the depot and the refinery of oil products (Fig. 2d; Luy et al., 2012).

Thus, TESVI values, associated to their corresponding graphs with proportional ordinate scaling, appear to be an efficient tool to compare the overall spatial variability of TE environmental levels throughout the whole of a studied area that index is applied to. TE sorting in ascending order of their corresponding TESVI values also allows one to efficiently highlight TEs of main environmental concern, as properly illustrated in Fig. 2 for V along French Mediterranean coasts. For comparison purpose, this index was further applied at the scale of the entire Mediterranean (110 sites), monitored using *P. oceanica* as bioindicator species (Richir et al., accepted for publication). In that survey, studied TEs were sorted in ascending order of TESVI values as follows: Cd, Cu, Pb, As, Ag and Ni; the equivalent list for the French Mediterranean littoral (see above) was Ni, Ag, Cu, Cd, Pb and As. Ni therefore displayed a more important spatial variability of its environmental levels at the scale of the entire Mediterranean than at the scale of the

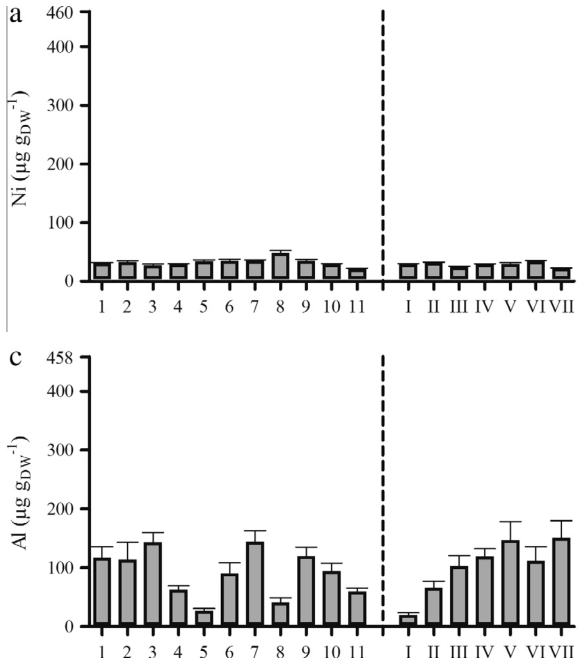

Fig. 2. Spatial overall variability of (a) Ni, (b) Pb, (c) Al and (d) V concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral and remote from one another of a few to hundreds of km (Luy et al., 2012). The graphical comparison of the overall spatial variability of trace element (TE) concentrations is based on the use of a proportional ordinate scaling between TEs, obtained by multiplying the minimum recorded mean concentration of each TE by the highest x_{max}/x_{min} mean concentration ratio (22.8 for Mo; see Table 1) calculated among the 19 studied TEs. Ni, Pb, Al and V histograms are ordered (a–d) according to the overall spatial variability of their concentrations (Trace Element Spatial Variation Index values; see Table 1) throughout the whole of the French Mediterranean littoral. Arabic and Roman numbers on the X-axis represent sites along continental Provence-Alpes-Côte d'Azur (1–11) or insular Corsican (I–VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud ; VII: Ajaccio Nord.

French Mediterranean littoral. However, since Ni contamination along French coasts was, on the whole, higher in comparison to the rest of the Mediterranean (Luy et al., 2012; Richir et al., accepted for publication), this TE could thus be regarded as a widespread contaminant of the French Mediterranean littoral. Such qualitative and quantitative information on specific threats posed by TEs may be of interest for environmental managers to design targeted monitoring surveys to precisely define their anthropogenic pollution sources. From these surveys, policy makers would have the ability to subsequently establish environmental protection measures in order to regulate their pollution inputs to coastal environments, and that at different spatial scales of protection (regional, national or international). Finally, because ranges of TESVI values were more important for TEs little monitored in *P. oceanica* compared to TEs classically monitored in that species (Table 1), and because abnormally high environmental levels of TEs previously little monitored in *P. oceanica* could be linked to specific anthropogenic activities (Mo: agriculture; Sb: mining; As: industry; V: transportation, storage and refinement of oil products; Sn, Bi, Ag: presence of important ports and urban centres; Luy et al., 2012), the list of TEs monitored along Mediterranean coasts should be broaden.

3.2. Trace element biomonitoring using P. oceanica and M. galloprovincialis

The 2nd objective of the present work was to study and compare the spatial resolution (the response sensitivity) of *P. oceanica and M. galloprovincialis* in the monitoring of environmental TE levels. Moreover, little has been published on the influence of their life styles (rooted primary producer or filter feeder) and their specific use as bioindicator species (passive – *P. oceanica* – or active – caged *M. galloprovincialis* – biomonitoring) in the modulation of their bioaccumulation behaviour when exposed to TEs.

Therefore, to meet this 2nd objective, the spatial variability in TE bioaccumulation in caged mussels (n = 48–49, except for Be: n = 24–25) immersed at 7–10 m depth from March to June 2010 in the 4 stations of Calvi Bay area (STARESO, aquaculture farm, Calvi city sewer and Punta Bianca; right crosses in Figs. 1b and 1c) was compared to the spatial variability in TE bioaccumulation in *P. oceanica* (n = 15) sampled between 13 and 22 m depth concomitantly when mussels were retrieved from water (circles in Figs. 1b and 1c). Tuckey and Dunn pairwise comparison test of means following one-way ANOVA or Kruskal-Wallis test showed 74 significant differences between stations, all TEs together, when using *P. oceanica* as bioindicator species, against 28 for *M. galloprovincialis* (Table 2; Fig. 3; Annex B). Thus, only *P. oceanica* could highlight the significant ($p < 0.05$) local impact of both Calvi city wastewater discharges and the fish farming activity on Sn (Fig. 3a) and Pb (Fig. 3b) environmental levels, the local impact of Calvi city wastewater discharges on Bi (Fig. 3c) environmental levels, or the general weak containment effect played by the Bay (lower Sn, Pb and Bi concentrations in *P. oceanica* from the Punta Bianca station). Moreover, differences in concentrations between stations were more marked when monitored in *P. oceanica* (from 7% for Sb to 954% for Al) rather than in *M. galloprovincialis* (from 7% for Zn to 120% for Al), except for Ag and Sb (Table 2). The same was true for the global TE pollution, given as Trace Element Pollution Index (TEPI) values, that differed up to two times more between stations when calculated for *P. oceanica* (38%) rather than for *M. galloprovincialis* (20%), respectively. This comparison further shows the relevancy of the TEPI to compare global TE pollution levels of monitoring surveys relying on the use of different bioindicator species.

Caged *M. galloprovincialis*, as filter feeders artificially maintained in the water column, bioaccumulated dissolved and particulate TEs. The more similar levels of bioaccumulated TEs in that

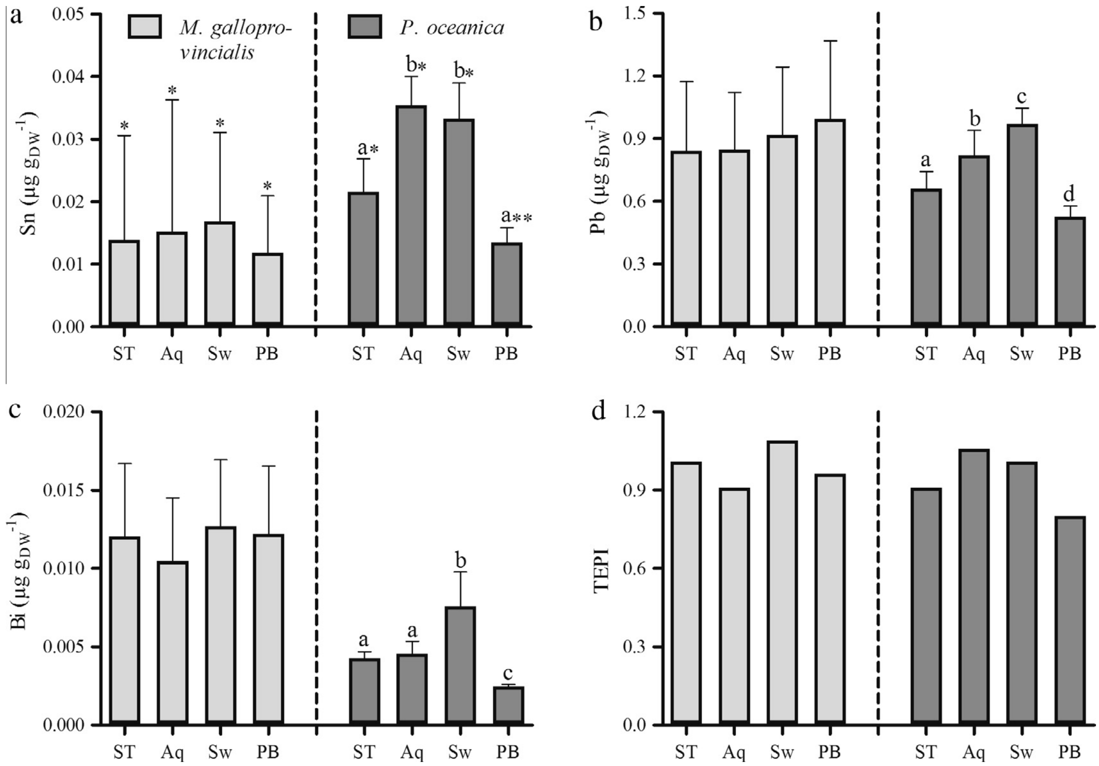

Fig. 3. Spatial variability of (a) Sn, (b) Pb and (c) Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* ($n = 48$–49) purchased from the Diane salty pond (eastern Corsica, France) and immerged between 7 and 10 m depth from March to June 2010 in 4 stations in Calvi Bay area (northwestern Corsica, France), and in *P. oceanica* ($n = 15$) sampled between 13 and 22 m depth in June 2010 concomitantly when mussels were retrieved from water. (d) Trace Element Pollution Index (TEPI) values were calculated for each station and bioindicator species from mean normalized concentrations of the 19 studied trace elements. The 4 sampled stations STARESO (ST), aquaculture farm (Aq), Calvi city sewer (Sw) and Punta Bianca (PB) were remote from one another by a distance of 1 to 3 km. Letters represent significant differences between stations for each bioindicator species. * and ** represent Sn concentrations $<L_Q$ or $<L_D$, respectively.

bioindicator species between stations reflected the relatively clean homogenous status of the water body of Calvi Bay area. Conversely, *P. oceanica*, as rooted organisms, bioaccumulated dissolved and sedimentary TEs. TEs, as non-degradable pollutants, accumulate in sediments (Navratil and Minarik, 2011; Pan and Wang, 2012); it is consequently possible to highlight long-term contaminations from weak point sources (*e.g.* fish farming activities, wastewater discharges) when using benthic organisms, even at the scale of a Bay. Results from the biomonitoring survey carried out in May 2010 along the Ajaccio Bay radial (western Corsica, France) adequately demonstrated the high sensitivity of *P. oceanica* to fine spatial scale monitoring mapping. Results showed that the more stations randomly sampled at 8–9 m depth ($n = 9$–10) were remote from the port and urban centre of Ajaccio city (from station A1 to station A9; squares in Fig. 1d), the more concentrations of numerous TEs decreased in seagrasses (Table 3). That pollution gradient along the Ajaccio Bay radial was further compared to the pollution recorded in the 18 sites sampled for *P. oceanica* ($n = 15$) at 15 m depth in April 2007 along the French Mediterranean littoral (TE concentrations detailed in Luy et al., 2012; Annex C), as shown in Fig. 4 for Fe and Bi concentrations as well as TEPI values.

Environmental Fe levels monitored in *P. oceanica* sampled along French Mediterranean coasts were low to very low (Fig. 4a′), for a mean concentration (104 ± 39 µg g_{DW}^{-1}) close to the critical value of 100 µg g_{DW}^{-1} of Fe-deficient seagrasses sampled from Fe-poor ecosystems (Duarte et al., 1995). The spatial variability observed at the scale of the French Mediterranean littoral could therefore be considered as an environmental heterogeneity inherent to sampled sites (Luy et al., 2012), such as the presence of carbonate-rich and Fe-poor sediments (Duarte et al., 1995; Marbà et al., 2007). In contrast, the significant ($p < 0.05$) rapid decrease of relatively high to below 100 µg g_{DW}^{-1} Fe levels with increasing distance from the port and urban centre of Ajaccio city (Fig. 4a″) likely highlighted its local impact at short distance. Bi contaminations were reported close to medium (*e.g.* Ajaccio city) to large-sized (*e.g.* Marseille city) urban centres (Fig. 4b′; Luy et al., 2012). Bi has been considered as a non-toxic replacement for other more noxious elements, particularly Pb (Filella, 2010). Bi concentrations measured in *P. oceanica* sampled along the Ajaccio Bay radial (Fig. 4b″) significantly ($p < 0.05$) decreased with increasing distance from their emission sources (port and urban centre of Ajaccio city), from contamination levels even higher than those reported by Luy et al. (2012) for the contaminated Plateau des Chèvres site (receiving wastewater from Marseille city; diamond 4 in Fig. 1a; Oursel et al., 2013) to relatively moderate ones. Furthermore, Bi concentrations in stations A6 to A9 (squares in Fig. 1d) were similar to levels of moderately contaminated Ajaccio Sud and Ajaccio Nord sites (diamonds VI and VII in Fig. 1a; Luy et al., 2012), remote of around 5 km of the radial. It can thus likely be assumed that Bi diffusely contaminated the overall Ajaccio Bay from specific punctual sources. The evolution of Fe and Bi levels was further consistent with the evolution of the global TE contamination (TEPI values; Fig. 4c″) along the Ajaccio Bay radial: it exponentially decreased with increasing distance from the port and urban centre of Ajaccio city. But TEPI histograms mainly highlighted that the global contamination of coastal environments with TEs, when monitored in rooted *P. oceanica* randomly sampled within areas of minimum 25 m^2, can varies as much at small spatial scale (*e.g.* a 116% variability in the Ajaccio Bay; Fig. 4c″) than at large spatial scale (*e.g.* a 85% variability at the scale of the French Mediterranean littoral; Fig. 4c′).

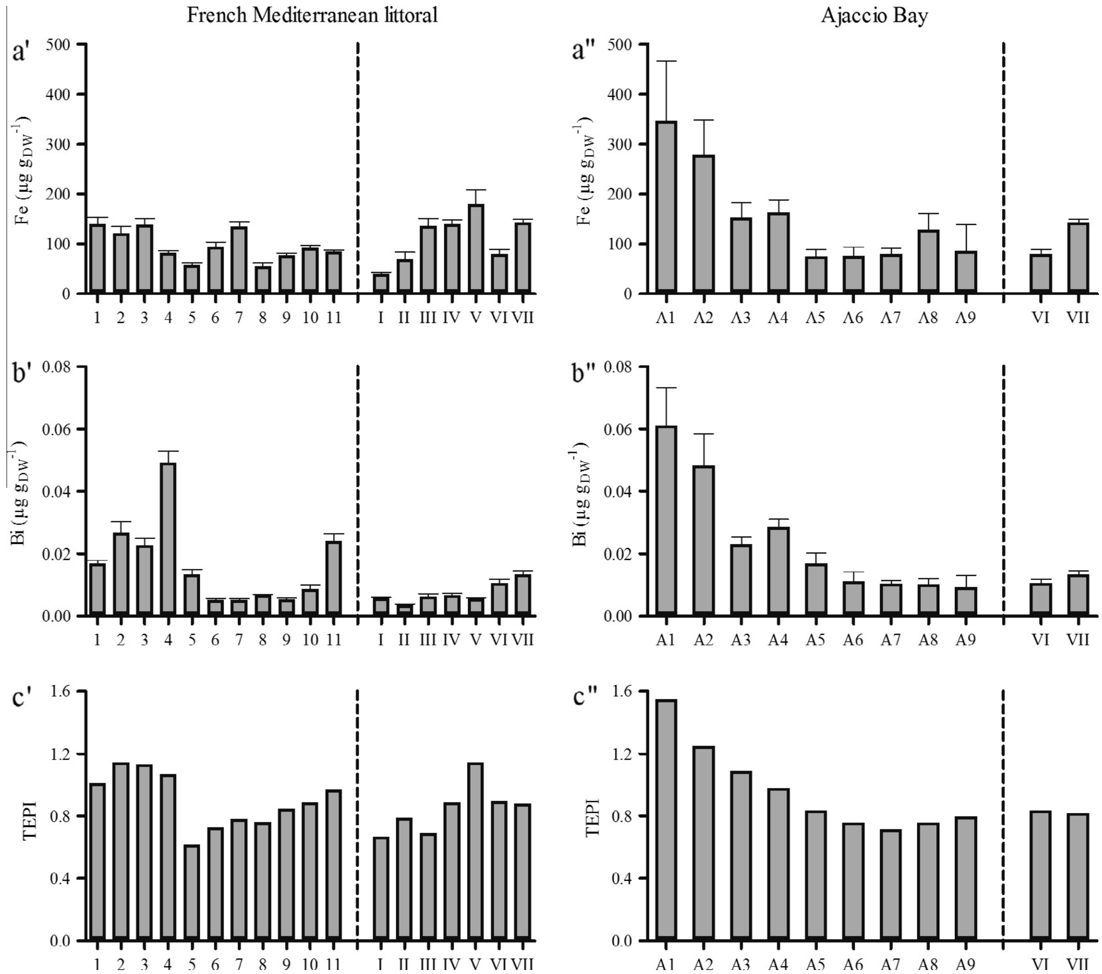

Fig. 4. Spatial variability of (a) Fe and (b) Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (a′, b′; *n* = 15; Luy et al., 2012) and in *P. oceanica* sampled at 8–9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; a″, b″; *n* = 9–10). Trace Element Pollution Index (TEPI) values (c′, c″) were calculated by site and station from mean normalized concentrations of the 19 studied trace elements. Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1–11) or insular Corsican (I–VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud ; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial.

The choice of representative sampling stations for a given site/area may consequently remain uncertain, all the more if the selected bioindicator is associated to sediments (*e.g.* seagrasses, but also naturally occurring benthic native Mytilidae populations) whose TE contents can significantly vary at small spatial scales in both pristine or contaminated sites (Birch et al., 2001; Scouller et al., 2006). However, benthic bioindicator such as rooted *P. oceanica* present the added benefit of allowing the fine-spatial scale mapping of coastal TE pollutions, since sediments offer a degree of time integration, contrary to the water column (Rainbow, 1995; Amiard, 2011). Since caged *M. galloprovincialis* can globally characterize the contamination status of a water body (*e.g.* a Bay) with less spatial variability (Fig. 3), this bioindicator could be preferred in large scale active monitoring surveys (Andral et al., 2011; Benedicto et al., 2011). If *P. oceanica* is nevertheless elected as bioindicator in large scale monitoring surveys (Salivas-Decaux et al., 2010), care will be taken when selecting sampling stations (*i.e.* remote from any punctual sources of contamination) in order not to overestimate the pollution status of areas *P. oceanica* will be sampled from (Figs. 3 and 4).

3.3. Trace element kinetics in P. oceanica and M. galloprovincialis

The 3rd objective of the present work aimed to study and compare TE kinetics in *P. oceanica* and *M. galloprovincialis*, with regard to their ecophysiology. *P. oceanica* biomass, foliar surface and leaf length are maximum in summer and minimum in winter. New juvenile leaves appear throughout the year, but a larger number of leaves are initiated from the end of September until November. Old adult leaves become necrotic throughout the year, but severe storms occurring in the winter favour their fall (Pergent and Pergent-Martini, 1991; Gobert, 2002). Sampling campaigns scheduled at the different key phases of *P. oceanica* growth cycle could give a good overview of the natural overall variation of TE concentrations in shoots, on annual basis. Such a seasonal monitoring survey was thus performed on *P. oceanica* (*n* = 15) sampled at 10 m depth in March, June and November of years 2008–2010 in the pristine seagrass bed in front of the oceanographic station STARESO (Luy et al., 2012; Richir et al., 2013), in the Calvi Bay (triangle in Fig. 1c). Results showed that TE concentrations varied seasonally according to the ecophysiological cycle of that perennial,

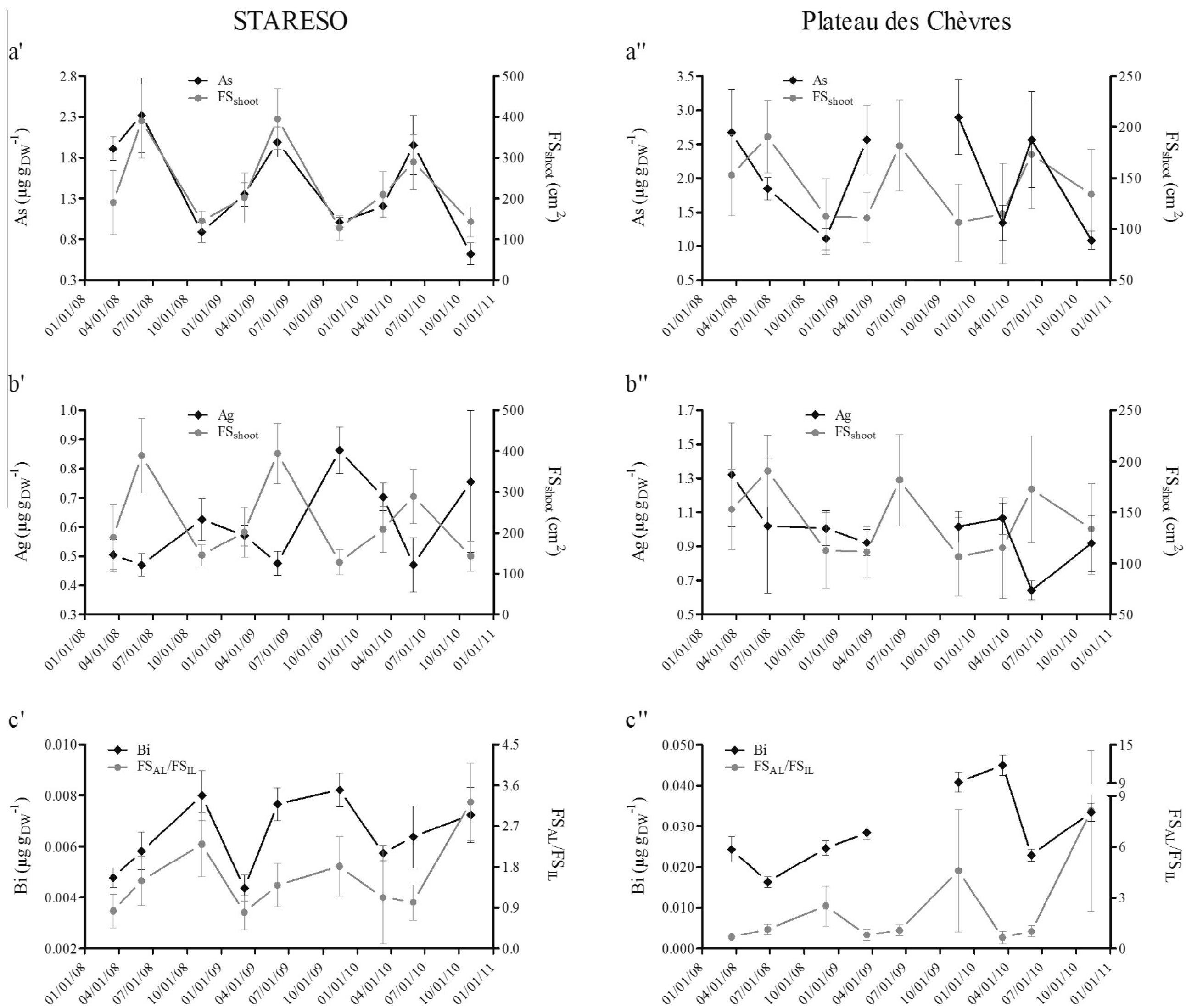

Fig. 5. Seasonal and pluriannual kinetics of (a) As, (b) Ag and (c) Bi concentrations (mean ± SD, in μg g^{-1}_{DW}) in *P. oceanica* ($n = 15$) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs a′–c′) and in the impacted seagrass bed of Plateau des Chèvres (Marseille, France; right graphs a″–c″), compared to the seasonal variation of shoot metrics. The foliar surface of shoots (FS_{Shoot}; mean ± SD, in cm^2) is an indicator of the cyclic growth of leaves. The ratio between the foliar surface of adult leaves and the foliar surface of intermediate leaves (FS_{AL}/FS_{IL}; mean ± SD) is an indicator of the cyclic aging of shoots. The sampling from June 2009 is missing for Plateau des Chèvres site. As *P. oceanica* leaf and shoot foliar surfaces did not significantly ($p < 0.05$) differ between June 2008 and 2010 in Plateau des Chèvres site, they were averaged to calculate the missing mean foliar surfaces of June 2009.

deciduous plant (Table 4; Annex D), as illustrated in Fig. 5 for As, Ag and Bi. As concentrations (Fig. 5a′) increased from November to June, as did the shoot foliar surface (an indicator of the cyclic growth of leaves): the more leaves grew, the more their exposure duration to As increased, the more the latter was bioaccumulated in shoots (Campanella et al., 2001; Luy et al., 2012; Cozza et al., 2013), and this until the next major leaf fall period. Ag is 7 times more concentrated in rhizomes than in leaves, on annual basis, and is also efficiently translocated from leaves towards rhizomes (Richir et al., 2013). This tissue distribution pattern could explain the decrease of Ag foliar concentrations, continuously transferred to rhizomes from November to June whereas the shoot foliar surface increased (Fig. 5b′). Bi levels are 1.7 times more concentrated in *P. oceanica* adult leaves than in intermediate leaves, on annual basis (Richir et al., 2013), as a result of both a longer exposure duration of old adult leaves to Bi (Campanella et al., 2001; Luy et al., 2012; Cozza et al., 2013) and a dilution effect in actively growing young intermediate leaves (Malea et al., 1994; Luy et al., 2012). Bi concentrations (Fig. 5c′) consequently increased from March to November following the trend of the ratio between adult and intermediate leaf foliar surfaces (an indicator of the cyclic aging of shoots). The winter drop in Bi concentrations corresponded to the renewal of leaves.

In an environment subjected to anthropogenic disturbances, these natural seasonal trends can however be deeply perturbed (Malea et al., 1994). Therefore, for comparison purpose with the seasonal monitoring survey performed in the pristine STARESO seagrass bed, an equivalent study was conducted in the impacted site of Plateau des Chèvres (diamond 4 in Fig. 1a), remote of around 3.5 km from the Cortiou outlet discharging TE contaminated wastewater from Marseille city (Oursel et al., 2013). *P. oceanica* ($n = 15$) were similarly sampled at 10 m depth in March, June and November of years 2008–2010 (except June 2009). In the impacted Plateau des Chèvres site, the seasonal cycle of numerous TE concentrations in *P. oceanica* did not follow the ecophysiological cycle of the plant (Table 4; Annex D), as illustrated in Fig. 5 for the 3 urban and industrial pollutants As, Ag and Bi. As concentrations (Fig. 5a″) showed a saw-tooth profile incompatible with the one of the plant growth cycle. Ag concentrations (Fig. 5b″) remained stable from June 2008 to March 2010, while Bi concentrations (Fig. 5c″) continuously increased during the same period of time; both TE concentrations dropped in spring 2008 and 2010. Thus, although TE kinetics in *P. oceanica* are naturally prone to temporal variations linked to the cyclic evolution of the plant leaf growth (*e.g.* STARESO station; Figs. 5a′–5c′), these natural variations can be hidden when seagrasses are exposed to environmental TE pollution sources (*e.g.* Plateau des Chèvres site receiving TE contaminated wastewater from Cortiou outlet; Figs. 5a″–5c″; Oursel et al., 2013).

Complementary to this field study, TE kinetics in *P. oceanica* exposed to contaminated seawater were experimentally monitored in 3 previous surveys. Ledent et al. (1992a, b) *in situ* contaminated *P. oceanica* with environmentally irrelevant high Cd levels, and so demonstrated that *P. oceanica* bioaccumuled this metal proportionally to experimental levels of exposure in a wide range of high to very high concentrations. Efficient TE uptakes were also reported by Warnau et al. (1996) who exposed *P. oceanica* acclimatized to laboratory conditions to a mix of radiolabelled Zn, Ag, Cd, Cs, and Am in concentrations 1–5 orders of magnitude lower than concentrations commonly reported for natural seawater. Recently, Richir et al. (2013) *in situ* experimentally exposed *P. oceanica* bed portions to environmentally relevant levels of a mix of 15 TEs (Cr, Fe, Co, Ni, Cu, Zn, Cd, Pb, Al, V, Mn, As, Mo, Ag and Bi). They reported that TE uptake kinetics depended of the nature of TEs (essential or not), of their concentrations, of interactions between TEs used as multielement solutions and of the duration of exposure times. Although *P. oceanica* leaves depurated rapidly once initial conditions were restored (Ledent et al., 1992a,b; Warnau et al., 1996; Richir et al., 2013), their short-term exposures (24 h or 5 days; Richir et al., 2013) to some TEs (*i.e.* Al, V, Mn, Cu, Cd, Bi, Zn, Ag) could nevertheless be recorded in below-ground rhizomes. This basipetal translocation of TEs from leaves towards rhizomes (superficial sediments in experimental setups had remained uncontaminated; Richir et al., 2013), recently evaluated by Sanz-Lazarro (2012) in the cycle of TEs within *P. oceanica* meadows, had to be fast and efficient (Richir et al., 2013; basipetal term of line 13, paragraph 2, section 4.4 and acropetal term of line 13, paragraph 2, section 4.5 of their manuscript must be inversed). *P. oceanica* below-ground tissues can thus effectively be used to bioassess the past pollution of coastal Mediterranean waters with TEs (Pergent-Martini and Pergent, 1994; Tranchina et al., 2005; Copat et al., 2012). However, the necessary destructive uprooting of *P. oceanica* raises again the question of its status of protected species (Boudouresque et al., 2006; Montefalcone et al., 2007).

Like *P. oceanica*, TE bioaccumulation in caged *M. galloprovincialis* relies on the ecophysiological status of mussels and ambient bioavailable TE levels (Casas and Bacher, 2006; Casas et al., 2008). To study these kinetics in caged mussels, rope grown *M. galloprovincialis* were purchased from the Diane pond (bold-thick right cross in Fig. 1a) on February 11, 2011, detached from ropes and placed in man-made pouches on February 12, left to acclimatize and to fix to pouches in the marina of the oceanographic station STARESO until February 14 and then placed on site (leaning cross in Fig. 1c) and regularly sampled until June 21 ($n = 12$, except for samplings dates 02/11/2011 – $n = 30$ – and 02/12/2011 – $n = 44$). The initial evolution of TE concentrations in mussels transferred from the productive Diane pond (Richir and Gobert, 2014) to the oligotrophic Calvi Bay (Richir et al., 2012) was typically asymptotic (*e.g.* V and Bi in Figs. 6a and 6b; Annex E), suggesting equilibrium between TE concentrations in mussels and water as previously reported by Casas and Bacher (2006) and Casas et al. (2008) for Hg, Cd, Pb, and Cu. Within 2 weeks (by February 25), TE concentrations in caged *M. galloprovincialis* had reached a new steady-state. In this new steady-state, bioaccumulation should be regarded as a stationary process, TE concentrations in mussel flesh being in pseudo-equilibrium with TE loads of their surrounding environment (Casas and Bacher, 2006; Casas et al., 2008). In addition to these general patterns, abrupt changes in flesh weight linked to the emission of gametes (Cossa, 1989; Richir and Gobert, 2014) were reported to provoke steep increases in TE concentrations at spawning time (Casas and Bacher, 2006; Casas et al., 2008). A first drastic mussel weight loss (*i.e.* CI dropping of 02/14/2011; Fig. 6; Table 5) likely linked to mussel gamete emission was measured after mid-February initial handling stress when mussels were detached from ropes, leading to the concentration peak observed for most TEs in mussels' remaining soft tissues (*e.g.* V and Bi in Figs. 6a and 6b; Table 5; Annex E). Indeed, physical stimulation by scraping the shell or cutting the byssus threads when detaching and sorting mussels prior to filing pouches may have stimulated mussel spawning (Gosling, 2003). The second mussel weight loss (*i.e.* CI dropping of 03/04/2011; Fig. 6; Table 5), and the resulting second TE concentration peak effect (*e.g.* V and Bi in Figs. 6a and 6b; Table 5; Annex E), was likely linked to a natural occurring spawning event. No further obvious increase of TE concentrations in mussels was observed during the rest of the caging experiment, except for V (Fig. 6a). This spring rise could presumably be linked to the resumption of the touristic period, the main notorious anthropogenic activity likely to contaminate the Calvi Bay (Vermeulen et al., 2011), and the consequent increase of marine shipping and recreational activities recognized as a major source of V pollution (Amiard et al., 2008; Pey et al., 2013). Finally, concentrations of essential Cu (Fig. 6c), Co, Se and Zn, as well as non-essential As (Table 5; Annex E), remained proportionally relatively constant during the overall deployment period of caged mussels. The temporal constancy of these 4 essential micronutrients could be indicative of a strong physiological regulation of their internal levels, as suggested by Richir and Gobert (2014) who observed similar Cu, Co and Zn contents in *M. galloprovincialis* having spawned or close to spawn and sampled at a 1-year time interval (physiological and temporal constancy).

TE uptake and loss kinetics in *P. oceanica* and *M. galloprovincialis* are thus under the influence of various parameters that interact such as environmental TE loads, the nature of TEs or the physiological status of bioindicator species. For *M. galloprovincialis*, a set of consensual protocol tools allow to compare results between sites and studies, *e.g.* adjusting TE concentrations according to the trophic heterogeneity of immersion sites, performing caging experiments during the period of sexual dormancy, or using homogenous starter batches of calibrated individuals (Andral et al., 2011; Benedicto et al., 2011), although this last consideration can be minimized (Saavedra et al., 2004; Richir and Gobert, 2014). In contrast, for *P. oceanica*, no general rule prevails, and shoots are collected at any time of the year without considering the seasonality and the small spatial scale sensitivity of this bioindicator species. However, Pergent-Martini and Pergent (2000) had already pointed out the importance of that seasonality and present results confirmed their statement. Malea et al. (2013), who recently seasonally monitored TE concentrations in the seagrass *Cymodocea nodosa* during one year, concluded the same. Consequently, scientists should develop consensual monitoring protocols in order to improve the use of seagrasses as bioindicators of the coastal pollution, as is the case for *M. galloprovincialis*.

3.4. Trace element compartmentalization in P. oceanica and M. galloprovincialis

Although we have extensively discussed different aspects of the biomonitoring of TEs using entire *P. oceanica* shoots of leaves or the

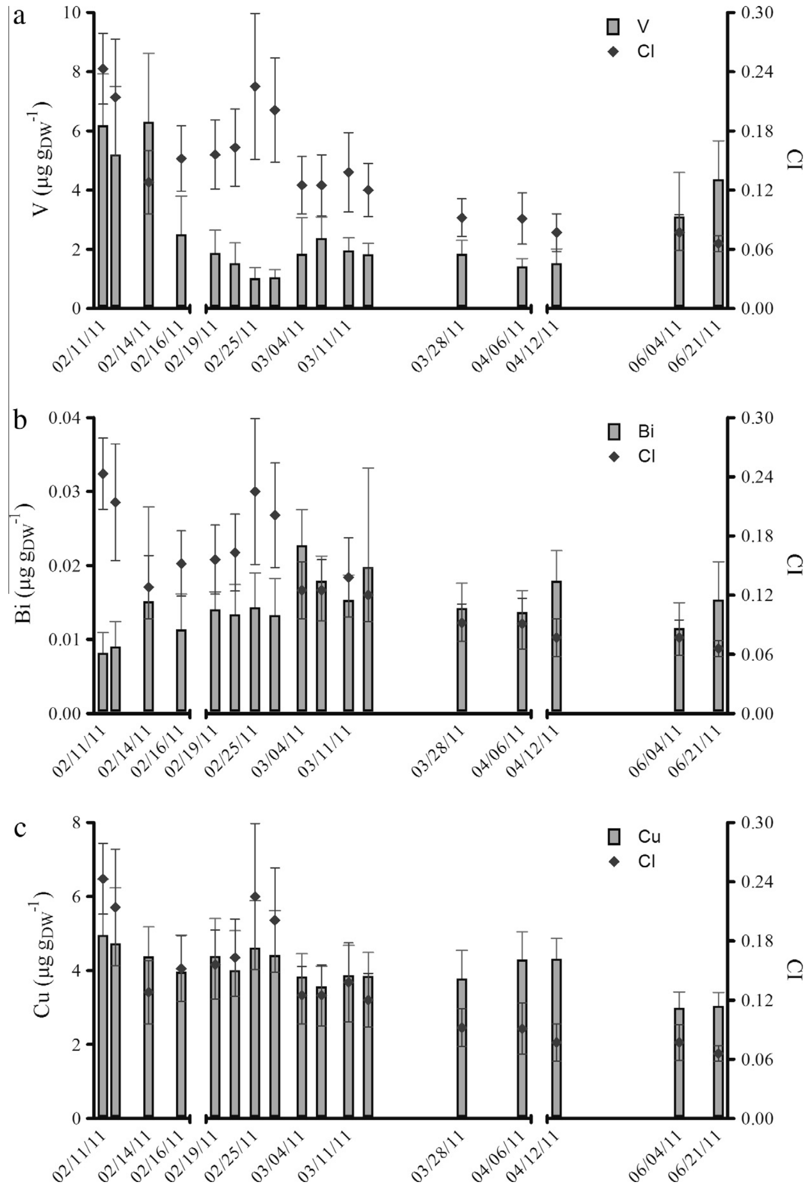

Fig. 6. Temporal kinetics of (a) V, (b) Bi and (c) Cu concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in caged rope-grown *M. galloprovincialis* ($n = 12$, except for sampling dates 02/11/2011 – $n = 30$ – and 02/12/2011 – $n = 44$) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). Mussel Condition Index values (CI ± SD; Andral et al., 2004) are also given. For clarity purpose, one in two sampling date is reported on the left half of the X-axis.

entirety of *M. galloprovincialis* body flesh, some previous studies have in contrast worked on specific body compartments (Adami et al., 2002; Roméo et al., 2005; Romero et al., 2007a, 2007b; Salivas-Decaux et al., 2010). The 4th objective of the present work therefore aimed, based on the compilation of observations reported in other studies, to discuss the reliability of using specific compartments of these two bioindicators instead of entire individuals in monitoring surveys, with regards to species-specific TE body distribution and compartment kinetics.

In *P. oceanica* monitored on annual basis, Richir et al. (2013) showed that TEs were either preferentially accumulated in above-ground shoots of leaves (*e.g.* As, V, Mn), either in below-ground rhizomes (*e.g.* Al, Fe, Ni) or indistinctly in above- and below-ground tissues (*e.g.* Cr, Cu, Mo). TE concentrations further differed between intermediate younger leaves and adult senescent ones, as a result of a longer exposure of adult leaves to ambient TEs (Campanella et al., 2001; Luy et al., 2012; Cozza et al., 2013), a higher retention rate of TEs in adult leaves (Warnau et al., 1996; Richir et al., 2013) and the dilution of accumulated TEs in actively growing intermediate leaves (Malea et al., 1994; Luy et al., 2012). In contrast, when *P. oceanica* shoots were experimentally contaminated with TEs, physiologically more active intermediate leaves took up most TEs more rapidly than adult leaves (Warnau et al., 1996; Richir et al., 2013). Both leaf types decontaminated rapidly when initial conditions were restored (Warnau et al., 1996; Richir et al., 2013), although some results showed that *P. oceanica* contaminated with radiotracers at levels several orders of magnitude lower than TE concentrations commonly reported for natural seawater could retained ^{110m}Ag, ^{134}Cs and ^{137}Cs for longer time (Calmet et al., 1991; Warnau et al., 1996). TE concentrations also varied along the same leaf, from its base to its tip (Campanella et al., 2001; Luy et al., 2012; Cozza et al., 2013), and accumulated TEs could further undergo redistribution processes between *P. oceanica* compartments (Richir et al., 2013). TE compartmentalization could also vary according to the contamination status of studied sites (Luy et al., 2012; Richir, 2012), but could also vary between reference sites (Richir et al., 2013) and with depth within the same reference site (Richir et al., unpubl. data).

Furthermore, for analytical or anatomical reasons, no compartment of *P. oceanica* is fully satisfactory for the monitoring of TE pollution. If *P. oceanica* 3rd intermediate leaf was shown to be globally representative of entire shoots (Luy et al., 2012), this leaf is however not systematically present all year round, as in summer when spring young intermediate leaves have aged to give adult leaves. Romero et al. (2007a, 2007b) selected, in a preliminary study, the 2nd youngest *P. oceanica* leaf instead of the 3rd one (juvenile leaves excluded) to measure physiological metrics of their environmental quality POMI index, of which TEs (Martínez-Crego, 2005; Martínez-Crego et al., 2008); but that tissue was regarded as not having had sufficient time to accumulate enough TEs (Martínez-Crego, pers. com.). Moreover, TE concentrations in *P. oceanica* compartments can evolve seasonally, as for *Cymodocea nodosa* (Malea et al., 2013), and that seasonality can differ from that of entire shoots (Richir et al., unpubl. data). As regards *P. oceanica*, the definition of water quality index based on different metrics of that protected species must further evolve toward non-destructive methods (Montefalcone, 2009). As an example, the non-destructive index of Gobert et al. (NDSM: Non Destructive Shoot Method; 2012) requires the measurement of several metrics on entire shoots of leaves cut close to their base to ensure their regrowth. When special care was given to the sampling, the storage and the processing of samples, it was possible to retrieve these shoots of leaves for further chemical analyses (*e.g.* TEs; Gobert et al., unpubl. data). Such an approach not only reduces the required amount of collected material, but also allows the optimization of sample preparatory work. From these observations, it can be concluded that the measurement of *P. oceanica* anatomical and physiological metrics, of which TEs, in entire shoots of leaves (cut with scissor and not uprooted) could be the most appropriate approach when using that species to biomonitor the health status of the coastal Mediterranean.

In *M. galloprovincialis*, there is a well-marked compartmentalization of TEs between organs, most of them being preferentially concentrated in the hepatopancreas (Richir and Gobert, 2014). The analysis of this organ has therefore been specifically privileged in some monitoring surveys (Adami et al., 2002; Gupta and Singh, 2011). Richir and Gobert (2014) reported that TE distribution remained similar between individuals sampled before or after spawning, from one year to the other. This conservative character of TE compartmentalization (physiological and temporal constancy) must imply the internal regulation of their levels and a quantitative redistribution between tissues (Gabbott, 1975; Lobel and Wright, 1982). TE concentrations are further lower in the mantle (Richir and Gobert, 2014) where the gonad follicles are dispersed (Torrado and Mikhailov, 2000). Caging experiments scheduled with regular samplings showed that when *M. galloprovincialis* spawned, most TE concentrations underwent a short-time increase in mussel flesh to recover thereafter a pseudoequilibrum with TE loads of their surrounding environment (Table 5; Figs. 6a and 6b; Annex E; Casas and Bacher, 2006; Casas et al., 2008). TEs must consequently be less concentrated in the reproductive material (Casas et al., 2008). Because of these important regulatory processes within the mantle, this tissue will not be used in biomonitoring surveys, and biomonitoring surveys will be performed with individuals in sexual dormancy (Andral et al., 2004).

Mytilus spp. are important shellfish products, which therefore raises certain health issues of food security (Stankovic and Jovic, 2012). In both passive (*e.g.* the Mussel Watch program in the USA: Goldberg, 1975; the RNO program in France: Chiffoleau et al., 2005) and active (*e.g.* RINBIO and MYTILOS programs in the Mediterranean: Andral et al., 2004; Benedicto et al., 2011) biomonitoring surveys, analysed *Mytilus* spp. may come from sites designated for the production of shellfish products (*e.g.* the Diane pond; Fig. 1a). In a risk assessment approach, it is the duty of ecotoxicologists to provide a maximum of relevant information on the incurred risks by the consumption of such products. Thus, regarding *M. galloprovincialis* purchased from the shellfish farm SARL Etang de Diane, Cd and Pb levels were reported to be well below the phytosanitary standards of 1 $\mu g\ g^{-1}$ of mussel fresh weight (Richir and Gobert, 2014), as defined by the European Union (EC, 2001). Because of this phytosanitary aspect, entire mussels should preferentially be analysed in biomonitoring surveys. In addition, existing TE kinetic models (*e.g.* Casas and Bacher, 2006; Casas et al., 2008) apply to entire *M. galloprovincialis*, not to specific organs. From these observations, it can be concluded that the health status of the coastal Mediterranean should be monitored in entire mussels.

4. Conclusion

The calculation of Trace Element Spatial Variation Index (TESVI) values, combined to the comparative graphical representation of TE concentrations by using a proportional ordinate (concentration) scaling, appeared to be an efficient tool to order and to compare TEs according to the overall spatial variability of their environmental levels throughout the whole of a studied area. The complementary calculation of weighted Trace Element Pollution Index (TEPI) values further allowed one to accurately compare the global TE pollution between monitored sites, whatever the bioindicator species considered. The calculation of TESVI values also highlighted that the overall spatial variability of TEs of previous little environmental concern could be higher than that of TEs classically biomonitored. The corresponding abnormally high concentrations of these contaminating TEs could further be linked to specific anthropogenic activities. In addition, the more the number of TEs being studied increases, the more TEPI values are probative. For these reasons, the list of Cr, Ni, Cu, Zn, Cd, Pb and Hg classically monitored along Mediterranean coasts should be broaden.

P. oceanica and *M. galloprovincialis* efficiently bioaccumulated TEs from their environment. If *M. galloprovincialis* appeared to be a good indicator of the overall quality of a water body, *P. oceanica* also allowed the fine-spatial scale mapping of the coastal pollution, since this rooted organism reflected the long-term integration of weak pollution sources in sediments. As both species are relevant bioindicators, and because they complement one another, they could consequently be used concomitantly to biomonitor the coastal pollution of the Mediterranean with TEs.

Both bioindicators rapidly equilibrated with TE loads of their ambient environment; both bioindicators therefore properly reflected the contamination status of their sampling environment within days to weeks, depending on the nature and kinetics of each TE. But because of this fast balancing, some punctual pollutions of importance could be missed. Although the efficient basipetal translocation of TEs from contaminated *P. oceanica* leaves towards rhizomes could record these punctual events, the necessary destructive uprooting of that protected species should limit below-ground organs sampling to specific case studies.

The bioaccumulation behaviour of both species was influenced by their biological cycle. In *M. galloprovincialis*, the gametogenic cycle played an important role by concentrating TEs in spawning individuals. For *P. oceanica*, the seasonal aging of their deciduous leaves modulated TE concentrations within shoots. A consensual use of these bioindicators is thenceforth essential to furnish relevant and comparable information. If this is largely the case for *M. galloprovincialis*, no common rule prevails for *P. oceanica*.

Body compartments of both species accumulated more or less TEs according to their age, their function, their exposure to TEs, etc. It can therefore be interesting to study the compartmentalization of TEs to better understand their dynamics and physiological regulation within organisms. However, none of these compartments properly reflected the bioaccumulation behaviour of entire organisms. Furthermore, detailed kinetic models incorporating environmental variables, which in return modulate TE bioaccumulation processes in organisms, are designed for entire individuals, as is the case for *M. galloprovincialis*. For these reasons among others, the monitoring of the coastal Mediterranean should therefore be performed in entire organisms.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.08.030.

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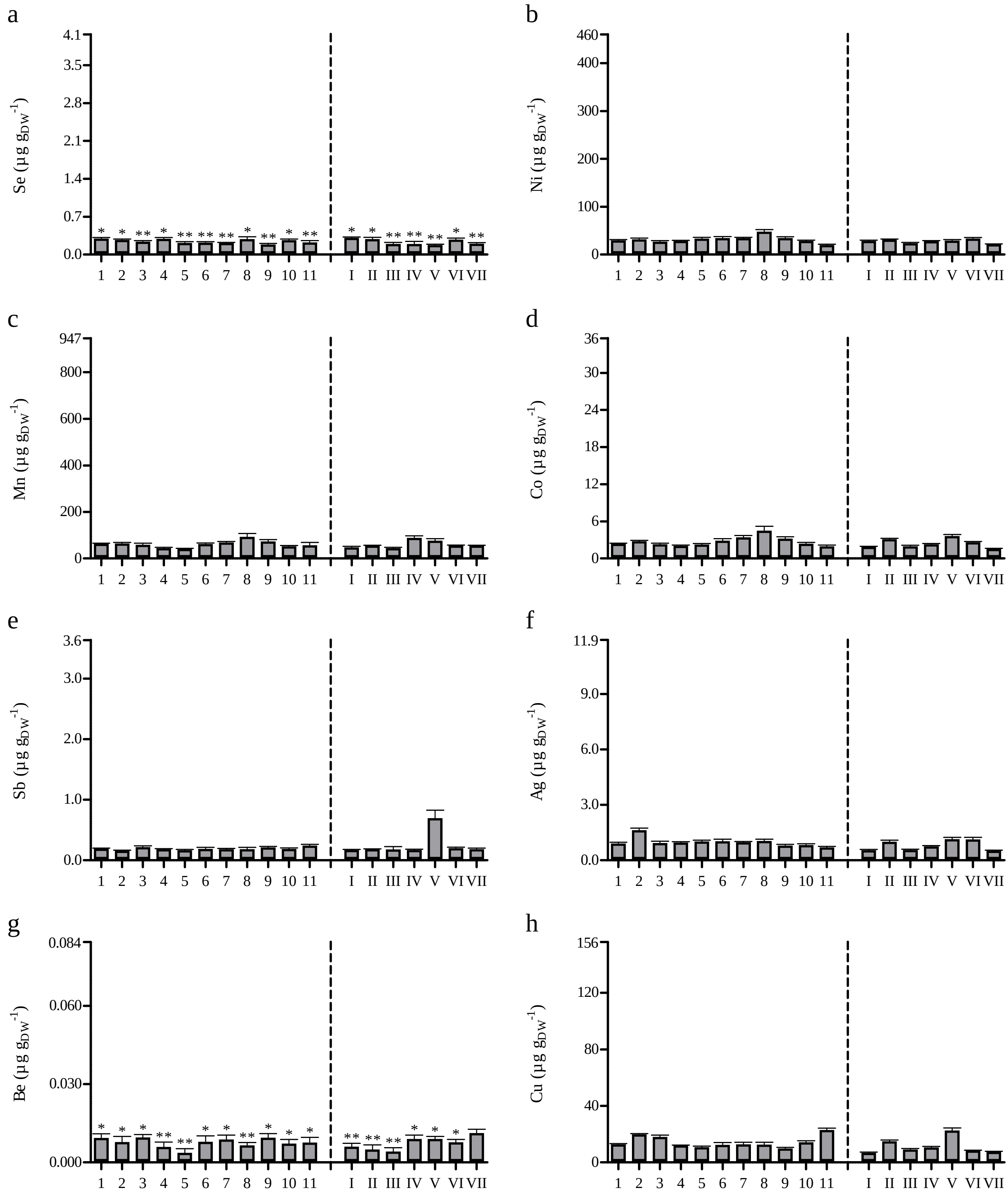
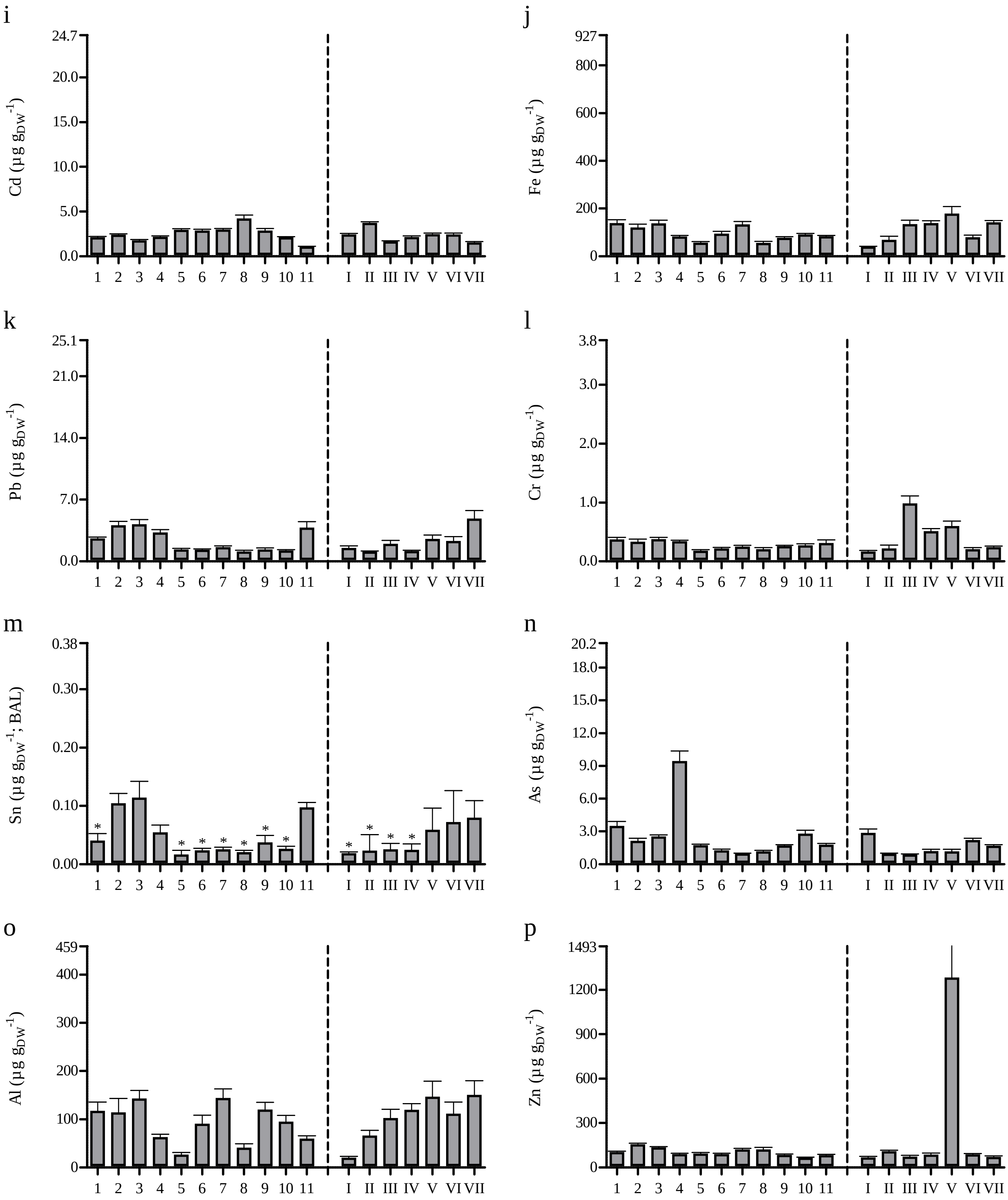
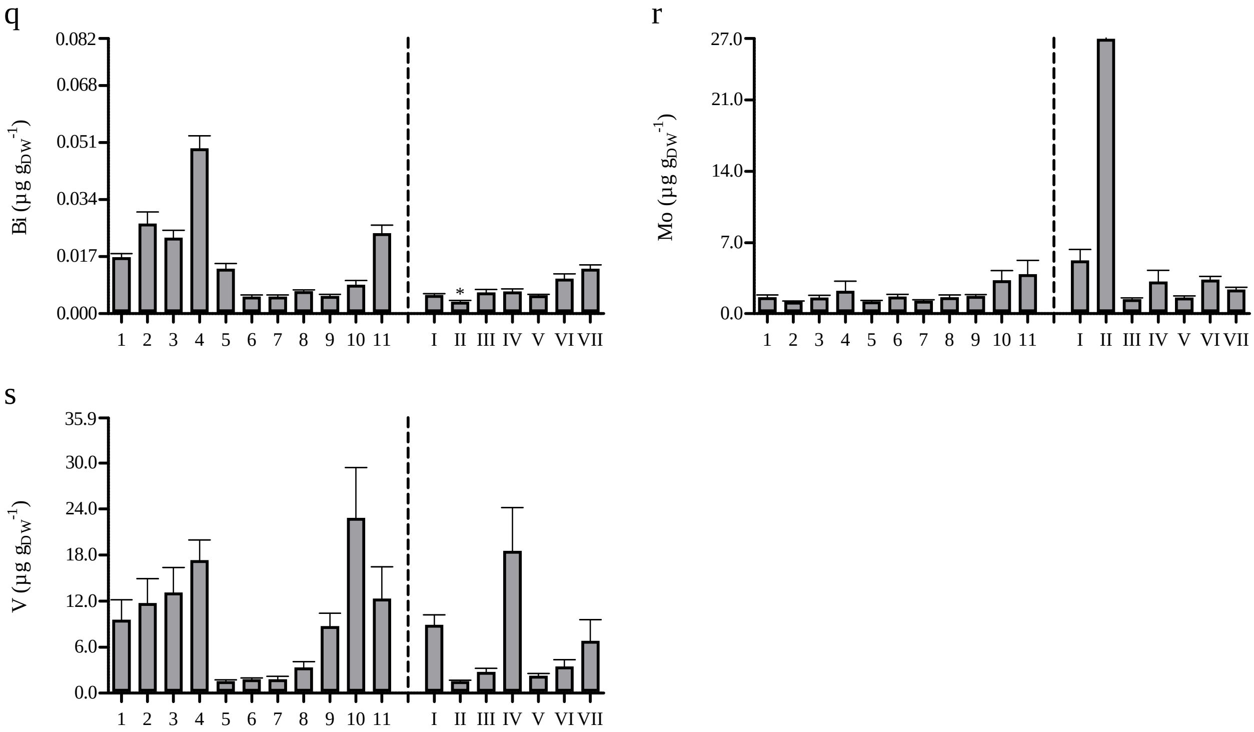


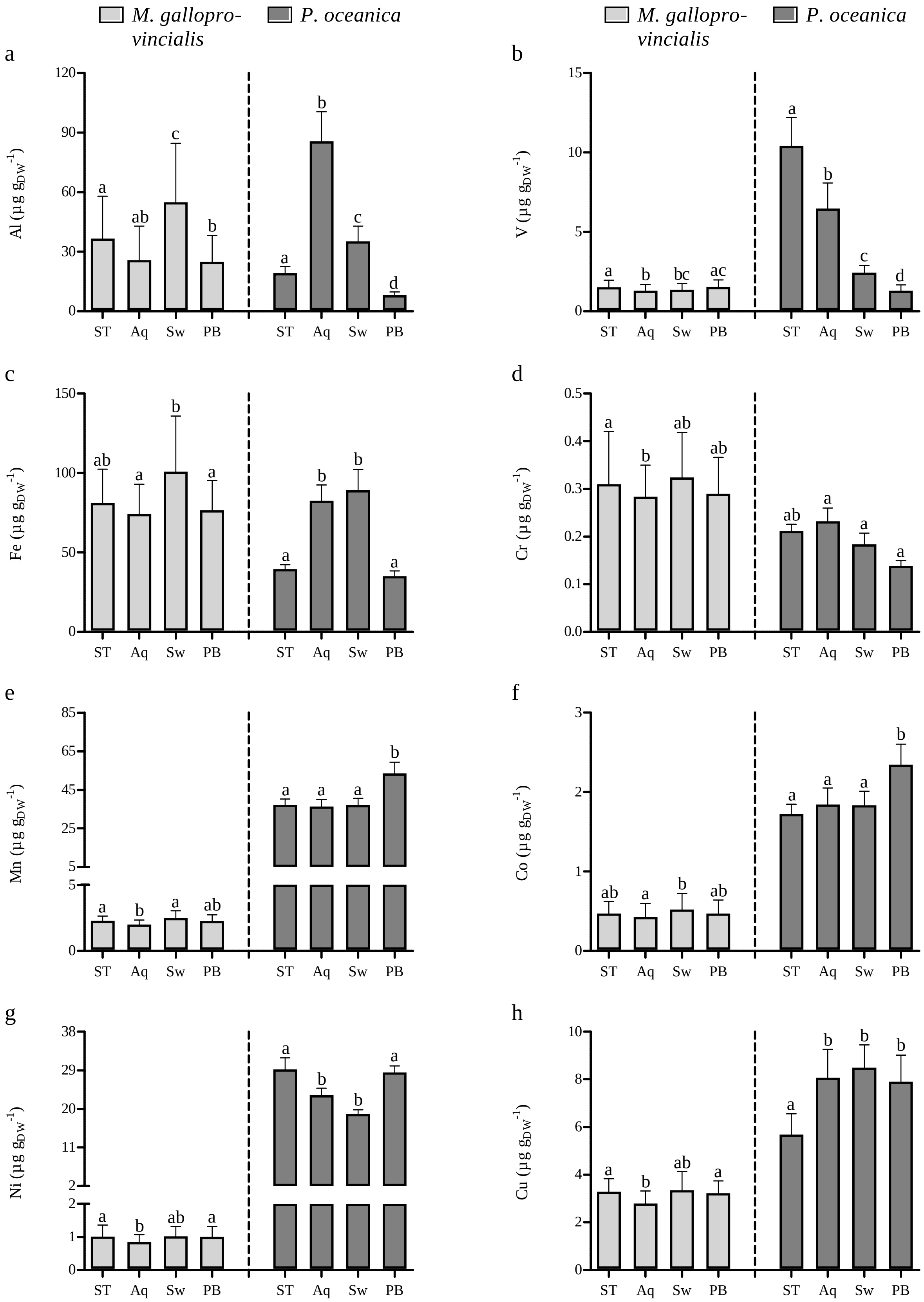
Annex A. Overall spatial variability of (a-h) Se, Ni, Mn, Co, Sb, Ag, Be and Cu concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral and remote from one another of a few to hundreds of km. The graphical comparison of the overall spatial variability of trace element (TE) concentrations is based on the use of a proportional ordinate scaling between TEs, obtained by multiplying the minimum recorded mean concentration of each TE by the highest x_{max}/x_{min} mean concentration ratio (22.8 for Mo) calculated among the 19 studied TEs. TE histograms are ordered (a-s) according to the overall spatial variability of their concentrations (Trace Element Spatial Variation Index values) throughout the whole of the French Mediterranean littoral. Arabic and Roman numbers on the X-axis represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud ; VII: Ajaccio Nord. * and ** represent TE concentrations < L_Q or < L_D, respectively.



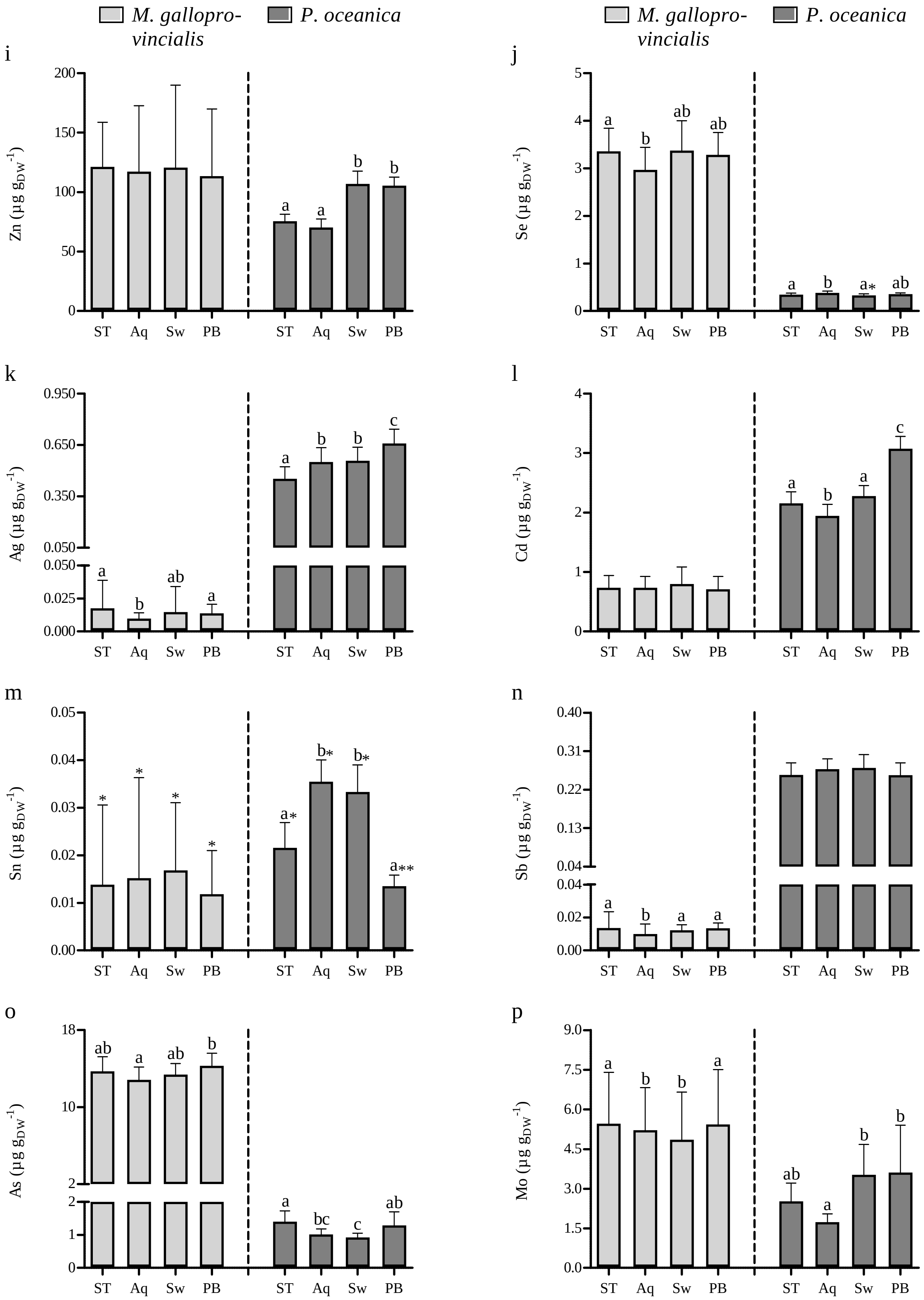
Annex A ***(Continued)*****.** Overall spatial variability of (i-p) Cd, Fe, Pb, Cr, Sn, As, Al and Zn concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral and remote from one another of a few to hundreds of km. The graphical comparison of the overall spatial variability of trace element (TE) concentrations is based on the use of a proportional ordinate scaling between TEs, obtained by multiplying the minimum recorded mean concentration of each TE by the highest x_{max}/x_{min} mean concentration ratio (22.8 for Mo) calculated among the 19 studied TEs. TE histograms are ordered (a-s) according to the overall spatial variability of their concentrations (Trace Element Spatial Variation Index values) throughout the whole of the French Mediterranean littoral. Arabic and Roman numbers on the X-axis represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud ; VII: Ajaccio Nord. BAL = blades of *P. oceanica* adult leaves. * represent TE concentrations < L_Q.



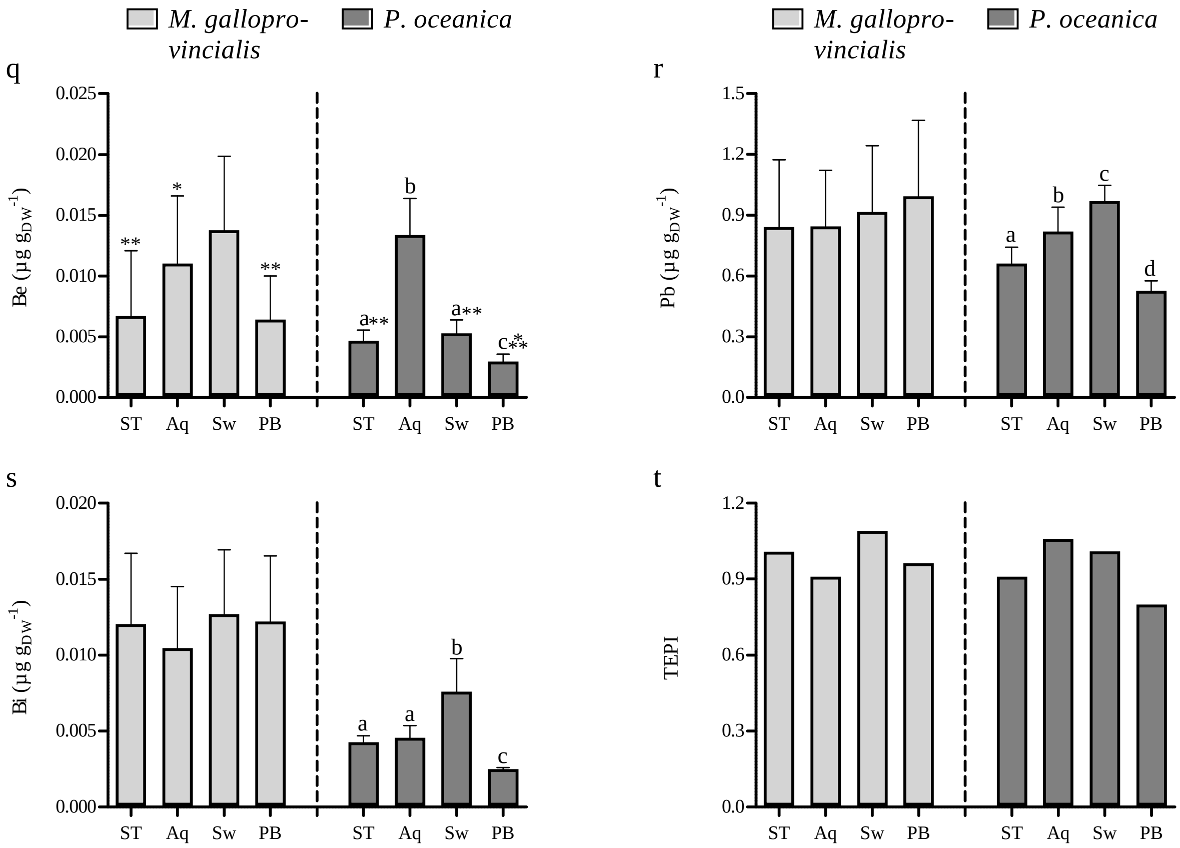
Annex A ***(Continued)*****.** Overall spatial variability of (q-s) Bi, Mo and V concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral and remote from one another of a few to hundreds of km. The graphical comparison of the overall spatial variability of trace element (TE) concentrations is based on the use of a proportional ordinate scaling between TEs, obtained by multiplying the minimum recorded mean concentration of each TE by the highest x_{max}/x_{min} mean concentration ratio (22.8 for Mo) calculated among the 19 studied TEs. TE histograms are ordered (a-s) according to the overall spatial variability of their concentrations (Trace Element Spatial Variation Index values) throughout the whole of the French Mediterranean littoral. Arabic and Roman numbers on the X-axis represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud ; VII: Ajaccio Nord. * represent TE concentrations < L_Q.



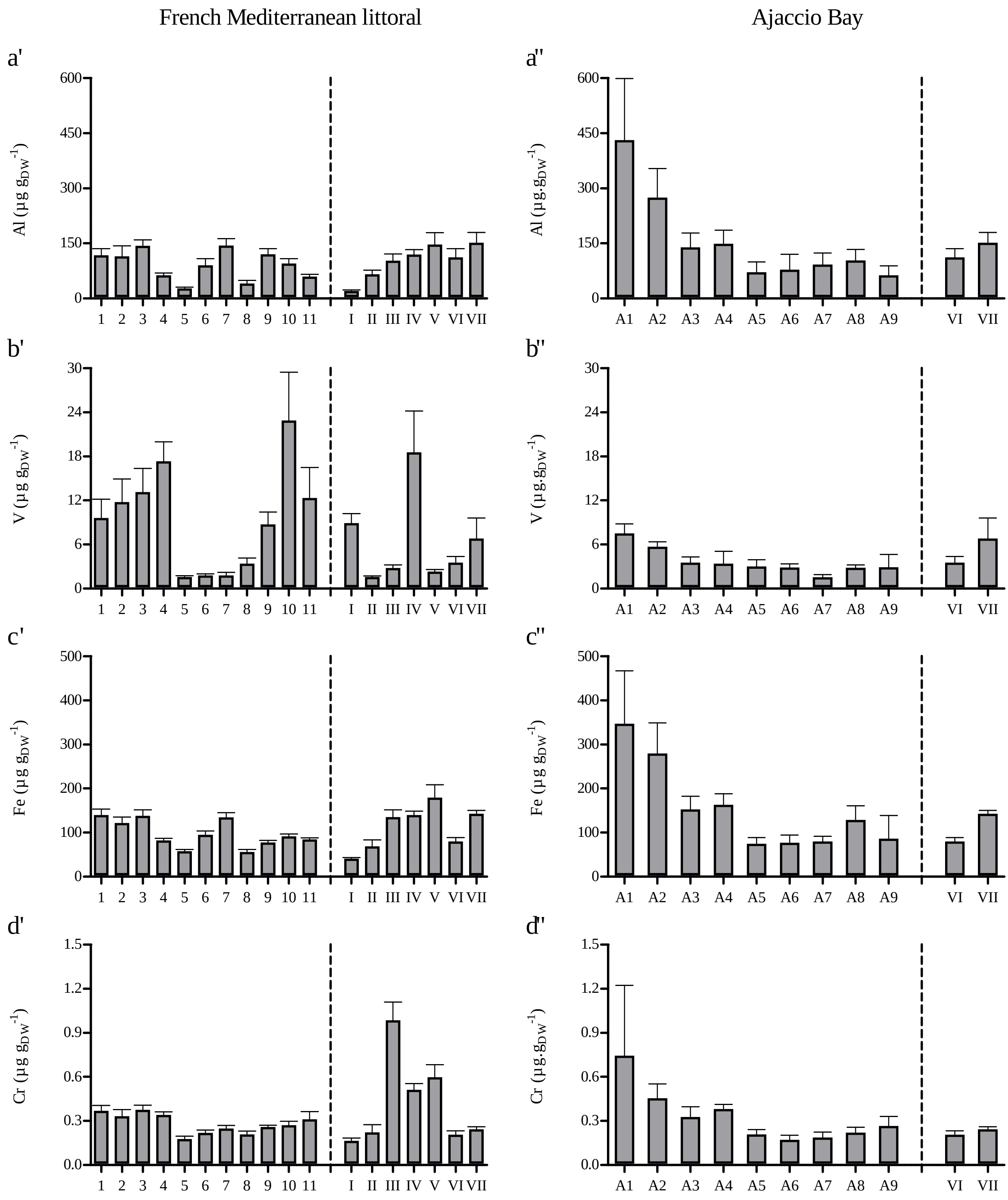
Annex B. Spatial variability of (a-h) Al, V, Fe, Cr, Mn, Co, Ni and Cu concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 48-49) purchased from the Diane salty pond (eastern Corsica, France) and immerged between 7 and 10 m depth from March to June 2010 in 4 stations in Calvi Bay area (northwestern Corsica, France), and in *P. oceanica* (n = 15) sampled between 13 and 22 m depth in June 2010 concomitantly when mussels were retrieved from water. The 4 sampled stations STARESO (ST), aquaculture farm (Aq), Calvi city sewer (Sw) and Punta Bianca (PB) were remote from one another by a distance of 1 to 3 km. Letters represent significant differences between stations for each bioindicator species.



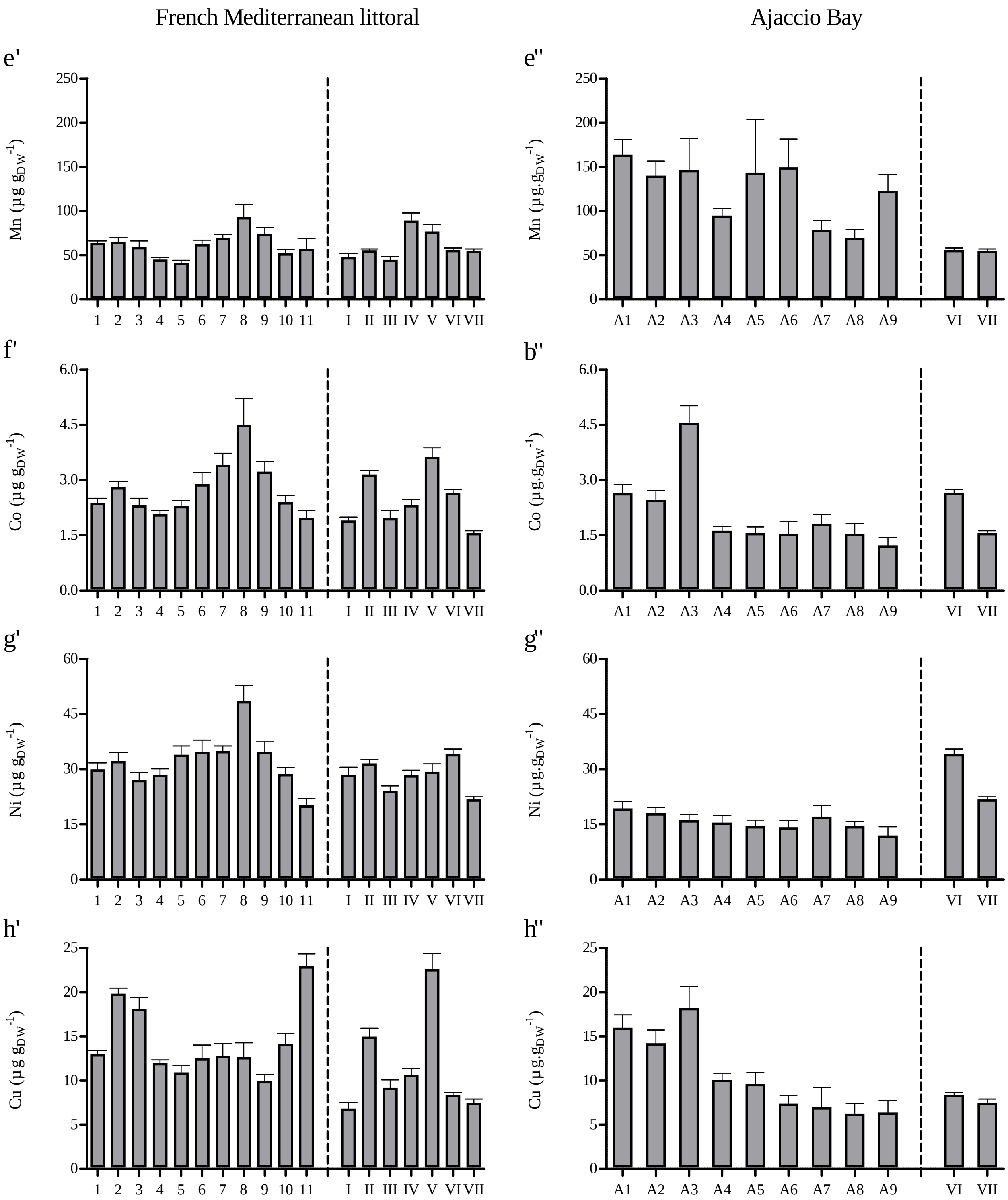
Annex B ***(Continued).*** Spatial variability of (i-p) Zn, Se, Ag, Cd, Sn, Sb, As and Mo concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 48-49) purchased from the Diane salty pond (eastern Corsica, France) and immerged between 7 and 10 m depth from March to June 2010 in 4 stations in Calvi Bay area (northwestern Corsica, France), and in *P. oceanica* (n = 15) sampled between 13 and 22 m depth in June 2010 concomitantly when mussels were retrieved from water. The 4 sampled stations STARESO (ST), aquaculture farm (Aq), Calvi city sewer (Sw) and Punta Bianca (PB) were remote from one another by a distance of 1 to 3 km. Letters represent significant differences between stations for each bioindicator species. * and ** represent trace element concentrations < L_Q or < L_D, respectively.



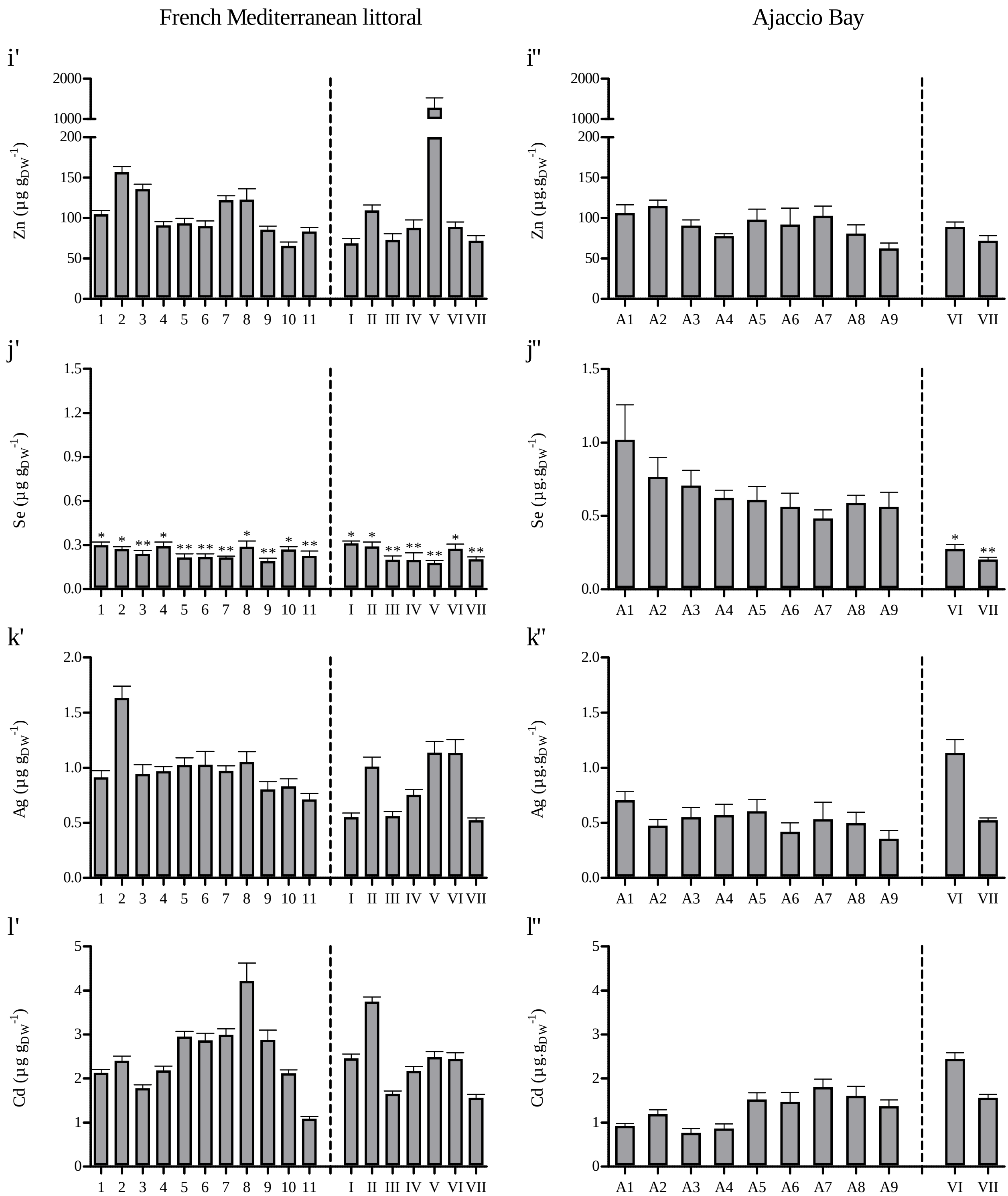
Annex B ***(Continued)*****.** Spatial variability of (q-s) Be, Pb and Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 48-49, except for Be: n = 24-25) purchased from the Diane salty pond (eastern Corsica, France) and immerged between 7 and 10 m depth from March to June 2010 in 4 stations in Calvi Bay area (northwestern Corsica, France), and in *P. oceanica* (n = 15) sampled between 13 and 22 m depth in June 2010 concomitantly when mussels were retrieved from water. (t) Trace Element Pollution Index (TEPI) values were calculated for each station and bioindicator species from mean normalized concentrations of the 19 studied trace elements. The 4 sampled stations STARESO (ST), aquaculture farm (Aq), Calvi city sewer (Sw) and Punta Bianca (PB) were remote from one another by a distance of 1 to 3 km. Letters represent significant differences between stations for each bioindicator species. *, ** and ⁎*⁎ represent trace element concentrations $< L_Q$, $< L_D$ or $< L_C$, respectively.



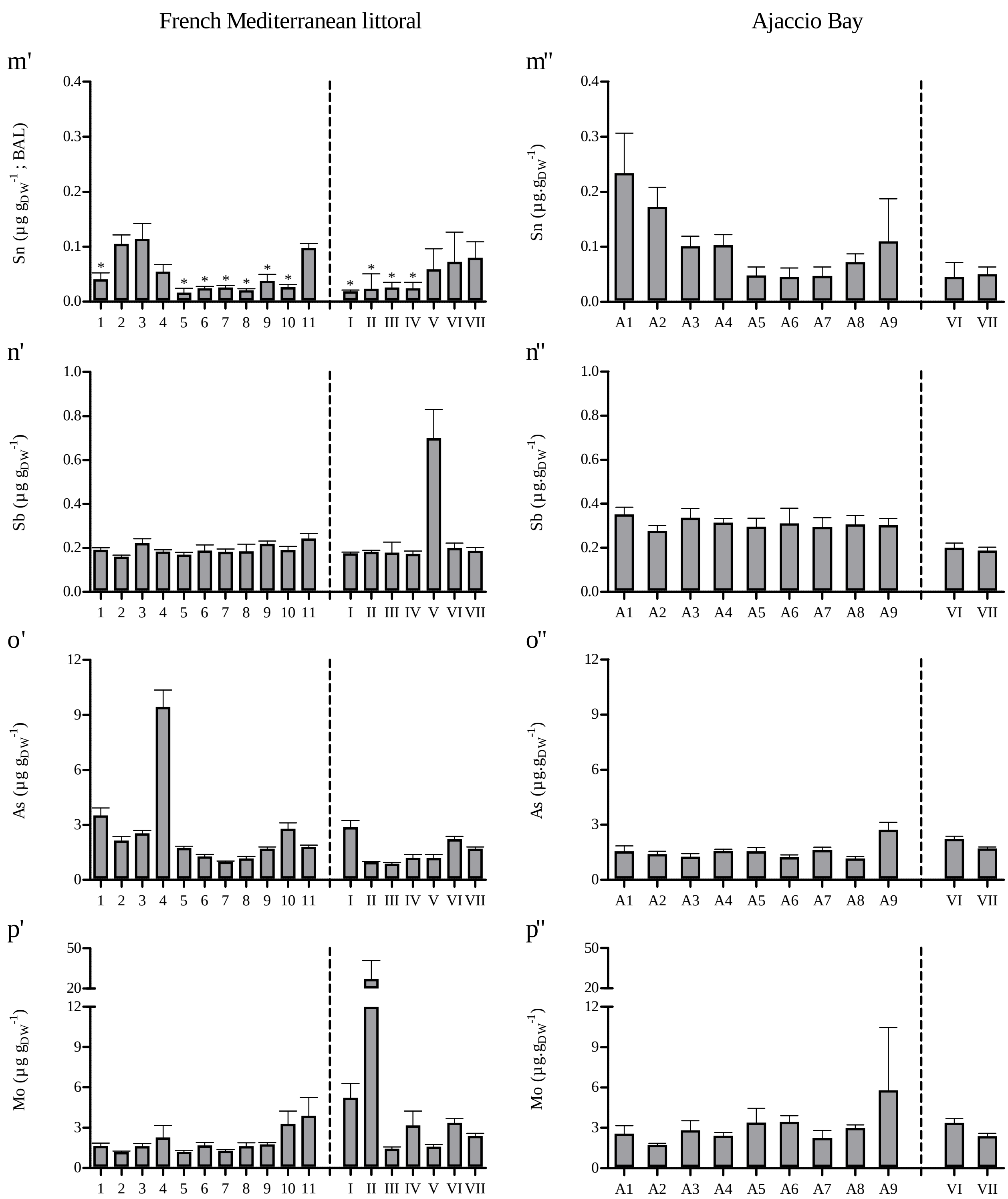
Annex C. Spatial variability of Al, V, Fe and Cr concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (a'-d'; n = 15) and in *P. oceanica* sampled at 8-9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; a''-d''; n = 9-10). Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial.



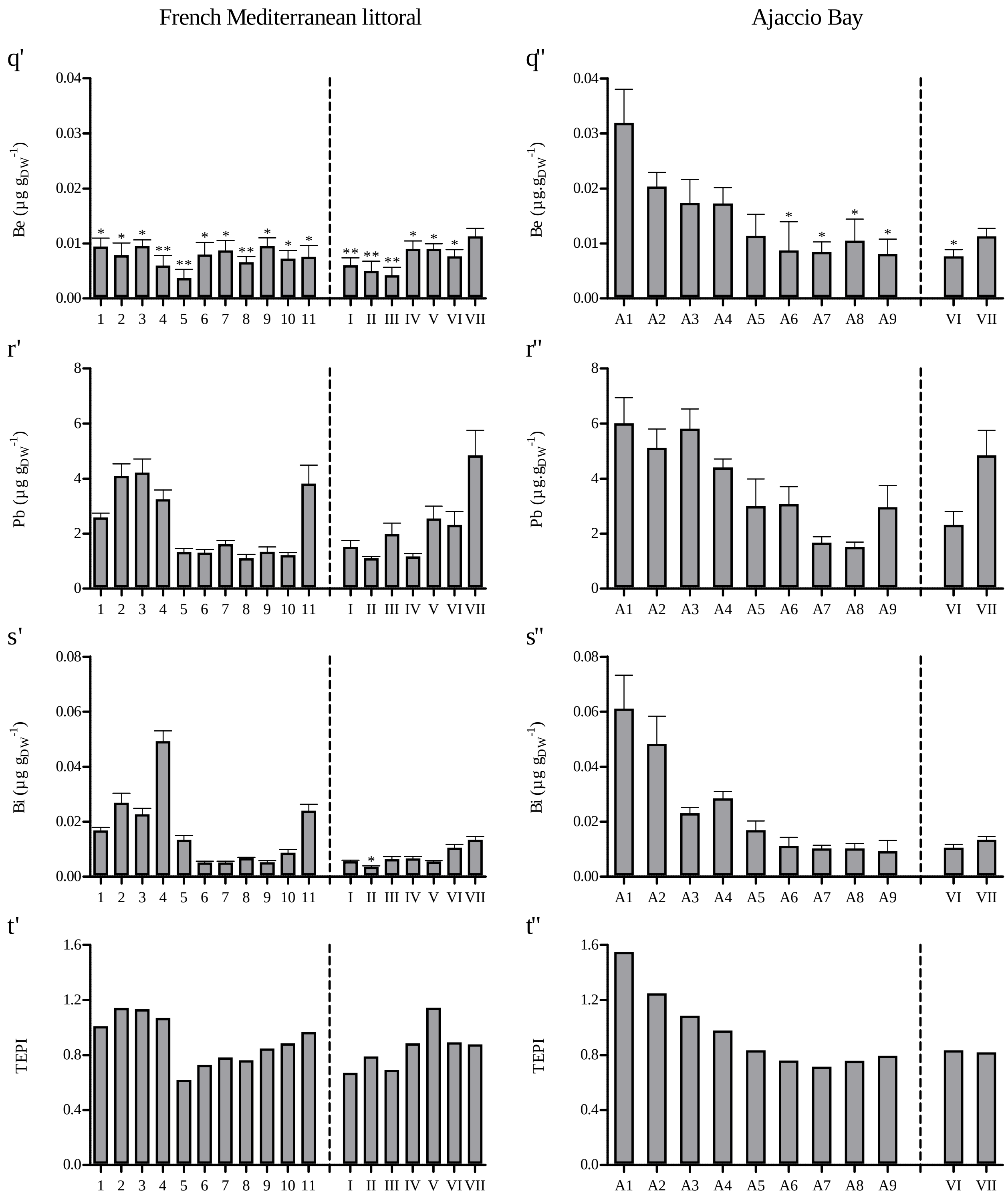
Annex C ***(Continued).*** Spatial variability of Mn, Co, Ni and Cu concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (e'-h'; n = 15) and in *P. oceanica* sampled at 8-9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; e''-h''; n = 9-10). Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial.



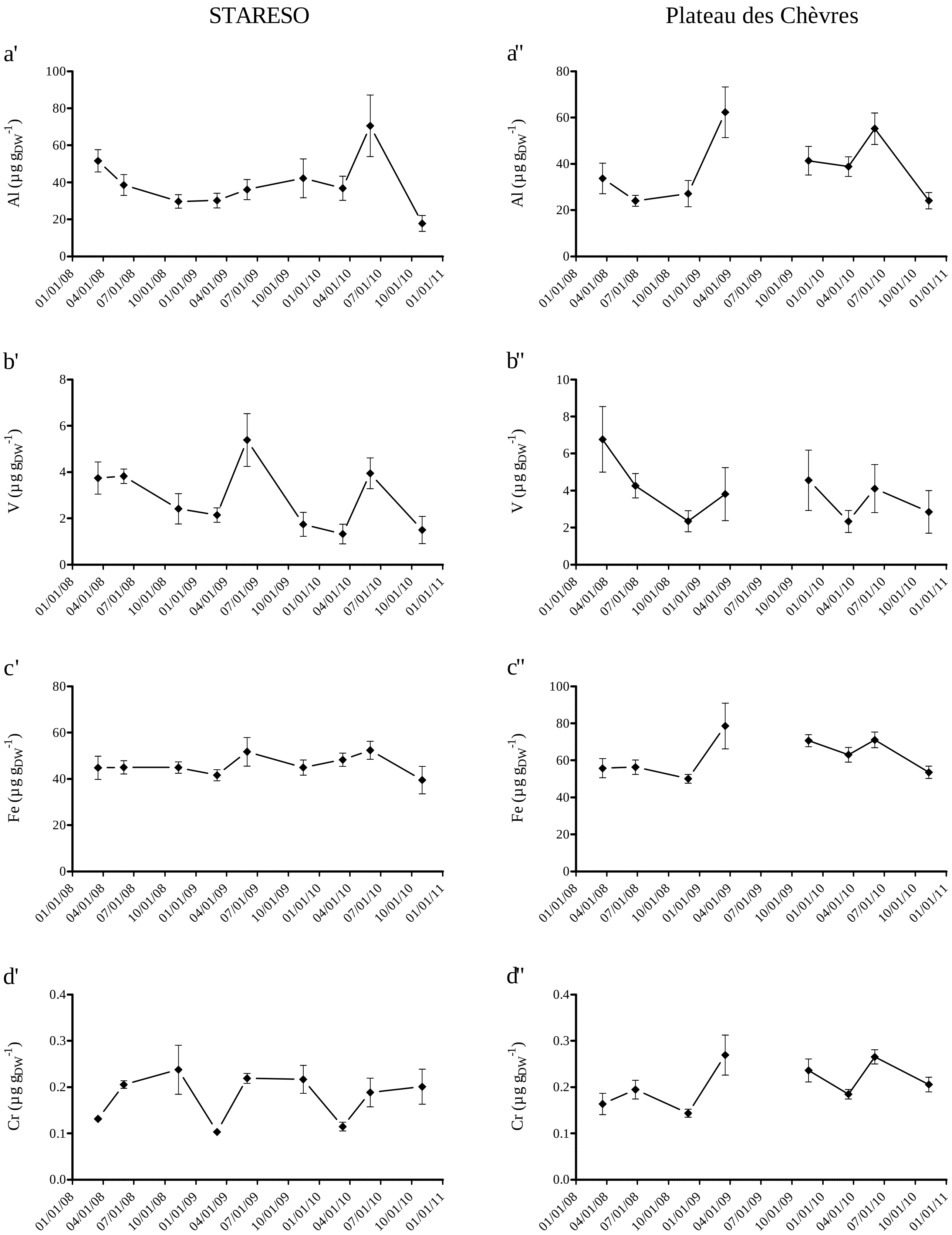
Annex C ***(Continued)*****.** Spatial variability of Zn, Se, Ag and Cd concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (i'-l'; n = 15) and in *P. oceanica* sampled at 8-9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; i''-l''; n = 9-10). Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial. * and ** represent trace element concentrations < L_Q or < L_D, respectively.



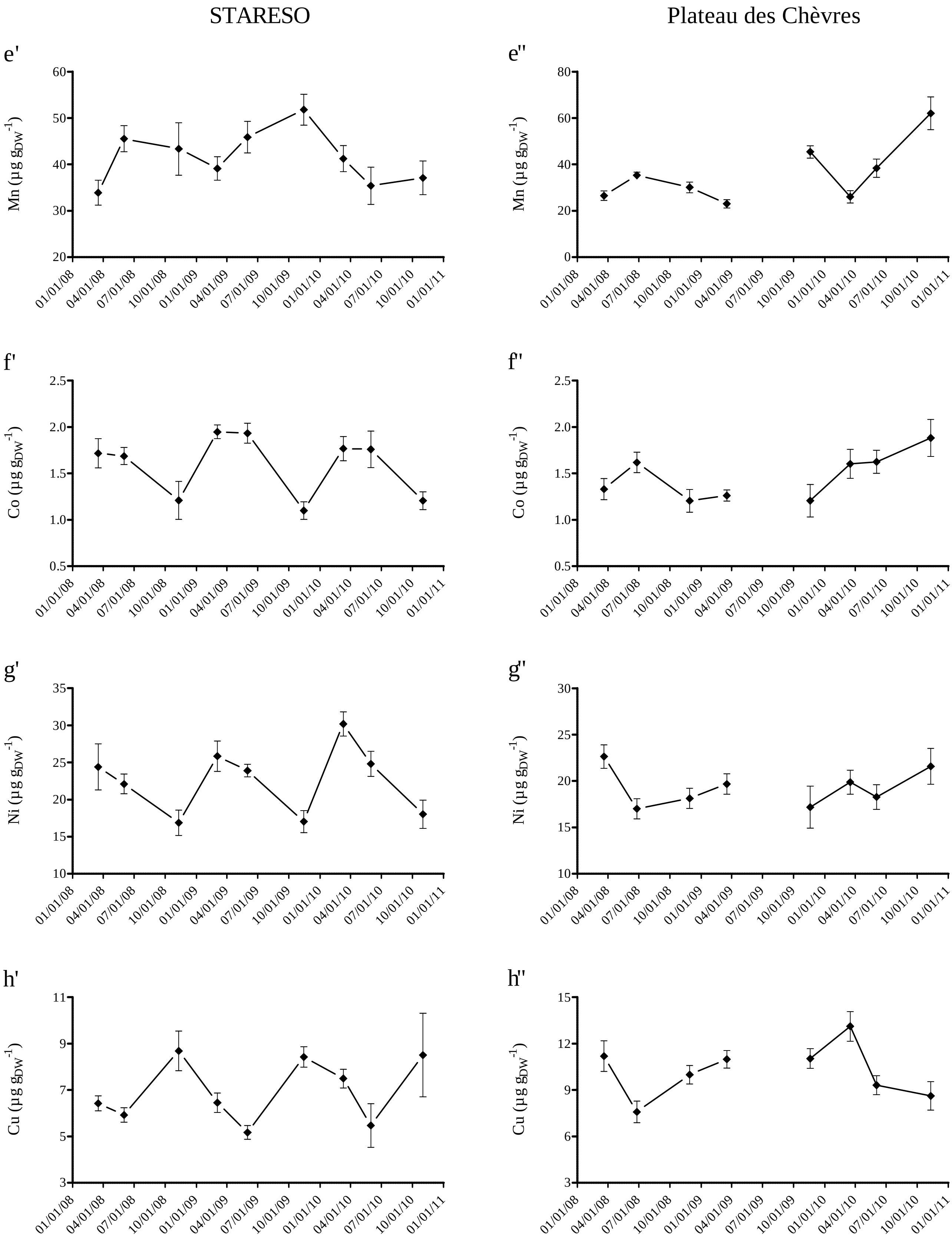
Annex C ***(Continued).*** Spatial variability of Sn, Sb, As and Mo concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (m'-p'; n = 15) and in *P. oceanica* sampled at 8-9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; m''-p''; n = 9-10). Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial. * represent trace element concentrations < L_Q. BAL = blades of *P. oceanica* adult leaves.



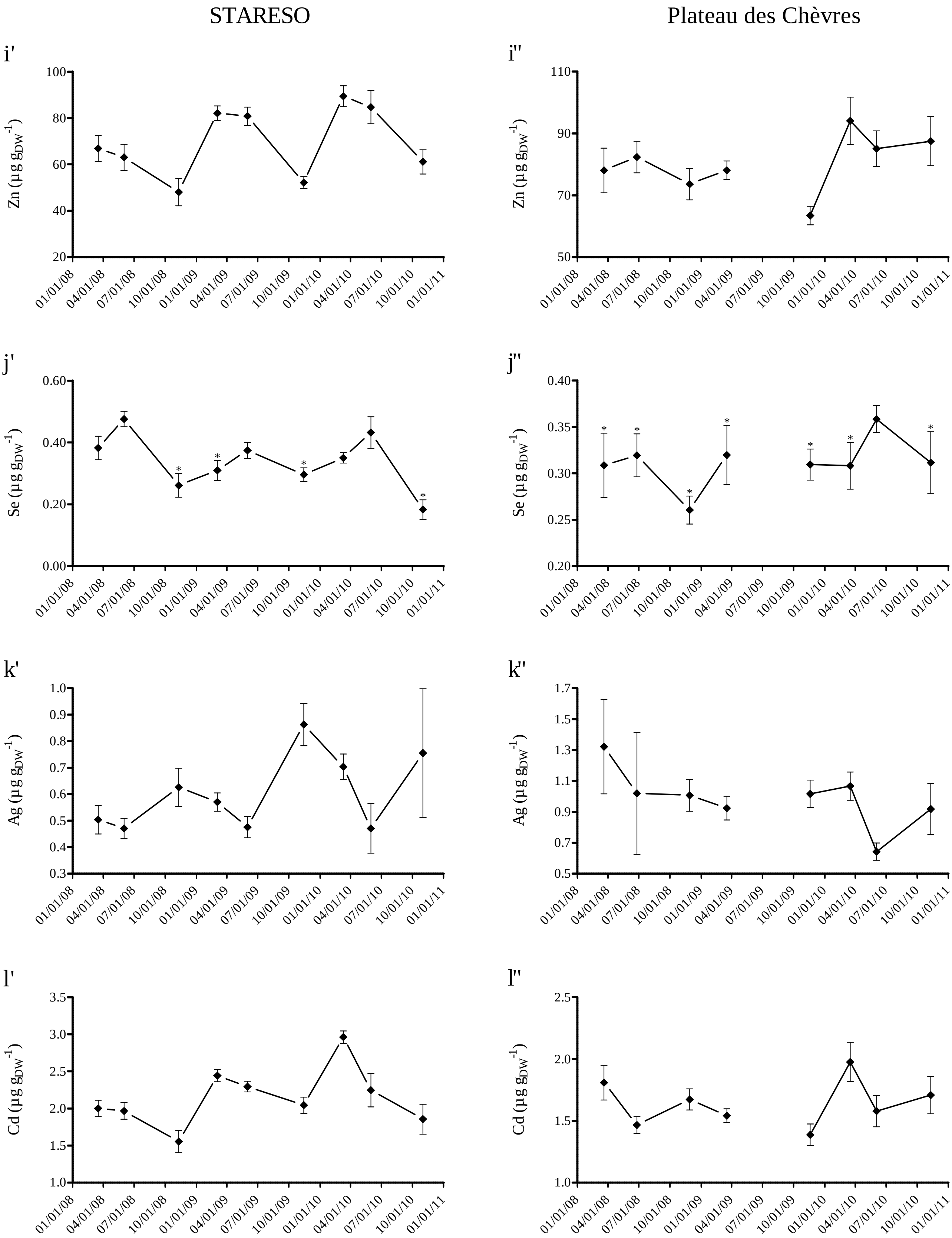
Annex C ***(Continued).*** Spatial variability of Be, Pb and Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* sampled at 15 m depth in April 2007 in 18 sites located along coasts of the French Mediterranean littoral (q'-s'; n = 15) and in *P. oceanica* sampled at 8-9 m depth in May 2010 in 9 stations located along a radial following the coastline at the back of the Ajaccio Bay (western Corsica, France; q''-s''; n = 9-10). Trace Element Pollution Index (TEPI) values (t', t'') were calculated by site and station from mean normalized concentrations of the 19 studied trace elements. Arabic and Roman numbers on the X-axis of left graphs represent sites along continental Provence-Alpes-Côte d'Azur (1-11) or insular Corsican (I-VII) coasts. 1: Ensuès; 2: La Vesse; 3: Corbière; 4: Plateau des Chèvres; 5: Riou; 6: Bénat; 7: Giens; 8: St Raphaël; 9: Cap Roux; 10: Antibes; 11: Villefranche; I: Calvi; II: Aregno; III: St Florent; IV: Taglio Isolaccio; V: Bravone; VI: Ajaccio Sud; VII: Ajaccio Nord. Numbered A letters A1-9 on the X-axis of right graphs represent stations along the Ajaccio Bay radial, with increasing distance from the port and urban centre of Ajaccio city. The two supplementary sites VI: Ajaccio Sud and VII: Ajaccio Nord were also reported on right graphs for comparison purpose at the scale of the Ajaccio Bay. The 18 sites located along coasts of the French Mediterranean littoral were remote from one another of a few to hundreds of km; the 9 stations located along the Ajaccio Bay radial were remote from one another of around 300 m, while the two Ajaccio Sud and Ajaccio Nord sites were remote of around 5 km of the radial. * and ** represent trace element concentrations < L_Q or < L_D, respectively.



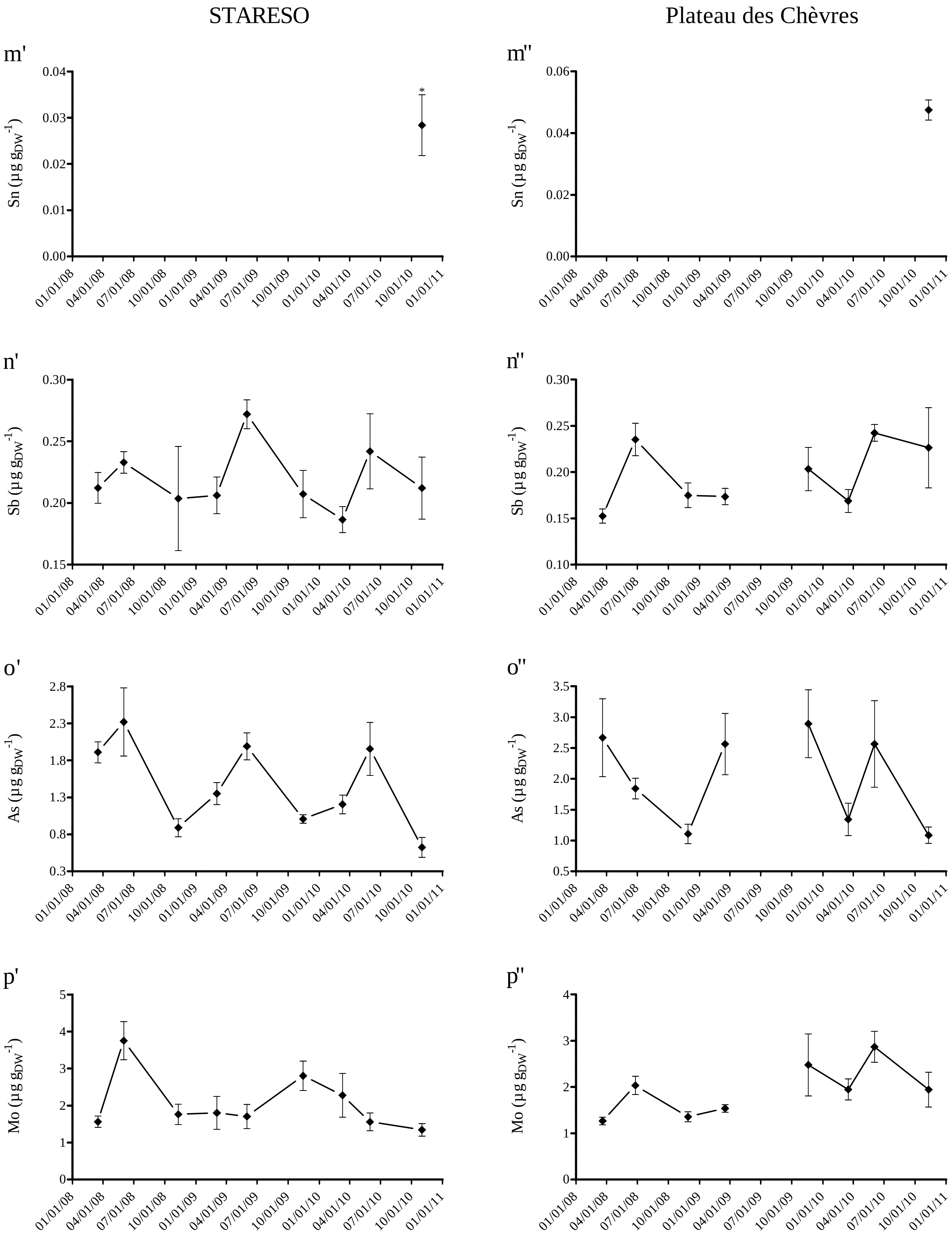
Annex D. Seasonal and pluriannual kinetics of Al, V, Fe and Cr concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs a'-d') and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France; right graphs a''-d''). The sampling of June 2009 is missing for Plateau des Chèvres site.



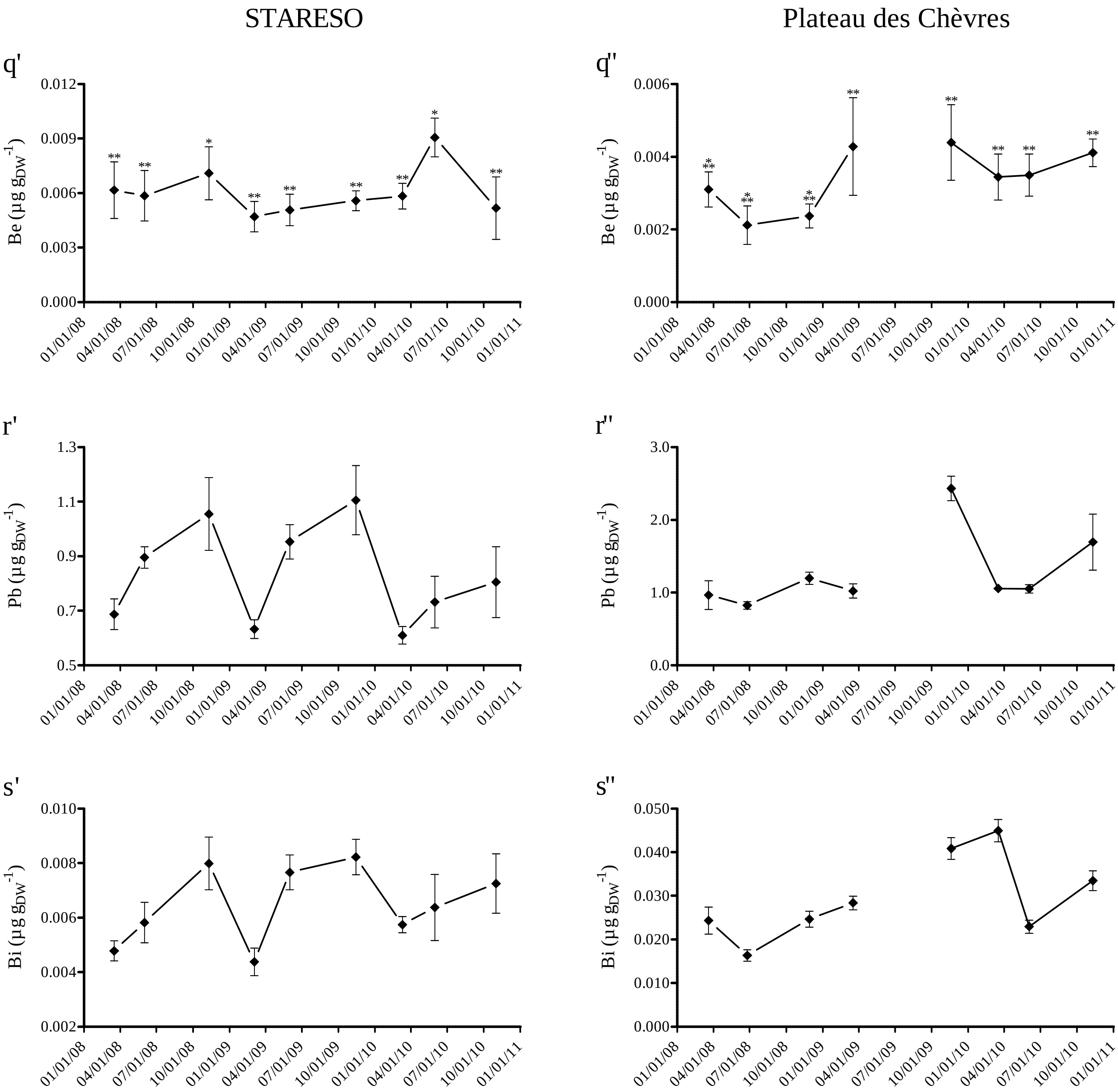
Annex D *(Continued)*. Seasonal and pluriannual kinetics of Mn, Co, Ni and Cu concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs e'-h') and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France; right graphs e''-h''). The sampling of June 2009 is missing for Plateau des Chèvres site.



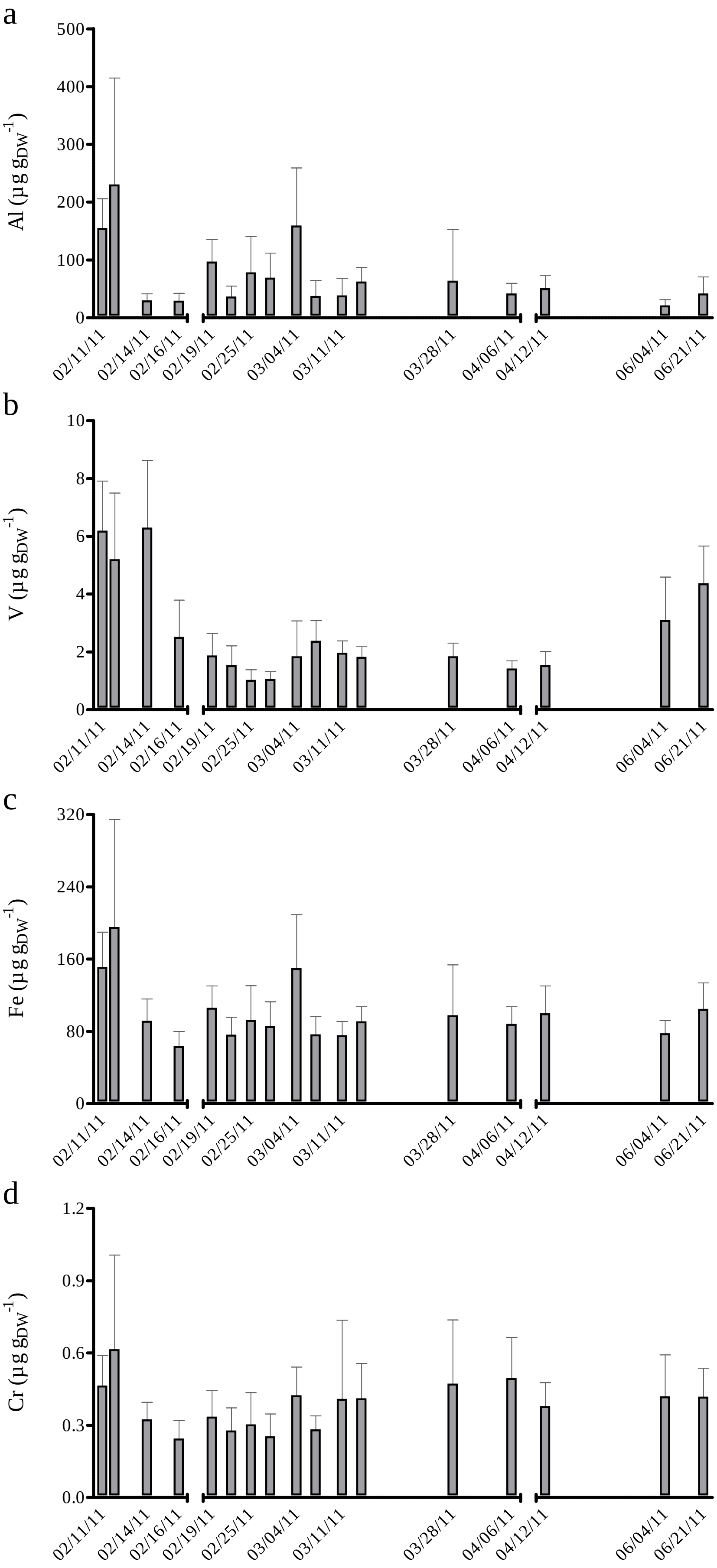
Annex D ***(Continued)*****.** Seasonal and pluriannual kinetics of Zn, Se, Ag and Cd concentrations (mean ± SD, in $\mu g\ g_{DW}^{-1}$) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs i'-l') and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France; right graphs i''-l''). The sampling of June 2009 is missing for Plateau des Chèvres site. * represent trace element concentrations < L_Q.



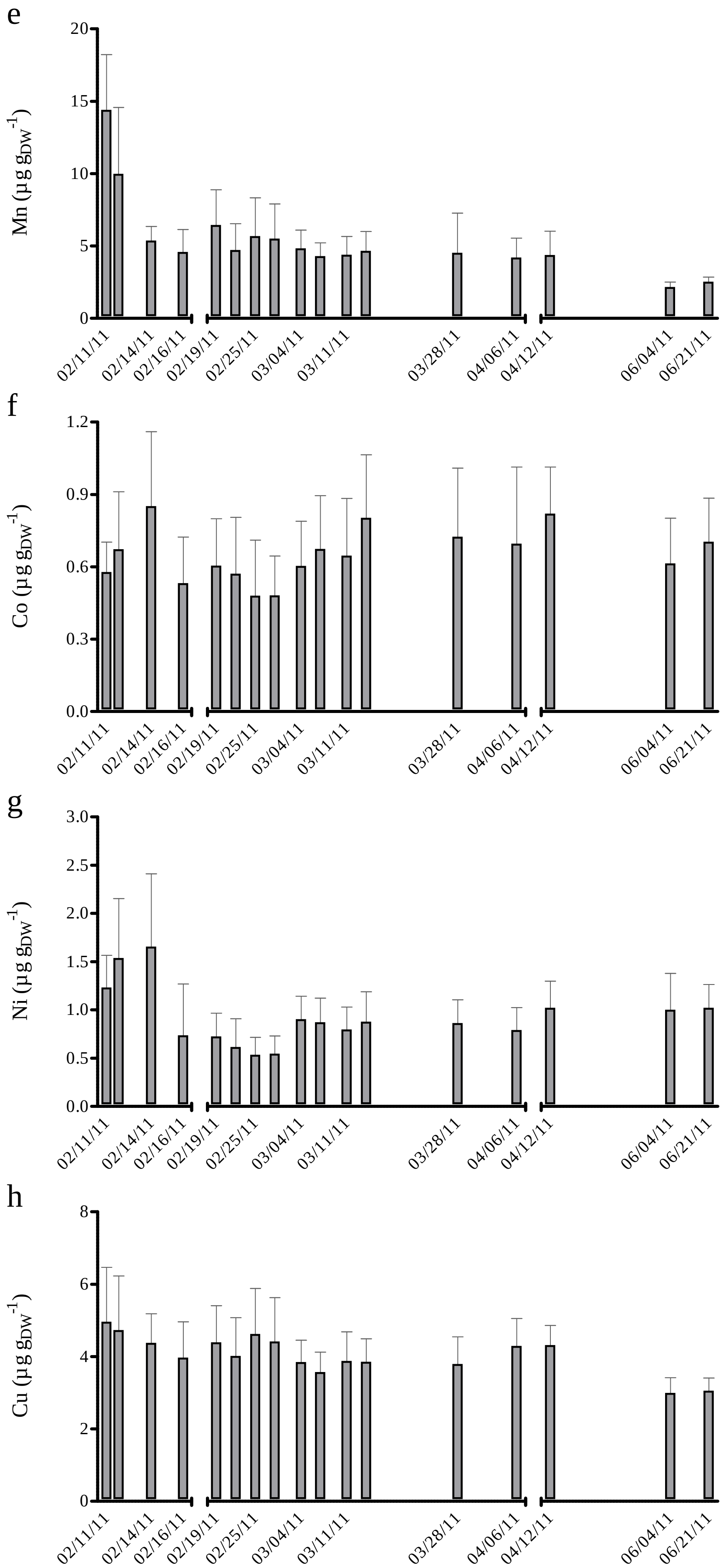
Annex D ***(Continued)*****.** Seasonal and pluriannual kinetics of Sn, Sb, As and Mo concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs m'-p') and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France; right graphs m''-p''). The sampling of June 2009 is missing for Plateau des Chèvres site, and Sn concentrations are only available for samplings of November 2010 in both *P. oceanica* beds. * represent trace element concentrations < L_Q.



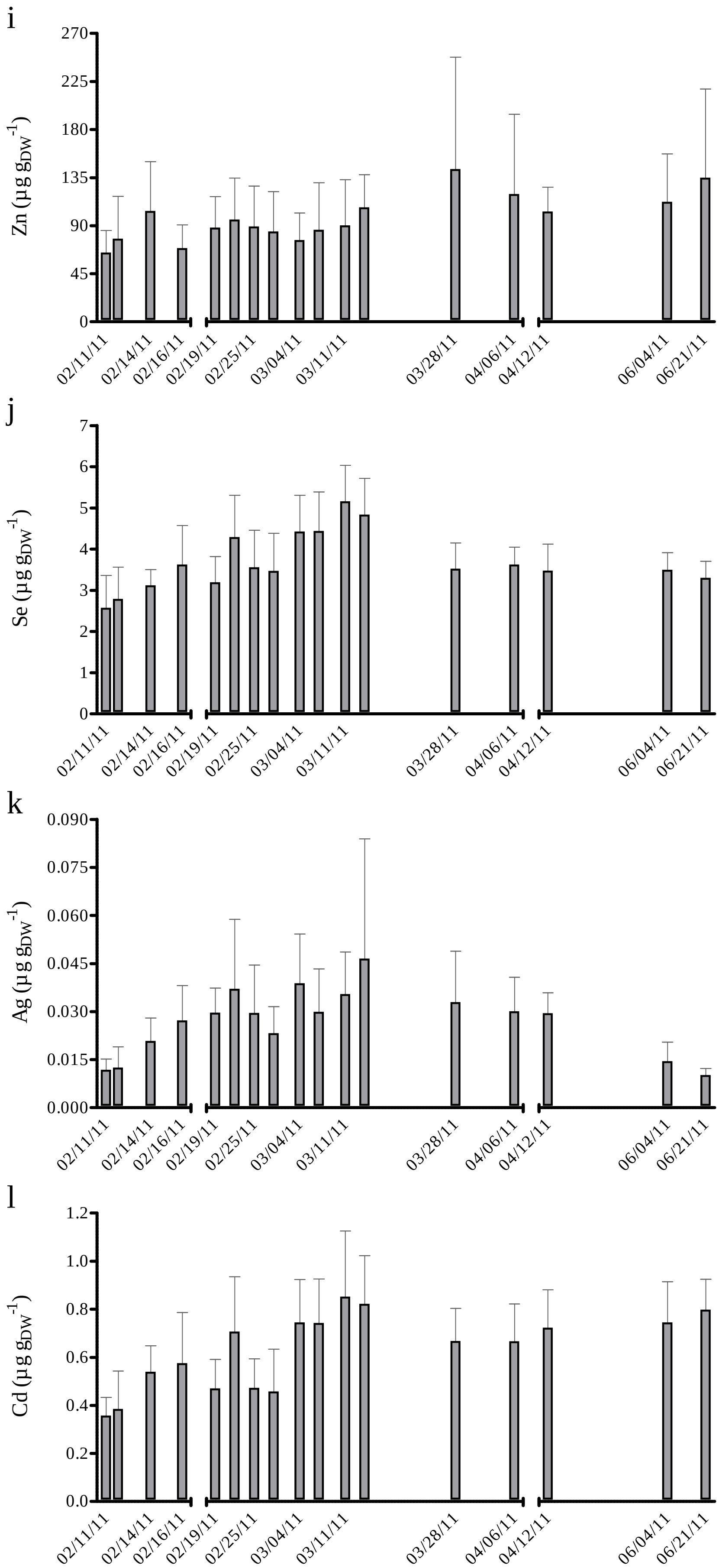
Annex D ***(Continued)*****.** Seasonal and pluriannual kinetics of Be, Pb and Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in *P. oceanica* (n = 15) sampled at 10 m depth from March 2008 to November 2010 in the pristine seagrass bed of STARESO (Calvi Bay, northwestern Corsica, France; left graphs q'-s') and in the impacted seagrass bed of Plateau des Chèvres (Maseille, France; right graphs q''-s''). The sampling of June 2009 is missing for Plateau des Chèvres site. *, ** and ∗*∗ represent trace element concentrations < L_Q, < L_D or < L_C, respectively.



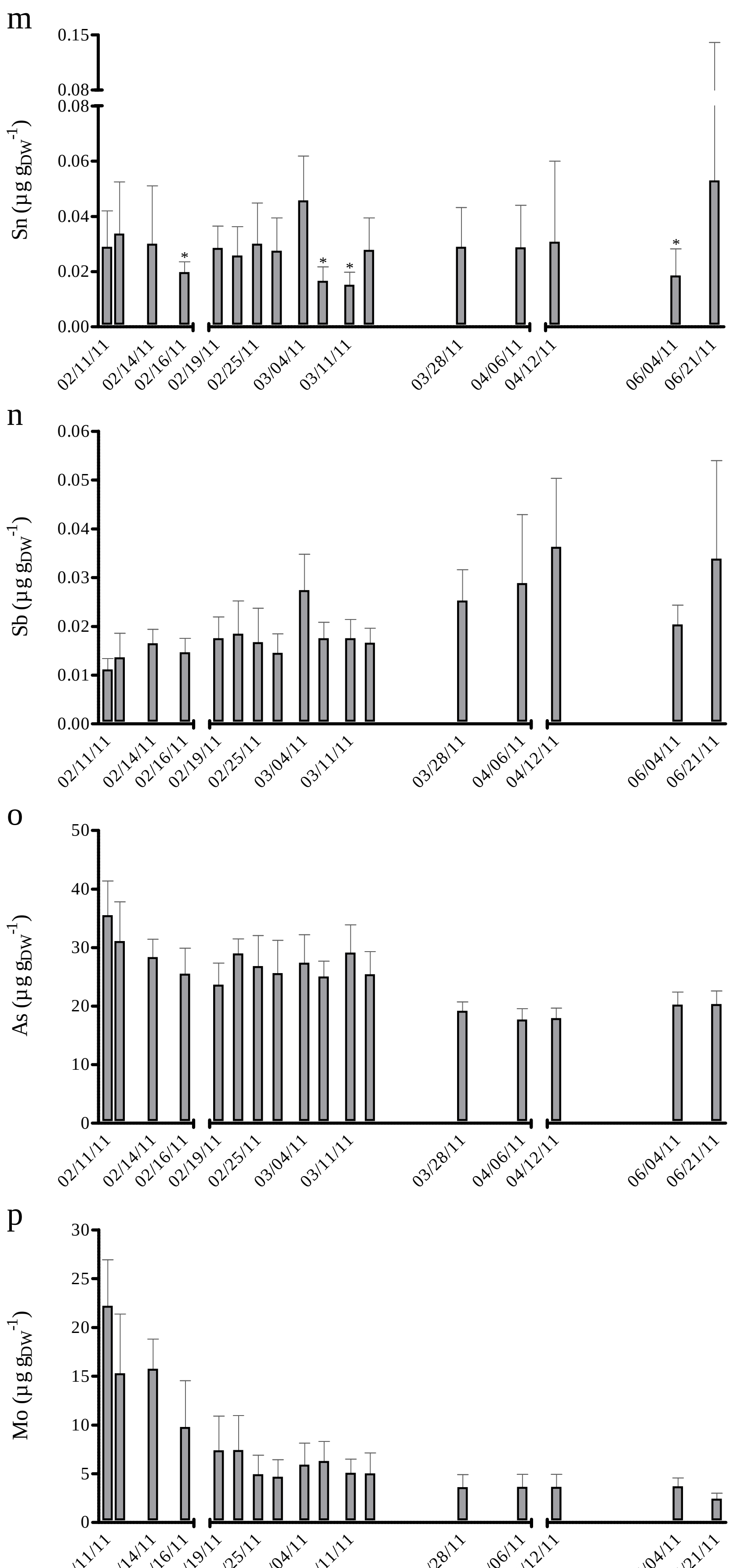
Annex E. Temporal kinetics of (a-d) Al, V, Fe and Cr concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 - n = 30 - and 02/12/2011 - n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). For clarity purpose, one in two sampling date is reported on the left half of the X-axis.



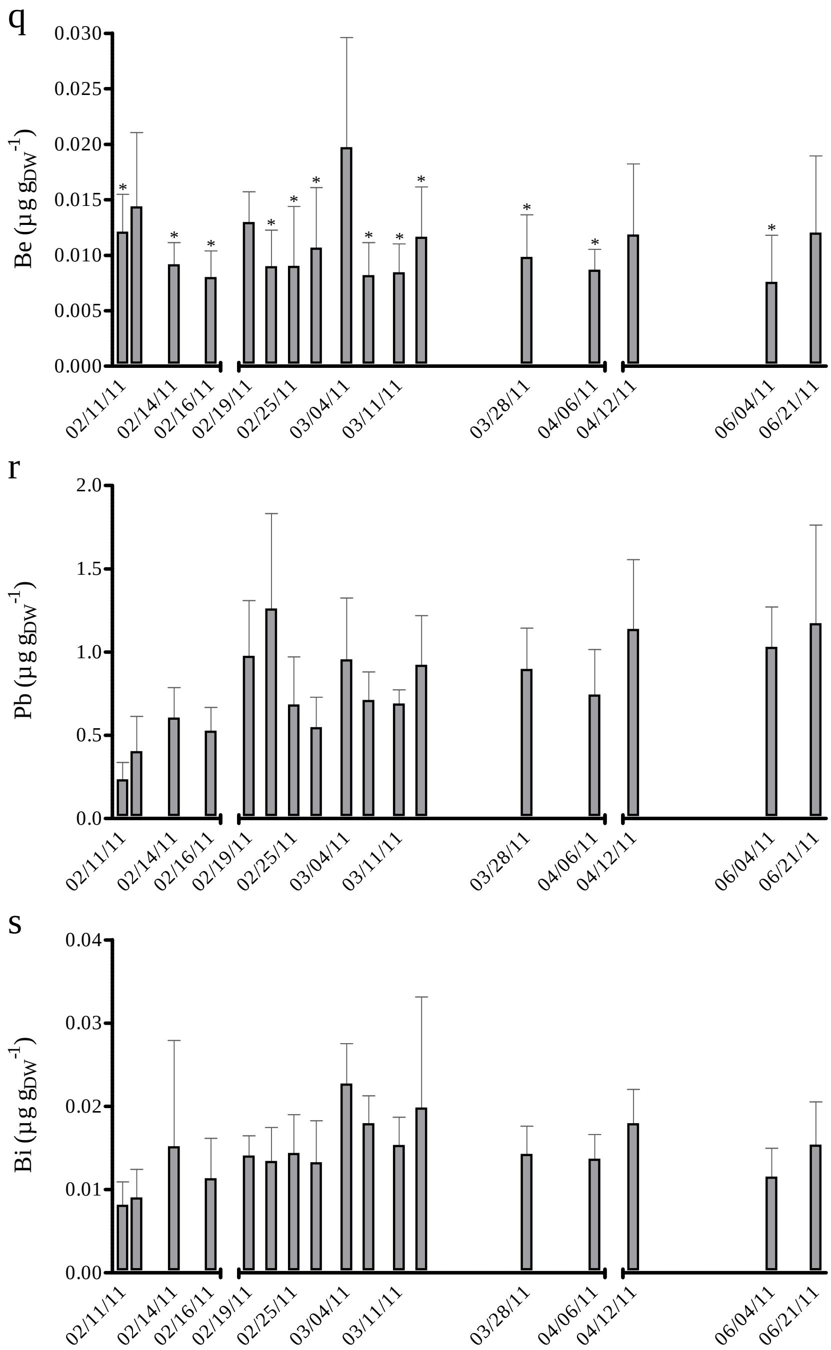
Annex E *(Continued)*. Temporal kinetics of (e-h) Mn, Co, Ni and Cu concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 - n = 30 - and 02/12/2011 - n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). For clarity purpose, one in two sampling date is reported on the left half of the X-axis.



Annex E *(Continued)*. Temporal kinetics of (i-l) Zn, Se, Ag and Cd concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 - n = 30 - and 02/12/2011 - n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). For clarity purpose, one in two sampling date is reported on the left half of the X-axis.



Annex E *(Continued)*. Temporal kinetics of (m-p) Sn, Sb, As and Mo concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 - n = 30 - and 02/12/2011 - n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). * represent trace element concentrations < L_Q. For clarity purpose, one in two sampling date is reported on the left half of the X-axis.



Annex E ***(Continued)*****.** Temporal kinetics of (q-s) Be, Pb and Bi concentrations (mean ± SD, in µg g_{DW}^{-1}) in caged rope-grown *M. galloprovincialis* (n = 12, except for sampling dates 02/11/2011 - n = 30 - and 02/12/2011 - n = 44) purchased from the Diane salty pond (eastern Corsica, France) and immerged from February to June 2011 near the oceanographic station STARESO after a 3 days acclimatization period (02/11-14/2011) in STARESO marina (Calvi Bay, northwestern Corsica, France). * represent trace element concentrations < L_Q. For clarity purpose, one in two sampling date is reported on the left half of the X-axis.