University of Liège
Faculty of Sciences
Department of Environmental Sciences and Management
Laboratory of Oceanology

Coastal pollution of the Mediterranean and extension of its biomonitoring to trace elements of emerging concern

Dissertation written by Jonathan Richir
for the fulfilment of the degree of PhD in Sciences
Academic year 2012-2013

Cover Image: Plume at the « Emissaire de Cortiou » discharging wastewaters from Marseille, France.

By Ballesta L., « La Méditerranée est souffrante mais pas mourante »

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RICHIR Jonathan

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Résumé

La Méditerranée, mer semi-fermée aux côtes densément peuplées, est soumise à de nombreuses pressions anthropiques, dont la pollution chimique par les éléments traces. Ces polluants, issus de nos activités continentales et transportés par voies fluviales, aériennes, ou directement rejetées dans les eaux côtières, s'accumulent dans le réceptacle final que sont les mers et océans, et touchent tout particulièrement les zones littorales. Dans le courant des années 70, des chercheurs suggérèrent d'utiliser des organismes, plus particulièrement les moules du genre *Mytilus*, pour évaluer le statut de contamination chimique des écosystèmes littoraux: le biomonitoring était né. Depuis, de très nombreux travaux utilisant diverses espèces animales et végétales ont été publiés.

Deux des indicateurs biologiques, ou bioindicateurs, les plus largement étudiés en Méditerranée sont la magnoliophyte marine *Posidonia oceanica*, formant de vastes et denses prairies sous-marines appelés herbiers, et la moule méditerranéenne *Mytilus galloprovincialis*. Les études portant sur ces deux espèces se sont essentiellement intéressées à la contamination par le Cr, le Ni, le Cu, le Zn, le Cd, le Pb (moules et posidonies), le Fe (posidonies), l'As, le V et l'Ag (moules). De nombreux autres éléments traces dont le Be, l'Al, le Mn, le Co, le Se, le Mo, le Sn, le Sb et le Bi n'ont cependant fait l'objet que de peu voir d'aucun suivi écotoxicologique chez ces deux espèces. Or, l'évolution de nos technologies et de nos modes de consommations et la récente augmentation de l'extraction et de la production de ces éléments en réponse aux besoins croissants des pays en voies de développement, font de la pollution environnementale par les éléments traces un sujet d'actualité.

L'objectif global de ce travail était dès lors d'évaluer le potentiel bioindicateur respectif de la posidonie et de la moule méditerranéenne dans le monitoring du Be, de l'Al, du Mn, du Co, du Se, du Mo, du Sn, du Sb, du Bi, du Fe, de l'As, du V, et de l'Ag, éléments pour la plupart catégorisés de "préoccupation environnementale nouvelle". Le biomonitoring intégré des éléments traces classiquement investigués nécessite un suivi continu de leurs niveaux de concentrations environnementales ; nous avons dès lors également mesurés leurs teneurs dans les deux bioindicateurs sélectionnés.

La posidonie et la moule méditerranéenne se complémentent l'une l'autre dans les études de monitoring de la pollution côtière. Si les deux espèces accumulent les polluants dissouts dans la colonne d'eau, la posidonie, enracinée sur le fond, accumule en outre les

polluants stockés à plus long terme dans le sédiment ; la moule, bivalve filtreur, accumule quant à elle les polluants particulaires en suspension dans la colonne d'eau. L'utilisation combinée de ces deux bioindicateurs fournit donc une vue d'ensemble de l'état de santé de l'environnement dont elles sont issues (eau, sédiments, matières en suspension).

Notre premier objectif était de mesurer, à l'échelle du littoral méditerranéen français, la variabilité spatiale des concentrations en éléments traces dans la posidonie et de définir dans quelle mesure les éléments de préoccupations environnementales nouvelles menaçaient l'intégrité chimique des écosystèmes côtiers. Nous avons observé d'une part que la majorité des éléments trace peu/pas étudiés présentaient une variabilité spatiale équivalente – voir supérieure – à celle des éléments classiquement dosés, et que cette variabilité spatiale pouvait être associée à des sources spécifiques de contaminations anthropiques tel que l'agriculture (Mo), l'activité minière (Sb), le stockage et le raffinage de produits pétroliers (V), ou la présence de ports et centres urbains d'importance (Sn, Bi). Leur monitoring, au même titre que celui des éléments traces classiquement suivis en Méditerranée, s'avère dès lors indispensable. De plus, l'étude approfondie de l'état de contamination de la Baie de Calvi, côte nord-ouest de la Corse (France), nous a par ailleurs permis de définir (ou redéfinir) ce site comme site de référence en Méditerranée nord-occidentale pour le monitoring de la pollution chimique par les éléments traces.

Nous nous sommes ensuite intéressés aux mécanismes physiologiques d'accumulation, de stockage et d'excrétion des éléments traces chez la posidonie. La contamination *in situ* de portions d'herbier permit de modéliser les cinétiques d'accumulation rapide des contaminants par la posidonie. Les compartiments de la plante répondirent différemment à l'exposition aux polluants. Ainsi, les feuilles âgées et sénescentes de la plante assimilèrent moins rapidement les contaminants que les jeunes feuilles en croissance. Les éléments traces, une fois accumulés, pouvaient ensuite être redistribués entre les différents compartiments de la plante, notamment vers le système rhizomes-racines du sédiment jouant ainsi le rôle d'archive biologique. Après l'arrêt des contaminations, les cinétiques de décontamination des faisceaux de feuilles furent relativement rapides et dépendaient notamment du temps d'exposition aux éléments traces, de leur caractère essentiel ou toxiques et du compartiment suivi. La posidonie s'avère donc être un bioindicateur sensible pour le monitoring de la pollution côtière par les éléments traces présente et passée.

Nous avons montré que la moule méditerranéenne accumulait efficacement les éléments traces, tant ceux de préoccupation environnementale nouvelle que ceux classiquement étudiés chez ce bioindicateur. De même que pour la posidonie, la physiologie de la moule conditionne sa réponse à l'exposition aux polluants. Ainsi, son cycle reproductif dilue, lors la production massive de gamètes, les concentrations en éléments traces, et conduit à des différences plus ou moins marquée entre individus de sexes opposés. Le caractère conservatif de la distribution des éléments traces entre les différents compartiments corporels des individus suggèrent une importante régulation physiologique de leurs niveaux internes. Enfin, la taille des moules utilisées dans le présent travail, issue de l'aquaculture, n'influence que peu les concentrations interindividuelles, toutes les moules d'un même lot ayant approximativement le même âge.

En conclusion, le présent travail a permis d'améliorer et d'élargir l'état des connaissances relatives au monitoring de la pollution par les éléments traces, en particuliers par des éléments peu étudiés de préoccupation environnementale avérée, via l'utilisation des deux principaux bioindicateurs utilisés en Méditerranée, soit la posidonie et la moule méditerranéenne.

Summary

The Mediterranean Sea, a semi-enclosed sea with densely populated coasts, is submitted to numerous anthropogenic pressures: among them, the chemical pollution by traces elements. These pollutants, coming from our continental activities, are transported through rivers or by air and accumulate in seas and oceans where they mainly affect coastal areas. During the 70^{ies}, scientist suggested to use organisms, in particular mussels of the genus *Mytilus*, in order to evaluate the status of chemical contamination of coastal ecosystems. Biomonitoring was born. Since, many monitoring studies were published using various animal and vegetal species.

Two of the most studied bioindicators species in the Mediterranean are the marine magnoliophyte *Posidonia oceanica* and the Mediterranean mussel *Mytilus galloprovincialis*. Monitoring studies with these two species have mainly focussed on contaminations by Cr, Ni, Cu, Zn, Cd, Pb (*Mytilus galloprovincialis* and *Posidonia oceanica*), Fe (*Posidonia oceanica*), As, V and Ag (*Mytilus galloprovincialis*). However, other trace elements like Be, Al, Mn, Co, Se, Mo, Sn, Sb and Bi have been subject to nearly no ecotoxicological survey. Furthermore the worldwide evolution of our technologies and of our lifestyle increases the extraction and production of trace elements (notably to answer needs of developing countries). The biomonitoring of the pollution by trace elements is henceforth a topical subject.

The overall objective of this work was therefore to evaluate the potential use of *Mytilus galloprovincialis* and *Posidonia oceanica* as bioindicators to monitor the Mediterranean coastal pollution by Be, Al, Mn, Co, Se, Mo, Sn, Sb, Bi, Fe, As, V, and Ag. These trace elements, mostly little studied, can be categorized as elements of “environmental emerging concern”. A time-integrated efficient monitoring of trace elements requires the continuous survey of their environmental levels; we therefore also measured levels of trace elements classically monitored with these two species.

Mytilus galloprovincialis and *Posidonia oceanica* complement each other in monitoring surveys. Both species accumulate pollutants dissolved in the water column. *Posidonia oceanica*, rooted in the seafloor, accumulates moreover pollutants stored in sediments in the long term. *Mytilus galloprovincialis*, as a filter feeder, further accumulate particulate pollutants suspended in the water column. The combined use of both bioindicators

therefore provides a global view of the health status of the coastal environment (water, sediments, suspended mater).

Our first goal was to measure, at the scale of the French Mediterranean littoral, the spatial variability of trace element contents in *Posidonia oceanica*, and to determine if trace elements of environmental emerging concern threaten the chemical integrity of coastal ecosystems. We observed that the large majority of trace elements little or no monitored with *P. oceanica* showed an equivalent to higher spatial variability than elements classically monitored with that species. We also showed that the spatial variability could be associated to specific anthropic activities like agriculture (Mo), mining (Sb), storage and refinement of oil products (V), or the presence of harbours and major urban centres (Sn, Bi). Their monitoring, along with the one of trace elements classically studied in the Mediterranean, turns out to be essential. In addition, the in-depth study of the contamination state of the Calvi Bay (Northwestern coast of Corsica, France), enabled us to define (or re-define) this site as a reference site for the monitoring of the chemical pollution by trace elements in the Northwestern Mediterranean.

We further studied the physiological mechanisms of accumulation, storage and excretion of trace elements by *Posidonia oceanica. In situ* contamination of seagrass bed portions allowed us to model the rapid kinetics of accumulation of contaminants by *Posidonia oceanica* shoots. Compartments of the plant answered differently to pollutants exposure. So, adult and senescent leaves assimilated pollutants less rapidly than young actively growing leaves. Trace elements, once accumulated, could be redistributed between the plant compartments, notably towards the rhizomes-roots systems buried in sediments. Our results experimentally showed that these below-ground organs could therefore play the role of biological archives for many elements. At the end of periods of exposure to pollutants, kinetics of decontaminations of *Posidonia oceanica* shoots were relatively fast and depended notably on the duration of exposure to trace elements, on their toxic or essential character and on the studied compartment. We concluded that *Posidonia oceanica* was a sensitive bioindicator for the monitoring of the past and present coastal pollution by trace elements.

We showed that *Mytilus galloprovincialis* efficiently accumulated trace elements of environmental emerging concern as well as elements classically studied with this bioindicators species. The physiology of mussels further conditioned their answers to pollutant exposures. Their reproductive cycle dissolved trace element concentration during the

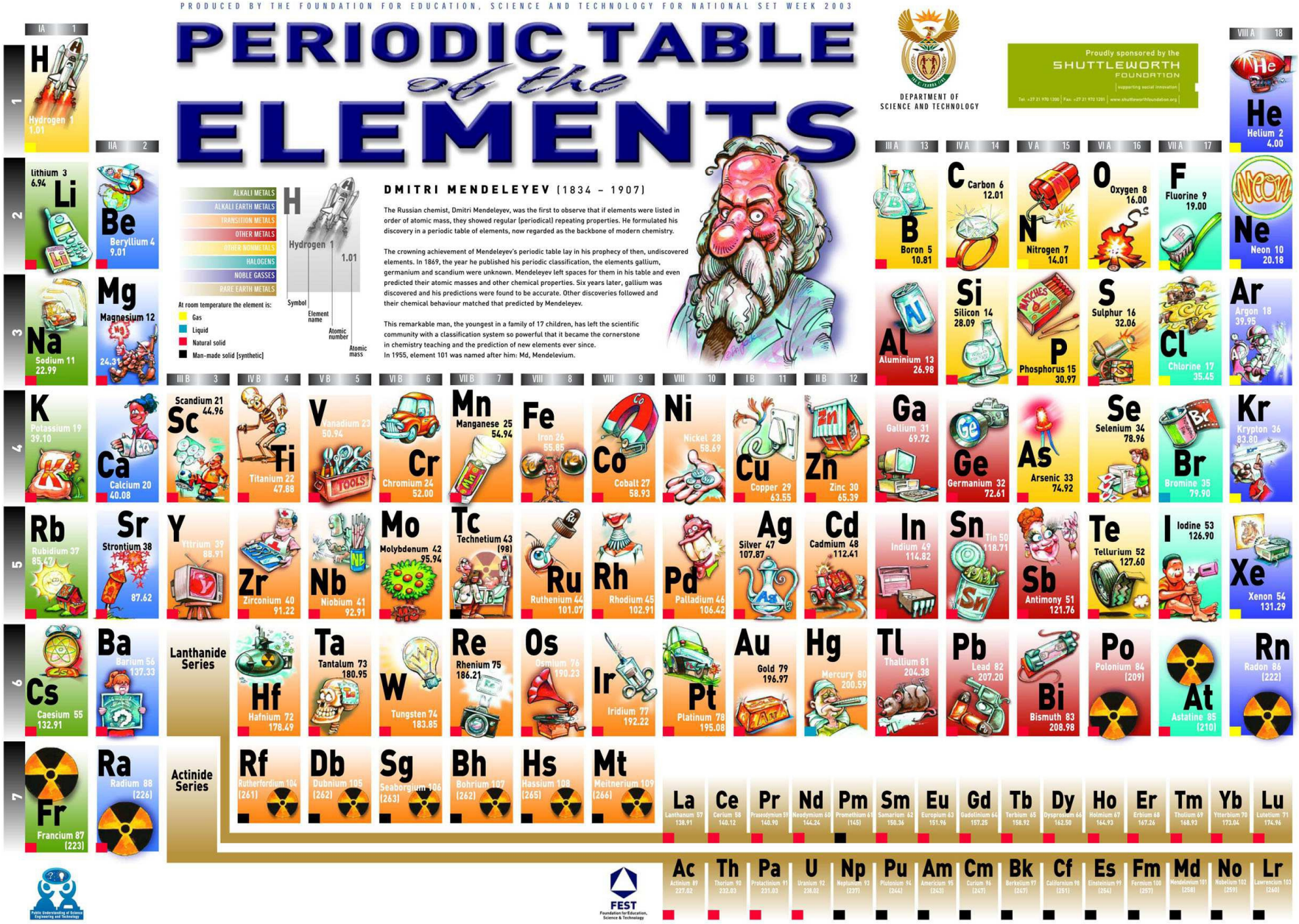
massive production of gametes and conducted to differences more or less important between individuals of both sexes. The conservative character of the distribution of trace elements between the different body compartments of *M. galloprovincialis* suggested an important physiological regulation of their internal levels. Finally, the size of mussels used in this study, harvested from an aquaculture farm, did not noticeably influence inter-individual concentrations, all mussels of a same rope having approximately the same age.

In conclusion, this study enabled to improve and enlarge our state of knowledge about the monitoring of the pollution of the Mediterranean coastal environment by trace elements. In particular, both *M. galloprovincialis* and *P. oceanica* showed to be good candidates for the monitoring of trace elements of environmental emerging concern.

List of abbreviations

3IL: 3rd Intermediate Leaves
ASTM: American Society for Testing and Materials
BAL: Blades of Adult Leaves
BiPO: Biotic index using *Posidonia oceanica*
BQE: Biological Quality Element
CGDD: Commissariat Général au Développement Durable
CI: Condition Index
COT: Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CRM: Certified Reference Material
DGT: Diffusion Gradient in Thin Films
DL: detection limit
DM: dry matter
DNA: deoxyribonucleic acid
DOC: dissolved organic carbon
DRC ICP-MS: Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology
EC50: the median effect concentration
EDTA: ethylenediaminetetraacetic acid
EEA: European Environment Agency
EPA: United States Environmental Protection Agency
ERL: effects range-low
ERM: effects range-median
EROD activity: Ethoxyresorufin-O-deethylase activity
Fm: maximum fluorescence
Fm': light-adapted maximal fluorescence
Fv: variable fluorescence
GSH: glutathione
GST: glutathione S-transferase
HC: Health Canada
IFREMER: Institut Français de Recherche pour l'Exploitation de la Mer
L_C: critical limit
LC50: the median lethal concentration
L_D: detection limit
L_Q: quantification limit
MN: micronucleus
mRNA: messenger ribonucleic acid
NRR: neutral red retention
OIL: Other Intermediate Leaves
PACA: Provence-Alpes-Côte d'Azur
PAM fluorometry: pulse amplitude modulation fluorometry
PAM: Plan d'Action pour la Méditerranée

PNUE: Programme des Nations Unies pour l'Environnement
PoCMT1: one member of the *Posidonia oceanica* chromomethylase (CMT) family
POMI: *Posidonia oceanica* multivariate index
PoMT2k: *Posidonia oceanica* Metallothionein (MT) 2k
PREI: *Posidonia oceanica* rapid easy index
PSII: photosystem II
SAL: Sheaths of Adult Leaves
Sn_{TBT}: tin as tributyltin species
STARESO: Station de Recherche Océanographiques et sous-marines
TBT: tributyltin
TE: trace element
TSS: total suspended solids
TT Action Level: Treatment Technique Action Level
UN: United Nations
USSG: United States Geological Survey
WFD: Water Framework Directive
WHO: World Health Organization
WSH deaths: deaths from unsafe water, sanitation and hygiene
ΔF: fluorescence yield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ALKALI METALS
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At room temperature the element is:
Gas
Liquid
Natural solid
Man-made solid [synthetic]
Symbol
Element name
Atomic number
Atomic mass
DMITRI MENDELEYEV (1834 – 1907)
The Russian chemist, Dmitri Mendeleyev, was the first to observe that if elements were listed in order of atomic mass, they showed regular (periodical) repeating properties. He formulated his discovery in a periodic table of elements, now regarded as the backbone of modern chemistry.
The crowning achievement of Mendeleyev's periodic table lay in his prophecy of then, undiscovered elements. In 1869, the year he published his periodic classification, the elements gallium, germanium and scandium were unknown. Mendeleyev left spaces for them in his table and even predicted their atomic masses and other chemical properties. Six years later, gallium was discovered and his predictions were found to be accurate. Other discoveries followed and their chemical behaviour matched that predicted by Mendeleyev.
This remarkable man, the youngest in a family of 17 children, has left the scientific community with a classification system so powerful that it became the cornerstone in chemistry teaching and the prediction of new elements ever since.
In 1955, element 101 was named after him: Md, Mendelevium.
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Chapter 1
-
General introduction

1. The sick Ocean

1.1. Human pressures on oceans

Such as terrestrial ecosystems, marine ecosystems are submitted to increasing anthropogenic disturbances. Land-based activities affect the runoff of pollutants and nutrients into coastal waters and remove, alter, or destroy natural habitats, while ocean-based activities extract resources, add pollution and change species composition (Halpern et al. 2008).

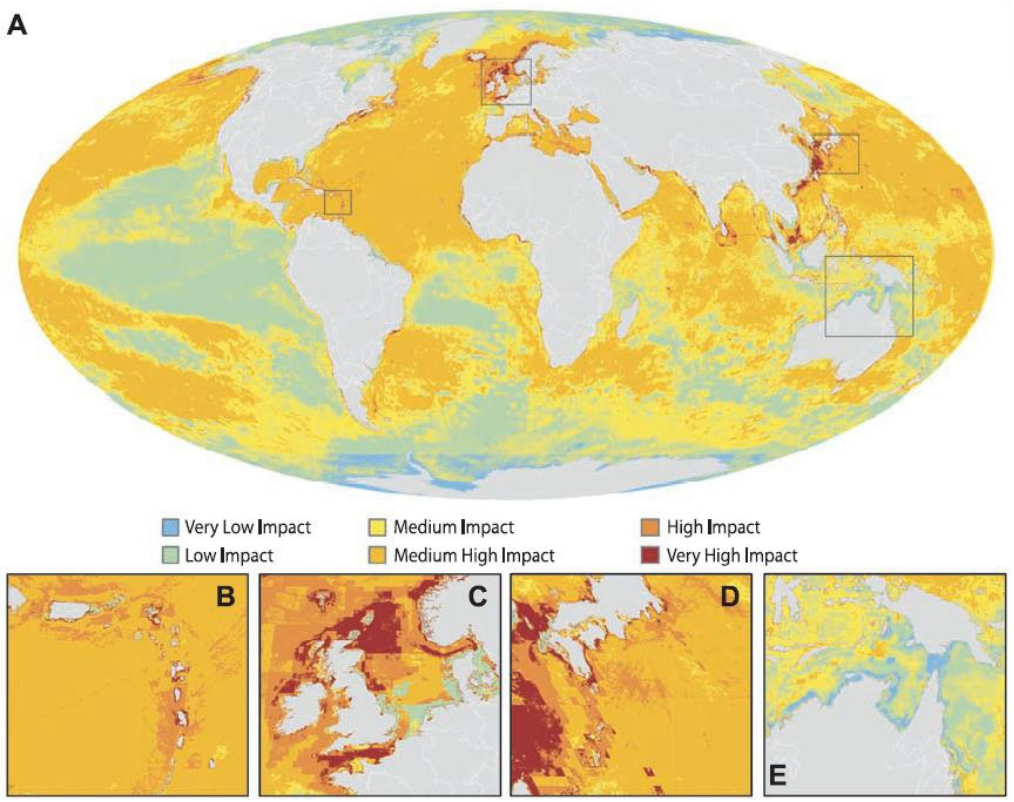

Fig. 1. Global map (A) of cumulative human impact across oceans. Insets: highly impacted regions in the Eastern Caribbean (B), the North Sea (C), and the Japanese waters (D) and one of the least impacted regions, in northern Australia and the Torres Strait (E) (modified after Halpern et al. 2008).

On the basis of expert judgment, Halpern et al. (2007, 2008) recently mapped the impact of 17 anthropogenic drivers of ecological change (e.g. pollution, fishing, ocean acidification, species invasion etc.) on marine ecosystems (Fig. 1), combined into a single comparable estimate of cumulative human impact (6 levels, from very low to very high impact). Their analysis indicated that no area is unaffected by human influence, and a large

fraction (41% of the oceans) is strongly affected (from medium high to very high scores) by multiple drivers. However, some large areas of relatively little human impact still remain (3.7% of the oceans), particularly near the poles were seasonal or permanent ice limits human access.

These increasing pressures upon the marine realm threaten ecosystems, especially seabed biotopes (Salomidi et al. 2012). The ecologic, economic and social importance of marine ecosystems being irrefutable (Costanza et al. 1997, Salomidi et al. 2012), a well-planned approach of managing the marine space is essential to achieve sustainability (Salomidi et al. 2012). Otherwise, entire ecosystems will stop functioning under their actual form, as is the case for the highly productive hotspots of biodiversity that are coral reefs (Hughes et al. 2003), which is likely to lead to the complete loss of goods and services derived from these ecosystems (Worm et al. 2006).

1.2. The polluted Mediterranean Sea

The intense human activities, particularly in coastal areas surrounding the enclosed and semi-enclosed seas, always lead in the long term to important environmental impacts which are reflected in the form of a marine and coastal degradation and an increase of the risk of more important damages (Papathanassiou and Gabrielides 1999). The Mediterranean is one of the richest regions of Europe in terms of diversity of marine species with a high rate of endemism (Bianchi and Morri 2000). In the Mediterranean, coastal ecosystems are dominated by macrophytes (magnoliophytes and algae; Boudouresque 2004), a globally net autotrophic system displaying many ecological benefits (e.g. primary production, habitats, source of food and oxygen, carbon well, stabilization of sediments etc.; Levin et al. 2001). Despite their environmental, economic and social importance, a growing number of reports document the occurring regression and/or ecofunctional changes of these ecosystems (e.g. Occhipinti-Ambrogi and Savini 2003, Ardizzone et al. 2006, Boudouresque et al. 2006)

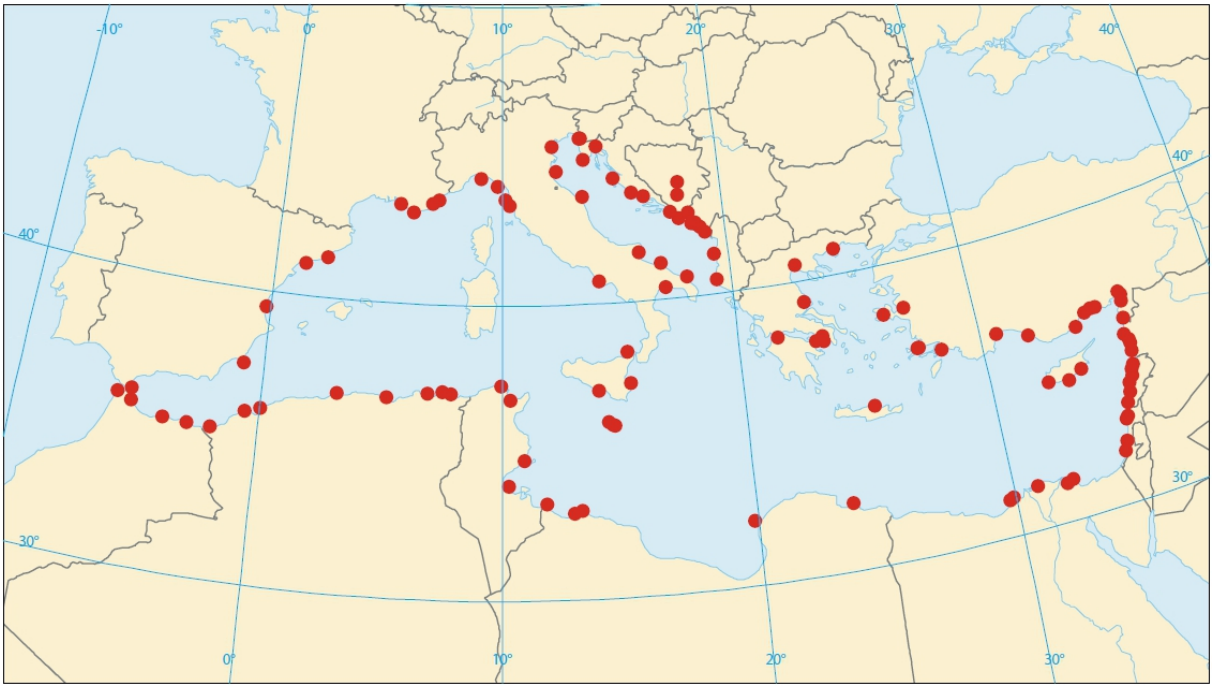

Fig. 2. Pollution hot spots (full red circles) along the Mediterranean coasts (EEA 2006).

Pressures suffered by the Mediterranean (e.g. pollution hot spots, Fig. 2) make it a vulnerable ecological unit (Turley 1999). This sea is of too small dimensions to ecologically self-counterbalance. The point of saturation of the pollutants discharged in the Mediterranean, whose area is the 35th of that of the Atlantic, will be more quickly achieved than in the oceans. In addition, the almost total absence of tide does not allow the dilution of pollutants and prevents the natural phenomena of depuration as encountered in larger bodies of water (i.e. in oceans). The Mediterranean also shows a deficiency in the movement of its deep water masses, and of its surface currents which "turn in circles" in this almost closed basin. As well, the answer of the Mediterranean to environmental disturbances is more rapid than in the larger oceans (Augier 2010). These specificities make therefore of the Mediterranean a privileged site of study of pressures and changes performed by men on the environment, and announced the scenarios that we could live on in the future in all the oceans of the world (Bethoux et al. 1999).

Major environmental problems along the French Mediterranean coasts are caused by river transported pollution, treated industrial and urban wastewater and intense urbanisation along its densely populated coastline. Areas of environmental concern are shown in Fig. 3; their major corresponding anthropogenic activities are listed below: • Marseilles and Nice are

relatively big coastal cities (density > 3000 persons per km^2) discharging mostly treated urban wastewater into the sea; • river Rhône transports significant loads of nutrients and other pollutants (organic matter, trace elements) from its drainage basin; • in the area of Fos-Étang de Berre are located the biggest French and the second largest European harbour hosting oil and methane terminals, as well as a large industrial complex; • rivers Hérault and Gard are considered as vectors of industrial pollution (hydroelectric and nuclear plants, petroleum processing, electronic, metal plants and chemicals); • in harbours of Marseilles, Sète, Port-la-Nouvelle, Port-Vendres, Toulon, Nice, Bastia and Ajaccio, petroleum hydrocarbon pollution occurs because of deballasting practices and accidental oil spills (EEA 2006).

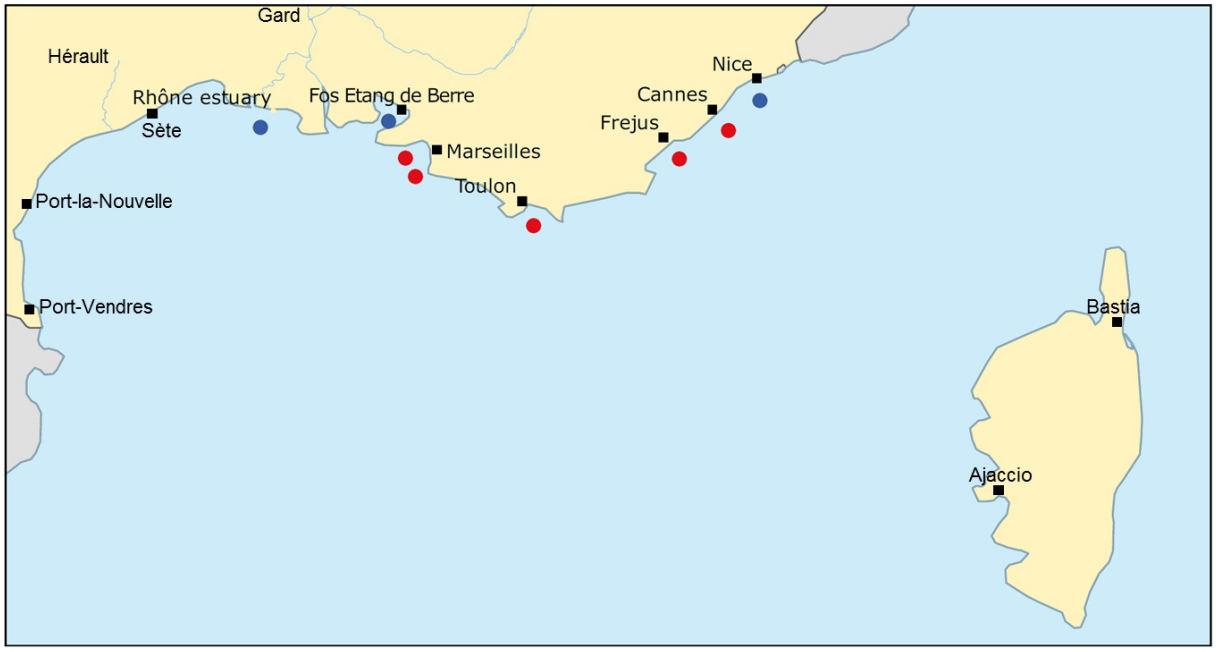

Fig. 3. Zoom on the Mediterranean coastlines of France. Full red circles: pollution hot spots; blue full circles: areas of major concern; full black squares: coastal cities (modified from EEA 2006).

As regards trace elements, the high levels currently measured in the Mediterranean indicate non-stationary geochemical cycles which result from an increase of external inputs (Saliot 2005). Effectively, although trace elements are present naturally in the environment, their current concentrations observed in coastal waters are linked to the growth of industrial, agricultural and urban activities since the early sixties (e.g. Bethoux et al. 1990, PNUE/PAM 2009). These anthropogenic activities generate the introduction of a considerable amount of chemicals in the marine coastal ecosystem; these substances show toxic properties likely to

cause multiple damage at the level of organisms, populations and ecosystems (Nordberg et al. 2007, Amiard 2011).

2. Trace elements

2.1. Definition

According to the International Union of Pure and Applied Chemistry (McNaught and Wilkinson 1997), trace elements (TEs) are any element having an average concentration of less than about 100 parts per million atoms (ppma) or less than 100 $\mu g.g^{-1}$ (Fig. 4). Such a precise definition does not exist in earth sciences, because the concentration of an element in a given phase can be so low that it is considered a trace element, whereas the same element can constitute a main part of another phase (e.g. Fe and Al; Navratil and Minarik 2011).

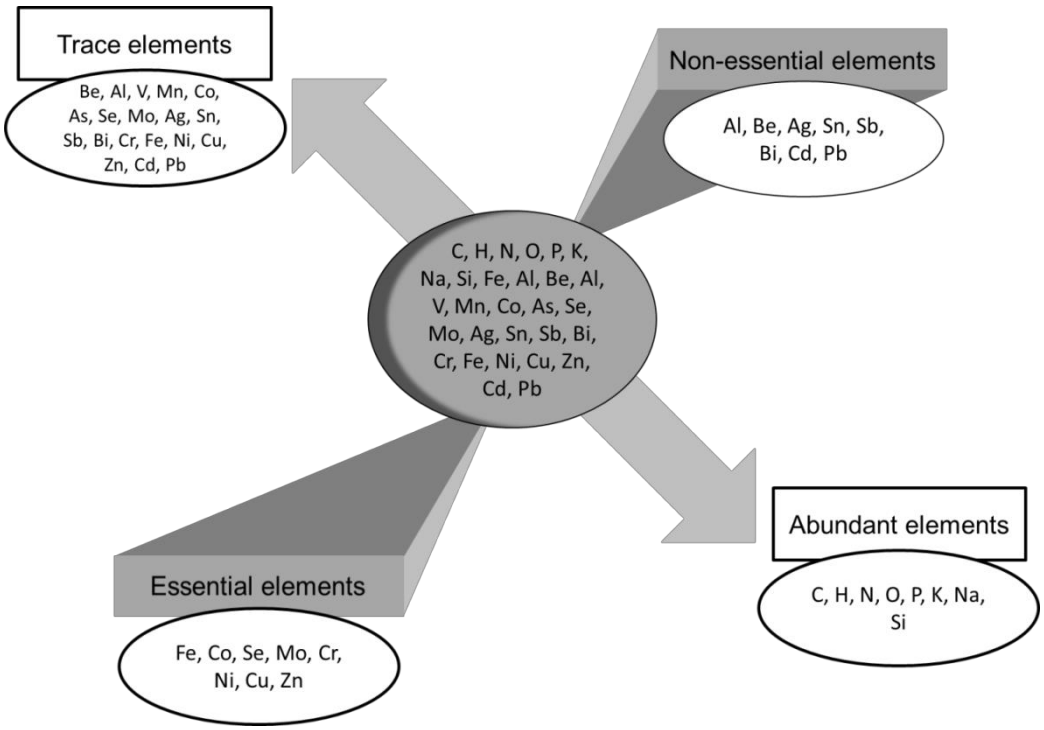

Fig. 4. Essential and non-essential elements, abundant or in traces (modified after Amiard 2011).

Previously, scientists used the generic term "heavy metals" when referring to trace elements. Today this appellation is discussed. Effectively, some metals are not particularly "heavy" (e.g. Al, Ni). In addition, some elements are not metals (e.g. As, Se). For these

reasons, the majority of researchers prefer today the name "metallic trace elements" (if it is indeed metals) to the appellation "heavy metals", or then the formula "trace elements" when they are not metals (As, Se, B) (Duffus 2002).

Trace elements can either be essential or non-essential (Fig. 4); the essential elements recognized by the World Health Organization (WHO) are I, Zn, Se, Fe, Cu, Cr and Mo, the latter playing an important role in biological systems (WHO 1996, 2004). Others TEs may/could also be essential, such as Mn, Co, As, Ni, V etc; the essentiality is a characteristic which evolves according to our knowledge and to the sensitivity of the authors who have a propensity more or less strong to classify an element among the essential or not (Amiard 2011). Non-essential TEs such as Hg, Pb or Cd play no physiological role, and are often toxic even in very small quantity (Nordberg et al. 2007). For these non-essential TEs, only a threshold of toxicity exists, while essential TEs can be either deficient in too small quantities, either toxic when they are absorbed in high concentrations (Amiard 2011).

2.2. Production and uses

If the world refinery and mine production of some trace elements (e.g. As, Cd, Pb, Sn) has proportionally little evolved since the 80^{ies}, most of them have known a substantial increase (e.g. Fe, Al, Mo), particularly since the beginning of years 2000 (Table 1, Fig. 5). World demand for minerals is affected by 3 general factors: (i) uses for mineral commodities, (ii) the level of population that will consume these mineral commodities, and (iii) the standard of living that will determine just how much each person consumes (Kesle 2007).

Today, with the integration of India, the People's Republic of China and other populous developing and emerging countries (e.g. Brazil and Russia) into the world economy, more than 50 % of the world's population (instead of the previous 20 %) account for the largest part of raw materials consumption (Tiess 2010); alone, the People's Republic of China's demand for Al, Pb, Cu, Ni, Zn and Sn accounted for 32 ± 5 % of the world consumption in 2008 (Sievers et al. 2010). This increasing demand for mineral raw materials further concerns numerous "emerging" elements. These elements can either be truly emerging as they have just gained entry to the environment or because they are "contaminants of emerging concern" (Daughton 2004, 2005), as is the case for V, Sb or Bi.

TE	Symbol	Year				
		1990	2000	↗(1990)	2010	↗(1990)
Aluminum	Al	17.817	24.400	37%	40.800	129%
Antimony	Sb	83,2	122,0	47%	167,0	101%
Arsenic	As	47,6	36,9	-23%	52,8	11%
Beryllium	Be	0,286	0,226	-21%	0,203	-29%
Bismuth	Bi	3,333	3,752	13%	8,467	154%
Cadmium	Cd	20,16	20,23	0%	21,40	6%
Chromium	Cr	12.846	4.320	-66%	7.290	-43%
Cobalt	Co	37,1	33,3	-10%	89,5	141%
Copper	Cu	8.815	13.200	50%	16.000	82%
Iron	Fe	543.000	1.061.148	95%	2.590.000	377%
Lead	Pb	3.367	3.100	-8%	4.140	23%
Manganese	Mn	27,2	20,2	-26%	42,7	57%
Molybdenum	Mo	112	129	16%	242	117%
Nickel	Ni	1.029	1.250	21%	1.590	54%
Selenium	Se	1.789	1.460	-18%	2.120	19%
Silver	Ag	17,7	18,4	4%	23,1	31%
Tin	Sn	219	238	9%	265	21%
Vanadium	V	31,0	43,0	39%	57,6	86%
Zinc	Zn	7.325	8.730	19%	12.000	64%

Table 1. World production of 19 trace elements of concern for the years 1990, 2000 and 2010, and percentage of increase by decades (data compiled from the Mineral Yearbooks published by the US Geological Survey website, http://www.usgs.gov/).

The use of these contaminants of emerging concern are multiple and diverse. V is regarded as one of the hardest of all metals. Since 1992, the world production of has V increased by 50 % per decade (Fig. 5a). This ubiquitous TE is employed in a wide range of alloys for numerous commercial applications extending from train rails, tool steels, catalysts, to aerospace (Moskalyk and Alfantazi 2003).

Antimony, whose mine production was 3 times higher in 2011 than in 1985 (Fig. 5b), greatly increases the hardness and the mechanical strength of lead, and is found in batteries, antifriction alloys, type-metal, small arms and tracer bullets, and cable sheathing. It further has many uses as a flame retardant (in textiles, papers, plastics and adhesives), as a paint pigment, ceramic opacifier, catalyst, mordant and glass decolouriser, and as an oxidation catalyst (Filella et al. 2002, Shtangeeva et al. 2011).

Bismuth consumption deeply decreased during the second half of the 80^{ies} (Fig. 5c), mainly because of a decline in usage by the chemical and pharmaceutical industries. Nowadays, Bismuth is anew largely consumed in low-melting alloys and metallurgical additives, including electronic and thermoelectric applications. The remainder is used for catalysts, pearlescent pigments in cosmetics, pharmaceuticals, and industrial chemicals (Fowler and Sexton 2007).

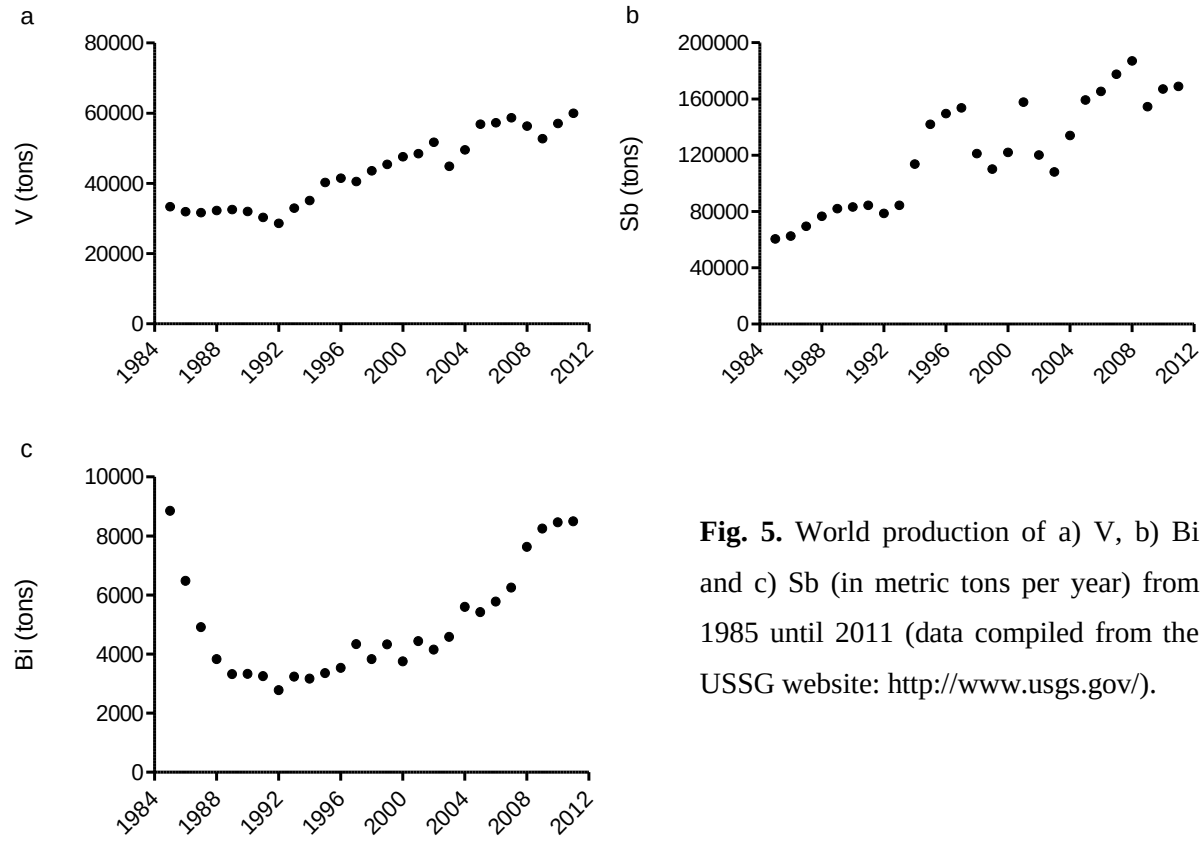

Fig. 5. World production of a) V, b) Bi and c) Sb (in metric tons per year) from 1985 until 2011 (data compiled from the USSG website: http://www.usgs.gov/).

2.3. Human toxicity

The major exposure media to TEs for humans are food, water and air (Beckett et al. 2007). The whole human kind needs water for sustaining life; the provision of a safe drinking water supply is a high priority issue for safeguarding the health and well-being of humans (Van Leeuwen 2000, WHO 2011) and is an important development issue at national, regional and local levels (WHO 2011). Although standards legislate the acceptable levels of contaminants in drinking water (Table 2), the supply of quality water remains a major challenge for humanity in the twenty-first century (Fig. 6; Schwarzenbach et al. 2010).

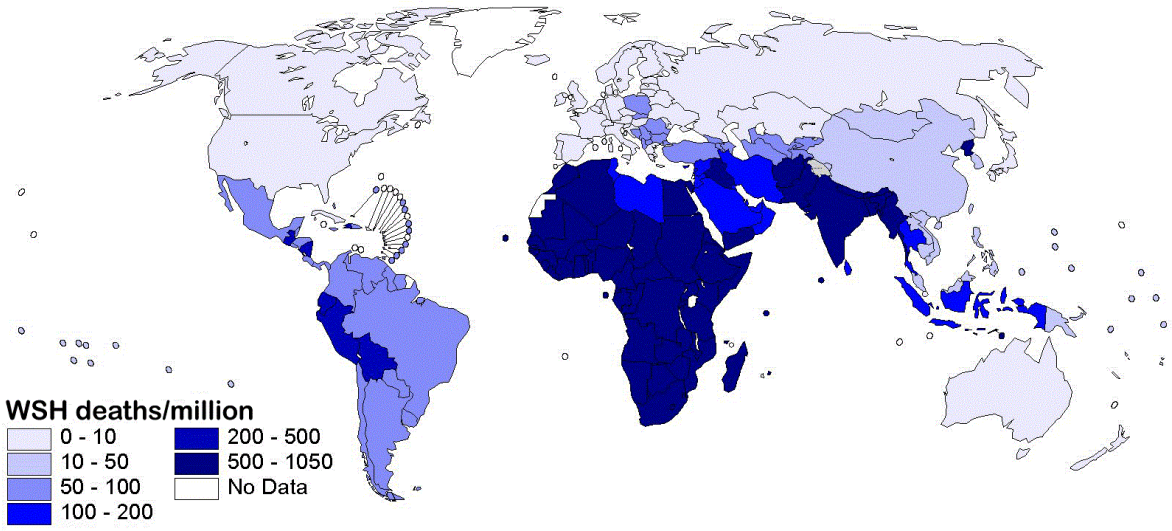

Fig. 6. Deaths from unsafe water, sanitation and hygiene (WSH) estimated by WHO sub-region for year 2000 (WHO 2002).

The spectrum of adverse effects caused by the consumption of contaminated water is huge and ranges from relatively harmless to life threatening (Table 2; Van Leeuwen 2000). Inorganic As is present in groundwater used for drinking in several countries all over the world (e.g. Bangladesh, Chile and China). Populations exposed to As via drinking water show excess risk of mortality from lung, bladder and kidney cancer, the risk increasing with increasing exposure. There is also an increased risk of skin cancer and other skin lesions, such as hyperkeratosis and pigmentation changes (Järup 2003).

Japanese who consumed Cd polluted rice and river water over a period of 30 years were found to have accumulated in their bodies a large amount of Cd that lead to a serious osteoporosis-like bone disease known to the Japanese as “itai–itai byo” or “ouch–ouch disease” (Pan et al. 2010). Industrial wastes and agriculture activities have released hazardous and toxic materials in the groundwater and thereby led to the contamination of drinking water of some of the great Cairo cities, Egypt. Strong relationships between contaminated drinking water and chronic diseases were identified: renal failure was related to Pb and Cd contaminations, liver cirrhosis to Cu and Mo, hair loss to Ni and Cr, and chronic anaemia to Cu and Cd (Salem et al. 2000).

Trace element	Guideline values (ppm) from the WHO, the USEPA and HC	Common sources of trace elements in drinking water	Potential human health effects
Al	WHO: 0.9 (< 0.1 in conventional treatment plants; < 0.2 in other treatment types)	Erosion of natural deposits; Al salts used as flocculents during the treatment of drinking water.	Little indication that orally ingested Al is acutely toxic to humans; no adverse health effect at levels found in drinking water; Al exposure is a risk factor for the development or acceleration of onset of Alzheimer disease.
Sb	WHO: 0.020; EPA, HC: 0.006	Erosion of natural deposits; discharge from petroleum refineries; fire retardants; ceramics; electronics; solders; contaminants from pipes and fittings.	Increase in blood cholesterol; decrease in blood sugar; microscopic changes in organs and tissues (thymus, kidney, liver, spleen, thyroid).
As	WHO, EPA: 0.01	Erosion of natural deposits (erosion and weathering of soils, minerals, ores); runoff from orchards; runoff from glass and electronics production wastes.	Skin damage or problems with the circulatory system; increased risk of getting cancer (lung, bladder, liver, skin - classified as human carcinogen); neurological effects (numbness and tingling of extremities).
Be	WHO: 0.010; EPA: 0.004	Discharge from metal refineries and coalburning factories; discharge from electrical, aerospace and defense industries.	Intestinal lesions; rarely found in drinking-water at concentrations of health concern.
Bi	No guideline value	Concentrations of Bi in drinking water have not been reported.	Doses used in medicines are very much larger than the estimated dietary exposure; dietary exposures to Bi are unlikely to be of toxicological concern.
Cd	WHO: 0.003; EPA, HC: 0.005	Erosion of natural deposits; corrosion of galvanized pipes; discharge from metal refineries; runoff from waste batteries and paints; leaching from solders or black polyethylene pipes; industrial and municipal waste.	Kidney damage; softening of bone; classified as human carcinogen.
Cr	WHO, HC: 0.05; EPA: 0.010	Erosion of natural deposits; releases or spills from industrial uses (steel and pulp mills).	Enlarged liver; irritation of the skin, respiratory and gastrointestinal tracts; kidney problems.
Co	No guideline value	Drinking water has a low-Co content, usually between 0.0001 and 0.005 ppm.	Cardiovascular effects (cardiogenic shock, sinus tachycardia, left ventricular failure, and enlarged hearts) observed in people who consumed large amounts of beer over several years time containing Co sulfate as a foam stabilizer; gastrointestinal effects (nausea, vomiting, and diarrhea), effects on blood, liver injury, and allergic dermatitis have also been reported in humans from oral exposure to Co.
Cu	WHO: 2; EPA: TT Action Level = 1.3; HC ≤ 1.0	Erosion of natural deposits (erosion and weathering of rocks and minerals); corrosion of household plumbing systems; contaminants from pipes and fittings; acidic mine water drainage; landfill leachates; sewage effluents; iron-related industries.	Short term exposure: gastrointestinal distress; long-term exposure: liver or kidney damages. Cu is an essential element in human metabolism; adverse health effects occur at levels much higher than the aesthetic objectives.
Fe	HC: aesthetic objectives ≤ 0.3	Erosion of natural deposits (erosion and weathering of rocks and minerals); use of Fe coagulants; corrosion of steel and cast iron pipes.	Not of health concern at levels causing acceptability problems in drinking-water.
Pb	WHO, HC: 0.010; EPA: TT Action Level = 0.015	Erosion of natural deposits; corrosion of plumbing systems (pipes, solders, brass fittings and lead service lines); contaminants from pipes and fittings.	Infants and children (under 6 years): delays in physical or mental development; neurobehavioural effects; children could show slight deficits in attention span and learning abilities. Adults: Kidney problems; high blood pressure. Others: anaemia; central nervous system effects; in pregnant women, can affect the unborn child; classified as probably carcinogenic to humans.
Mn	WHO: 0.4; HC: aesthetic objectives ≤ 0.05	Erosion and weathering of rocks and minerals; naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions (the most important source for drinking-water).	Not of health concern at levels causing acceptability problems in drinking-water.
Mo	WHO: 0.02	Contaminations may occur in areas where Mo ore is mined.	Occurs in drinking-water at concentrations well below those of health concern.
Ni	WHO: 0.07	Ni levels in natural waters ranges from 0.002 to 0.010 ppm in fresh and tapwater; water is generally a minor contributor to the total daily oral intake; the Ni contribution from water may be significant where there is heavy pollution, where there are areas in which Ni occurs naturally in groundwater or where there is use of certain types of kettles of non-resistant material in wells or of water that has come into contact with Ni-plated taps; released from fittings; released from industrial Ni deposits.	Lack of evidence of a carcinogenic risk from oral exposure to Ni.
Se	WHO: 0.04; EPA: 0.05; HC: 0.01	Naturally occurring (erosion and weathering of rocks and soils); discharge from petroleum and metal refineries; discharge from mines.	Toxic effetcs: hair or fingernail losses at extremely high levels of exposure; numbness in fingers or toes; circulatory problems.
Ag	WHO: available data inadequate to permit derivation of health-based guideline value; EPA: 0.1	Naturally occurring (erosion and weathering of rocks and soils); drinking water, not treated with Ag for disinfection purposes, usually contains extremely low concentrations of Ag.	Water-soluble Ag compounds such as nitrate have a local corrosive effect and may cause fatal poisoning if swallowed accidentally.
Sn	No guideline value	Drinking-water is not a significant source of Sn; increasing use of Sn in solder, which may be used in domestic plumbing, and proposed for use as a corrosion inhibitor.	Occurs in drinking-water at concentrations well below those of health concern; excessive levels of Sn in canned beverages or other canned foods has been acute gastric; no evidence of adverse effects in humans associated with chronic exposure to Sn.
V	No guideline value	Typical values of V concentrations in drinking water are below the detection limit.	The main source of V intake is food; V little absorbed in the gastro-intestinal tract and mainly eliminated unabsorbed with the faeces.
Zn	HC: aesthetic objectives ≤ 0.05	Naturally occurring; industrial and domestic emissions; leaching may occur from galvanized pipes, hot water tanks and brass fittings; Zn concentrations in water from active or inactive mines can be substantial.	Not of health concern at levels found in drinking-water; effects on human health by contamination on water supplies must be rare.

Table 2. Trace element guildeline values (ppm) for drinking water, common sources of trace elements in water and potential effects on human health. Data compiled from the World Health Organization (WHO, 2011), the United States Environmental Protection Agency (EPA, 2009), Health Canada (HC, 2012), Nordberg et al. (2007) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT, 2008). Treatment Technique (TT) Action Level: US drinking water requiring a treatment process in order to reduce the level of a contaminant.

Essential micronutrient may also show toxic effects when ingested at too high levels, as reviewed by Goldhaber (2003) and widely detailed in Nordberg et al. (2007). The ingestion of very high doses of Cr causes liver and kidney problems. Abdominal pain, cramps, nausea, diarrhea, and vomiting have been caused by the consumption of beverages containing high levels of Cu, and liver damage has been seen in individuals with diseases of Cu metabolism. Although exposure to Mn is not of health concern at levels causing acceptability problems in drinking-water, the consumption of Mn-containing well water has caused lethargy, tremor, and mental disturbances in Japan, and neurologic symptoms were reported in individuals exposed to Mn-contaminated drinking water in Greece.

The rivers, estuaries, coastal seas and oceans form an ecological continuum where pollutants pass in transit (CGDD 2011). According to the United Nations (UN 2004), more than 80 % of the pollution of the seas comes from inland via the rivers or through runoff and discharges from the coastal areas. As at least 60 % of the world's population live within 100 km of the coast, the contaminations of coastal waters may pose serious risks to human health as well as marine ecosystems (Tanaka 2006). It is therefore essential to monitor the chemical pollution of coastal areas.

2.4. TEs in the sea: sources and speciation

Continental runoff and atmospheric deposition are the primary inputs of TEs in the marine environment (Callender 2003): crustal material is either weathered on (dissolved) and eroded from (particulate) the Earth's surface or injected into the Earth's atmosphere by volcanic activity; forest fires and biogenic sources are of a lesser importance (Nriagu 1989, 1990). In addition to these natural sources, there exists a multitude of anthropogenic emission sources, the major ones resulting from mining and smelting activities. Mining releases metals to the fluvial environment as tailings and to the atmosphere as metal-enriched dust whereas smelting releases metals to the atmosphere as a result of high-temperature refining processes (Callender 2003). Other important land-based anthropogenic sources of TEs proceed from various municipal, industrial and agricultural activities (Tanaka 2006). The occurrence of TEs in effluents stemming from notorious anthropogenic sources is summarized in Table 3.

Source	Trace element																		
	Al	Ag	As	Be	Bi	Cd	Co	Cr	Cu	Fe	Mn	Mo	Pb	Ni	Sb	Se	Sn	V	Zn
Mining operations and ore processing	X		X			X					X	X	X					X	
Metallurgy and electroplating		X	X	X	X	X		X	X				X	X		X			X
Chemical industries	X		X			X		X	X	X			X			X	X		X
Dyes and pigments	X		X			X			X	X			X		X				
Ink manufacturing							X		X	X				X					
Pottery and porcelain			X					X							X				
Alloys				X															
Print								X					X						X
Photography		X				X		X				X				X			
Glass			X				X							X		X			
Paper mills	X							X	X						X				
Leather training	X		X					X	X	X									X
Pharmaceuticals	X								X	X						X			
Textiles	X		X			X			X	X				X	X				
Nuclear technology						X													
Fertilizers	X		X			X		X	X	X	X			X		X			X
Chlor-alkali production	X		X			X		X		X	X						X		X
Petroleum refining	X		X			X		X		X			X	X					X

Table 3. Occurrence of trace elements in effluents and associated land-based human sources (modified after Nagajyoti et al. 2010).

Trace elements are present in seawater under different oxidation states and chemical forms including free solvated ions, inorganic complexes (e.g. with Cl^-, OH^-, CO_3^{2-}, SO_4^{2-} etc.), organometallic compounds and organic complexes (e.g. with phytoplankton metabolites, proteins, humic substances etc; Donat and Bruland 1994). This distribution of elements amongst defined chemical species is termed "speciation" (Templeton et al. 2000). Speciation affects bioavailability, and bioavailability is determined by both external environmental conditions and the physiological/biological characteristics of organisms (Chapman 2008).

Addressing the chemical form of an element instead of its total concentration renders thenceforth the gained information much more valuable. The underlying reason for this is that the characteristics of just one species of an element may have such a radical impact on living systems (even at extremely low concentrations) that the total element concentration becomes of little value in determining its impact. A good example is Sn: the inorganic form of this element is much less toxic (or even does not show toxic properties), but the alkylated form is highly toxic (Cornelis and Nordberg 2007).

2.5. Removal processes from seawater

Unlike organic pollutants which can be degraded to less harmful components by biological or chemical processes, TEs are considered as non-degradable pollutants (Navratil and Minarik 2011, Pan and Wang 2012). This persistent character of TEs can alter, sometimes quite strongly, their natural biogeochemical balance in contaminated environments (Doney 2010). Processes removing TEs from seawater include active biological uptake or passive scavenging onto either living or non-living particulate material (Bruland and Lohan 2003). From the biological point of view, the most important uptake pathway is the mass transport through plasma membrane (Ahlf et al. 2009).

TEs are mainly transported into biological cells in ionic form due to the fact that ionic channels are involved (Fig. 7). In addition, specific transport mechanisms cross the membrane barrier like binding with membrane carrier proteins or transport through hydrophilic membrane channels. Lipid-soluble (non-polar) metal forms, including alkyl-TE compounds and neutral, lipophilic, inorganically complexed TE species can cross biological membrane by diffusion. TEs bound to very fine particles can also be engulfed by endocytosis (McGeer et al. 2004, Ahlf et al. 2009). Following absorption, metals are transported to internal organs for utilization, storage, toxic effects, and possibly release (Ahlf et al. 2009).

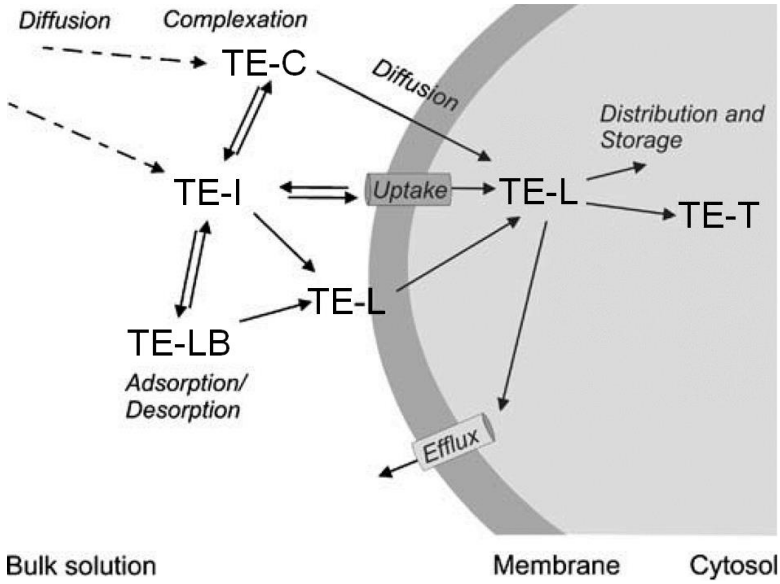

Fig. 7. Conceptual model of the main processes and sources for uptake of trace elements at a biological membrane. TE-C: TE complex; TE-I: TE ion; TE-LB: labile particle-bound TE; TE-L : TE bound to a biological ligand; TE-T : TE at target site (modified after Worms et al. 2006)

TEs can be removed from seawater through passive adsorption onto a wide variety of relatively high affinity surface sites on both living and dead particulate material (Bruland and

Lohan 2003). The combined process of surface adsorption, followed by particle settling, is termed scavenging (Goldberg 1954, Turekian 1977). Thereafter, labile bound TEs can also be desorbed from particles and resupply free metal ions to the dissolved metal pool (Fig. 7; Ahlf et al. 2009).

Much of the particulate material (along with its associated TEs) is internally recycled either in the water column or in surficial sediments. Marine sediments are usually considered as the ultimate sink of TEs where they can heavily accumulate (Bruland and Lohan 2003); marine sediments can also act as a source of TEs by releasing chemicals back to the overlying water column (Burton 2010, Pan and Wang 2012). The primary flux processes between sediments and the water column are resuspension and deposition, bioturbation, advection, upwelling/downwelling, diagenesis reactions, and diffusion (Burton 2010). Because of these remobilization processes, the effects of metal pollution on local environments and organisms can be substantial and long lasting in spite of years of restoration efforts (Pan and Wang 2012).

2.6. TE toxicity on marine biota

2.6.1. TE metabolism and toxicity testing

The potential impacts of contaminants on aquatic biota depend on the total concentrations of the contaminant, its speciation, interactions at receptors sites (e.g. at a fish gill membrane or on an algal cell surface), and uptake into the organism/cell, with either subsequent adverse effects or intracellular detoxification (Batley et al. 2004). Thenceforth, the sole exposure to a TE does not harm an organism; it has to be both absorbed and retained in sensitive portions of its life structure in toxic amounts (Chapman 2008).

The toxicity of each TE is related to a metabolically available threshold concentration (Rainbow 2002). TEs accumulated by biota occur in a bioreactive fraction that is metabolically active and available, and a fraction that has been detoxified and is unavailable (Fig. 8; Chapman 2008). TEs bound to inducible TE-binding proteins such as metallothionein or phytochelatin or precipitated into insoluble concretions consisting of TE-rich granules comprise biologically detoxified TEs, whereas TEs bound to sensitive fractions such as

organelles and heat-sensitive proteins can be metabolically active; the higher the proportion of TE-sensitive fractions, the greater the vulnerability to TE toxicity (Wallace et al. 2003, Wallace and Luoma 2003). The TE-sensitive fractions and TE bound to metallothionein represent TE available for trophic transfer to predators (Wallace and Luoma 2003). This compartmentalization of metal as defined by Wallace and Luoma (2003) and Wallace et al. (2003) is a useful tool to interpret multiple ecotoxicological consequences of the subcellular partitioning of metals within organisms.

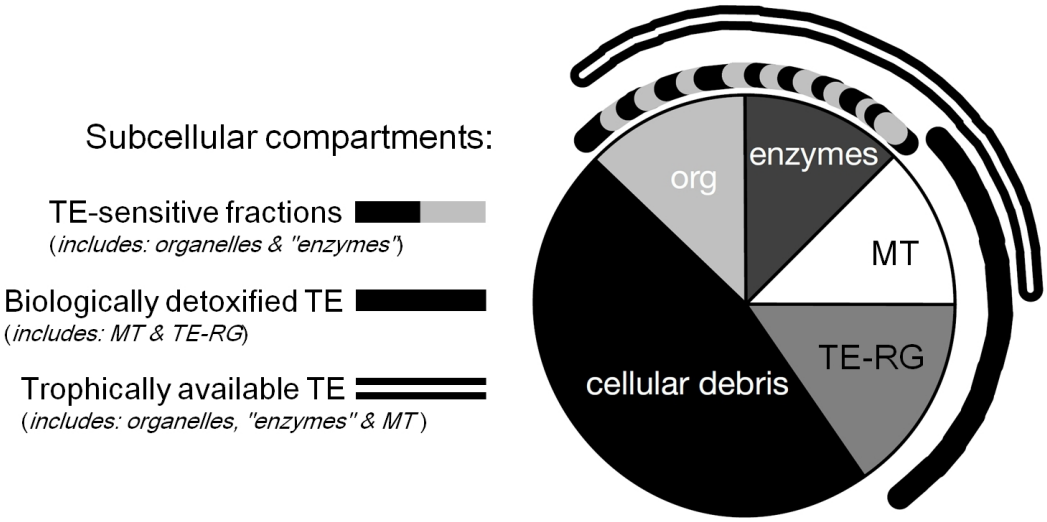

Fig. 8. A generalized ecotoxicological pie chart depicting subcellular compartments based on the biological significance of the various subcellular fractions in clams. Clams were homogenized, and differential centrifugation and tissue digestion techniques were used to obtain the following subcellular fractions (detailed procedure in Wallace and Luoma, 2003): TE-rich granules (TE-RG), cellular debris, organelles (org), heat-sensitive proteins ("enzymes") and heat-stable proteins (metallothioneins, MT). Subcellular fractions that are potentially vulnerable to TE exposure (i.e. organelles and "enzymes"; dashed arc) constitute TE-sensitive fractions. Those fractions that are involved with TE detoxification (i.e. metallothionein, MT, and TE-rich granules, TE-RG; solid arc) constitute biologically detoxified TE. Fractions containing TE that is readily available to predators (i.e. organelles, "enzymes" and MT; double arc) constitute trophically available TE (modified after Wallace et al. 2003).

Mayer-Pinto et al. (2010) critically reviewed studies done on effects of TEs on aquatic assemblages and/or populations of invertebrates. These authors selected a sub-set of papers; the selection was done using an explicit randomisation process to ensure that a representative sample was taken from the scientific literature. Results from this random selection brought

out that most studies in the field had been descriptive: they generally demonstrated that the diversity of an assemblage tends to decrease with an increase of TE concentrations and that there are differences in the structure of the assemblages in the presence of large concentrations of TEs. Such descriptive studies are, however, unable to demonstrate any causal relationship between the TE contamination and the changes in the assemblages (Mayer-Pinto et al. 2010).

Toxicity testing methods are therefore required as a tool for predicting and assessing the impacts of anthropogenic environmental stressors on organisms and ecosystems (Amiard 2011). Laboratory-based toxicity testing has the obvious advantage to be unaffected by habitat or natural disturbances. This allows for experimental evaluation of various parameters and conditions, such as temperature, toxicant threshold effect levels, mixture interactions, life stages, and exposure duration under strictly controlled conditions (Burton 2010). Despite laboratory studies show lethal and sub-lethal effects of TEs on organisms, extrapolating such findings to the field is little reliable (Ralph et al. 2006): the majority of contaminant exposures of aquatic organisms are episodic in nature due to changing water and sediment quality; in addition, physicochemical characteristics of aquatic ecosystems (e.g., temperature, pH, water hardness and dissolved organic carbon) greatly influence bioavailability, toxicity, and bioaccumulation of contaminants (Chappie and Burton 2000, Liber et al. 2007, Clements et al. 2012). Naturally occurring factors in water and sediments affecting TE chemical bioavailability cannot be easily simulated in the laboratory (Lewis and Devereux 2009). Additional field experiments are therefore necessary to validate laboratory results under relevant environmental conditions.

Complementary results from laboratory and field toxicity tests are useful for decision making, particularly if the responses of the test organisms are severe and occur in multiple species (Burton 2010). Within this perspective, detailed reviews on tested organisms from different taxonomic groups allow (i) to identify species that may be suitable candidates in a suite of toxicity test protocols, keeping in mind regional differences in species requirements and tolerances (Fairbrother et al. 2007), and (ii) to highlight knowledge gaps that require to be addressed (van Dam et al. 2008). Such a work of synthesis was recently performed for the coastal waters of northern Australia, recognised internationally as important strongholds for marine biodiversity and containing some of the least impacted marine habitats in the world

(Fig. 1; Halpern et al. 2008): Van Dam et al. (2008) reviewed the state of the science for toxicity testing methods for water column contaminants, summarizing data available for 16 taxonomic groupings, among them vascular plants (seagrasses and mangroves) and bivalve molluscs; Adams and Stauber (2008) reviewed concomitantly the status of whole sediment toxicity tests developed with numerous species of 7 taxonomic groupings, of which bivalve molluscs. The 2 following paragraphs will focus on TE toxic effects assessed on seagrasses and bivalves molluscs.

2.6.2. TE toxic effects on bivalve molluscs

Bivalve molluscs possess many physiological attributes (sensitive to contaminants, tolerant of a wide range of abiotic factors, easily cultured and maintained in laboratory etc.) that render them suitable for consideration as bioassay organisms for toxicity testing (Depledge and Hopkin 1995). They are moreover good accumulators of organometallic contaminants and TEs from their surrounding environment due both to their behaviour and mode of feeding (Bryan et al. 1985). A number of standardized toxicity test protocols have been developed for determining toxicity of single chemicals, complex effluents and ambient samples of water or sediment to marine bivalves (e.g. Cal/EPA 2004, Burton 2010; detailed guidance manuals available from the EPA and the American Society for Testing and Materials, ASTM). Toxicity tests established on bivalve embryo-larval development are among the most sensitive in the EPA's national toxicity dataset, which is used to derive water quality criteria (Rosen et al. 2005). Among bivalve molluscs, mussels from the genus *Mytilus* have been largely used.

TE toxicity on bivalve molluscs can be determined at different structural levels, from genes to individuals, and response parameters have included, in addition to larval-embryo developments, changes in growth rates, clearance rates and survival rates, DNA-damages, or changes in tissue morphology and in specific component immunoreactivity (Table 4). Toxicity varies greatly between TE. The median effects concentrations (EC50) for *Meretrix meretrix* embryogenesis is 188 higher for Cd than for Hg. Moreover, this difference in toxicity directly relies upon the response parameter of interest; so, the EC50 for *M. meretrix* larval growth is only 6 times higher for Cd than for Hg (Wang et al. 2009).

Species	Studied trace elements and sites	Measured parameters	Test duration	Experimented concentrations	Field or effect concentrations and measured effects	Supplementary comments	Ref.
Mytilus galloprovincialis (laboratory)	water exposure: Cu; San Diego Bay (CA, USA)	embryo-larval development	48 h.	unfiltered seawater; nominal tested concentration range ($\mu g.L^{-1}$): 0-50; filtered seawater used for reference toxicant tests	absence of ambient toxicity to bivalve embryos; reference toxicant test EC50 value = 6.43 ± 1.36 $\mu g_{Cu}.L^{-1}$; EC50 of Cu spiked ambient water samples: 1.7 to 3.4 times lower at sites located near the mouth of the Bay compared to sites near the back of the Bay.	Normally developed bivalve larvae possess a hinged D-shaped shell (prodissoconch); differences bewteen unfiltered Cu spiked water samples indicate a gradient in complexation capacity increasing from the mouth to the back of the Bay (consistent with similar increasing trends in DOC and TSS).	1
Mytilus trossolus (laboratory)	water exposure: Cu, Zn, Ni, Cd (tested separetely)	embryo-larval development	48 h.	filtered seawater; measured concentration ranges ($\mu g.L^{-1}$): Cu = 1.1-71.0; Zn = 5-576; Ni = <DL-760; Cd = <DL-1 200; effect of DOC addition on metal toxicity tested for Cu	EC50 (in $\mu g.L^{-1}$): 9.6 for Cu, 99 for Zn, 150 for Ni, and 502 for Cd; experimental addition of DOC reduced Cu toxicity.	Normally developed bivalve larvae possess a hinged D-shaped shell (prodissoconch); protective effects of DOC on Cu toxicity are influenced by their distinct physicochemical properties: protection appears to be related to higher fulvic acid and lower humic acid contents.	2
Mytilus galloprovincialis (laboratory)	water exposure: Cr	gill morphology and immunoreactivity to components involved in gill motility; total glutathione content; activities of GSH-related enzymes, of catalase, and of key glycolytic enzymes; mRNA expression of selected genes	96 h.	artificial seawater; nominal tested concentrations ($\mu g.L^{-1}$): 0.1, 1, 10	Morphological, biochemical and molecular changes in mussel gills when exposed to concentrations ranging from 0.1 to 10 $\mu g.L^{-1}$.	Progressive changes in gill morphology and in immunoreactivity to components involved in neurotransmission; increased activities of GSH-related enzymes and total glutathione content suggesting Cr detoxication/reduction at the site of metal entry; increased activity of glycolytic enzymes, indicating modulation of carbohydrate metabolism; significant changes in transcription of different genes (sex- and concentration-related differences).	3
Mytilus edulis (laboratory)	water exposure: Ni	clearance rate; haemolymph genotoxicity and cytotoxicity	120 h.	filtered seawater; nominal tested concentrations ($\mu g.L^{-1}$): 4.6 (control), 18, 56, 180	56 $\mu g.L^{-1}$: clearance rate decreases; 180 $\mu g.L^{-1}$: NRR decreases and % tail DNA increases in mussel haemocytes	Clearance rate is Ni concentration-dependent (decreased by 30 % at the highest concentration); NRR assays, designed to assess the viability of the cells based on the penetration of a weakly cationic dye across lysosomal membranes, indicate a cytotoxic response; Ni has a genotoxic effect on the integrity of the DNA in haemocytes.	4
Meretrix meretrix (laboratory)	water exposure: Cd, Pb, Hg (tested separately)	embryogenesis; survival, growth and metamorphosis of larvae	24 h.: embryogenesis; 48 h.: metamorphosis; 96 h.: growth and survival	unfiltered seawater; measured concentration ranges ($\mu g.L^{-1}$): Hg = 2-17 977; Cd = 1-10 167; Pb = 2-7 158	EC50 for embryogenesis ($\mu g.L^{-1}$): 5.4 for Hg, 1 014 for Cd and 297 for Pb; 96 h LC50 for D-shaped larvae ($\mu g.L^{-1}$): 14 for Hg, 68 for Cd and 353 for Pb; growth retardment ($\mu g.L^{-1}$): 18.5 for Hg, 104 for Cd and 197 for Pb; EC50 for metamorphosis: similar to 48 h. LC50; higher than 96 h. LC50.	Embryo toxicity: the % of normal D-shaped larvae decreases when Hg, Cd and Pb concentrations increase. Larval growth: dead larvae with extruded velum and granulated tissues in the more toxic treatments; injury to the velum and swimming inhibition at lower concentrations; reduction in growth rate following exposure to most concentrations from 24 h. Concentration-dependent survival inhibition of larvae. Hg most toxic and Pb least toxic to metamorphosing larvae.	5
Tellina deltoidalis (laboratory)	metal spiked sediment exposure: Cd, Cu, Ni, Pb, Zn (tested separetely); water exposure: Cu and Zn (tested separetely)	survival rate	10 d.	nominal metal concentrations in sediment tests ($mg.kg^{-1}$): 75, 1 300, 420, 1 000, and 4 000 for Cd, Cu, Ni, Pb, and Zn, respectively; measured metal ranges in seawater tests: 0-710 $\mu g.L^{-1}$ for Cu; 0-13 $mg.L^{-1}$ for Zn	Sediment exposure: absence of toxicity. Water exposures: at Cu concentrations of 50 and 200 $\mu g.L^{-1}$, time to LC50 = 7 d. and 5 d., respectively; at Cu concentration of 13 $mg.L^{-1}$, time to LC50 = 4 d.	Survival to metal spiked sediments: 88–100 %; the absence of toxicity is consistent with the low sensitivity of *T. deltoidalis* to these metals in the dissolved intertidal phase and indicated that the exposure from the ingestion of metal-contaminated particles was not sufficient to cause toxicity over the 10-day period. Survival to water exposure: concentration-dependent survival; Cu toxicity higher than Zn toxicity.	6
Mytilus galloprovincialis (field)	sediment exposure: Ni; Kaštela and Trogir Bays (Croatia)	MN test (toxin induced heightened MN frequency) and Comet assay (tail DNA) with mussel haemocytes	30 d.	na	Ni range in sediments: 48-420 $\mu g.g^{-1}$.	Increased % of MN (defined as small round structures in the cytoplasm smaller than 1/3 of the nucleus diameter) compared to mussels from the reference location evident for most of the contaminated locations; increase in the % of tail DNA in individuals collected from mostly all the polluted sites; the 2 methods complement each other and it is desirable to use them both in monitoring the impacts of genotoxic pollution.	7
Mytilus spp. (field)	sediment elutriate (water soluble fraction) exposure: Zn, Ni, Cr, Cu, As, Pb, Hg, Pb, Ag, Cd, Se; San Fransisco Bay (USA)	% normal larval development	48 h.	na	Sediment quality guidelines used to evaluate the potential toxicity of sediments: concentrations <ERL, between the ERL and ERM or >ERM are rarely (<11%), occasionally (16-18%) or frequently (48-52%) associated with toxicity, respectively; Ni usually above the ERM; As, Cr, Cu and Hg often exceed their respective ERLs.	Sediment elutriate toxicity varied spatially (decreasing from sites located at the back of the Bay near 2 main river mouths - no survival of mussel larvae - to sites located near the Bay entrance - no toxicity); no signicant trends in larval development over time at most sites; larval bivalve toxicity was associated with metals in bulk sediments.	8
Mytilus trossulus (field)	sediment exposure: As, Cr, Cu, Pb, Hg, Zn, Sn_{TBT}; Puget Sound (WA, USA)	juvenile mussel growth rate	82 d.	na	Trace element ranges in sediments ($\mu g.g^{-1}$): As: 8-57; Cr: 44-93; Cu: 66-965; Pb: 22-297; Hg: 0.13-1.95; Zn: 107-592; Sn_{TBT}: 0.18-18.41.	In contaminated sites, mussels had lower growth rates than the reference site mussels; Sn_{TBT} and Cu: contaminants of greatest concern; Pb and Zn: contaminant of additional concern; statistically significant inverse relationship between growth rate and toxicity-normalized sediment contamination.	9

Table 4. Biological responses in bivalves exposed to dissolved and particulate trace elements under laboratory or field conditions.
Corresponding references: 1 - Rosen et al. (2005); 2 - Nadella et al. (2009); 3 - Ciacci et al. (2012); 4 - Millward et al. (2012); 5 - Wang et al. (2009); 6 - King et al. (2010); 7 - Klobucar et al. (2008); 8 -Thompson et al. (1999) and Long et al. (1995); 9 - Salazar (1995).
h.: hour; d.: day; na: not applicable; EC50: the median effect concentration; LC50: the median lethal concentration; DOC: dissolved organic carbon; TSS: total suspended solids; DL: detection limit; Glutathione (GSH)-related enzymes; NRR: neutral red retention; MN: micronucleus; ERL: effects range-low; ERM: effects range-median.

TE toxicity also fluctuates spatially and over time with water properties, as demonstrated for Cu. The EC50, obtained by Cu spiking of ambient water samples, for mussel embryo development was lower at sites located near the mouth of the San Diego Bay (California, USA) compared to sites near the back of the Bay; this increase was consistent with similar increasing trends in dissolved organic carbon (DOC) and total suspended solids (Rosen et al. 2005). This protective effects of DOC on Cu toxicity, experimentally demonstrated with contaminated filtered seawater spiked with DOC, appeared related to higher fulvic acid and lower humic acid contents (Nadella et al. 2009).

2.6.3. TE toxic effects on seagrasses

The toxicity database for seagrasses largely consists of results determined for single TEs in aqueous tests (Table 5). Much of current understanding on TE toxicity is based on results for 8 species. Experimental designs have varied considerably due, in part, to the lack of standardized toxicity tests for marine vascular plants, contrary to bivalve molluscs. Test durations have been between 6 hours and 51 days and response parameters have included photosynthetic activity, amino acid concentrations, growth of tissues, pigment content or leaf cell mortality.

Of the TEs, Cu, Cd, Pb, and Zn have been more commonly used as test compounds. Cu is particularly toxic to seagrasses, leading to leaf necrosis and decay (e.g. Macinnis-Ng and Ralph 2002, Ambo-Rappe et al. 2011). Interspecific differences in sensitivity to the same TE have been reported. Prange and Dennison (2000) incubated 5 seagrass species (*Halophila ovalis*, *Halophila spinulosa*, *Halodule uninervis*, *Zostera capricorni* and *Cymodocea serrulata*) with Fe or Cu (1 ppm); seagrass responses to pollutants have been assessed by changes in PSII photochemical efficiency and free amino acid content. Fe addition experiments only affected *Halophila* spp, while Cu additions affected other seagrass species as well. TE sensitivities can even differ between populations of a same species. Macinnis-Ng and Ralph (2004) looked at the impacts of *in situ* exposure to Cu and Zn for 3 isolated populations of *Zostera capricorni* in the Sydney (Australia) region. Photosynthetic efficiency and chlorophyll pigment concentrations showed different sensitivities to Cu impacts at the 3 geographically isolated sites. Seagrasses from the least developed estuary were the most sensitive to Cu and the two more developed estuaries had more tolerant populations.

Species	Studied trace elements and sites	Measured parameters	Test duration	Nominal experimented concentrations	Effect concentrations and measured effects	Supplementary comments	Ref.
Zostera marina (laboratory)	Cu, Cr, Cd, Hg, Zn, Pb (tested separetely)	growth rate	0.5, 2, 5, 8, 12 and 19 d.	0.1, 0.5, 5 and 50 µM	Growth rate inhibition - Cd: after 12 d. at 5 µM and after 8 d. at 50 µM; Cu: after 5 d. at 5 µM and after 2 d. at 50 µM; Hg: rapid effect at all concentrations; Zn: after 2 d. at 50 µM.	Growth rate inhibited by Cd, Cu, Hg, Zn; no effect of Cr and Pb exposures; toxicity of metals decreases in the order: Hg ≥ Cu > Cd ≥ Zn > Cr and Pb; cellular substances are leached to the water and plants turne black in the Cu 50 µM experiment and in the Hg 5 µM and 50 µM experiments.	1
Halophila stipulacea (laboratory)	Al	leaf cell viability	12 d.	from 10^{-4} to 10^{-9} mol.L^{-1}	Cellular damages from 10^{-4} to 10^{-8} mol.L^{-1}.	Protoplast necrosis in all cell categories (except in the mid-rib cells); plasmatic resistance decreases in the order mid-rib, mesophyl, epidermal, teeth cells.	2
Halophila ovalis, *Halophila spinulosa*, *Halodule uninervis*, *Zostera capricorni* and *Cymodocea serrulata* (laboratory)	Fe, Cu (tested separetely)	changes in PSII photochemical efficiency (Fv/Fm) and free amino acid content	12 d. of exposure; 5 d. of recovery	1 mg.L^{-1} + EDTA	Fe and Cu: 1 mg.L^{-1}; decline in PSII photochemical efficiency and in amino acid contents; effects are species-specific.	Fe addition experiments: declines in PSII photochemical efficiency in *H. ovalis* and *H. spinulosa* correspond with the replacement of fresh seawater (12 days), suggesting that these species became acclimatized to the new environmental conditions; *Z. capricorni* exhibited a decline in total free amino acid contents (could be a precursor signal of Fe induced stress). Cu addition experiments: Fv/Fm ratio response was highly variable between the 5 seagrass species (death of *H. spinulosa*); decline in amino acid concentrations in *Z. capricorni* and *H. uninervi*.	3
Halophila ovalis (laboratory)	Cu, Cd, Pb, Zn (tested separately)	chlorophyll *a* fluorescence; pigments (chlorophyll *a*, *b*, and carotenoids)	4 d.	1, 5 and 10 mg.L^{-1}	Cd - 1 to 10 mg.L^{-1}: limited stress. Cu - 5 and 10 mg.L^{-1}: lethal effect. Pb - 1 to 10 mg.L^{-1}: limited effect on fluorescence; chl. *a*, *b* decrease. Zn - 1 to 10 mg.L^{-1}: changes to the chlorophyll *a* fluorescence responses; various effects on pigment contents.	Variety of effects on the photosynthetic processes, with Cu and Zn having greater effects than Pb and Cd; quantum yield is the most sensitive measure of the photosynthetic processes; pigment contents generally confirme the chlorophyll *a* fluorescence responses.	4
Zostera capricorni (field)	Cd, Cu, Pb, Zn (tested separetely); 1 reasonably pristine site at Pittwater (NSW, Australia)	photosynthetic efficiency (ΔF/Fm'); pigments (chlorophyll *a*, *b*, and carotenoids)	10 h. of exposure; 3 d. of recovery	0.1 and 1 mg.L^{-1}	Cu, Zn - 0.1 and 1 mg.L^{-1}: photosynthetic efficiency decreases during the exposure period; Cu - 0.1 and 1 mg.L^{-1}: after 96 h., carotenoid pigments decline, the chlorophyll *a*/*b* ratio is depressed and the chlorophyll/ carotenoid ratio is elevated.	Samples exposed to Zn recover to pre-exposure levels but those exposed to Cu do not; browning of leaves and some leaf loss occurred due to exposure to Cu; Cd and Pb do not impact on the chlorophyll *a* fluorescence and the pigment data support these findings.	5
3 isolated populations of *Zostera capricorni* (field)	Cu, Zn (tested separetely); 1 semi-pristine and 2 impacted sites in Sydney region (Australia)	photosynthetic efficiency (ΔF/Fm'); pigments (chlorophyll *a*, *b*, and carotenoids)	10 h. of exposure; 3 d. of recovery	0.1 and 1 mg.L^{-1}	Cu in the semi-pristine site - 0.1 mg.L^{-1}: fluorescence decreases; 1 mg.L^{-1}: chlorophyll concentration decreases. Cu in the 2 impacted sites - 1 mg.L^{-1}: fluorescence decreases.	Lack of response due to Zn exposure; different sensitivities to Cu: greater impact of Cu on the more naïve population (higher decrease of fluorescence during exposure and lower recovery).	6
Posidonia oceanica (laboratory)	Cd	DNA methylation and chromatin reconfiguration; expression of PoMT2k and PoCMT1; nuclear chromatin ultrastructure	6 h., 2 d., 4 d.	10 and 50 µM	PoMT2k expression - Cd 50 µM: increase in PoMT2k expression after 6 h. in apical tips and leaves; Cd 10 µM: increase in PoMT2k expression after 2 d. in leaves. Changes in DNA methylation and in PoCMT1 expression: Cd 10 µM after 6 h. Chromatin reconfiguration: Cd 50 µM after 2 d.	Cd treatment induces a DNA hypermethylation (time- and dose-dependent), as well as an up-regulation of CMT, indicating that *de novo* methylation occur; a high dose of Cd leads to a progressive heterochromatinization of interphase nuclei and apoptotic figures are observed after long-term treatment; Cd perturbs the DNA methylation status through the involvement of a specific methyltransferase; such changes are linked to nuclear chromatin reconfiguration likely to establish a new balance of expressed/repressed chromatin; the data show an epigenetic basis to the mechanism underlying Cd toxicity in plants.	7
Halophila ovalis (laboratory)	Pb, Cu (tesed separetely)	growth rate; leaf fluctuating asymmetry and dimension	51 d.	Pb: 10 and 50 mg.L^{-1}; Cu: 0.5, 2 and 4 mg.L^{-1}	Growth rate decrease - Cu: 0.5 mg.L^{-1}; Pb: 10 mg.L^{-1}. Reduced leaf dimension - Cu: 0.5 mg.L^{-1}; Pb: 50 mg.L^{-1}. Increased leaf asymmetry - Cu: 2 mg.L^{-1}.	Reduced growth rate of the seagrass observed both in Pb and Cu treatments; leaf size of the plant reduces as the metal concentrations increase and when the plants are exposed to the metal for longer duration; increased leaf asymmetry more apparent at the 2 ppm Cu treatment; no increase in fluctuating asymmetry in Pb treatments; the mortality of leaves is especially high in Cu treatments.	8

Table 5. Biological responses in seagrasses exposed to dissolved trace elements under laboratory or field conditions.
Corresponding references: 1 - Lyngby and Brix (1984); 2 - Malea and Haritonidis (1996); 3 - Prange and Denisson (2000); 4 - Ralph and Burchett (1998); 5 - Macinnis-Ng and Ralph (2002); 6 - Macinnis-Ng and Ralph (2004); 7 - Greco et al. (2012); 8 - Ambo-Rappe et al. (2011).
EDTA: ethylenediaminetetraacetic acid; PSII: photosystem II; PoMT2k: *Posidonia oceanica* Metallothionein (MT) 2k, an important metal tolerance gene; PoCMT1: one member of the *Posidonia oceanica* chromomethylase (CMT) family, a DNA methyltransferase. Fv: variable fluorescence; Fm: maximum fluorescence; ΔF: fluorescence yield; Fm': light-adapted maximal fluorescence.

Marine vascular plants are still rarely used in ecotoxicological testing, primarily because of difficulties in culturing/adapting and testing with such large, slow growing organisms (van Dam et al. 2008). To overcome these difficulties, and for greater environmental relevance, more recent toxicity tests have involved the measurement of photosynthetic endpoints (using PAM fluorometry) on wild plants in *in-situ* chamber experiments (e.g. Macinnis-Ng and Ralph 2002, 2004). Field measurements of photosynthetic efficiency can moreover be easily used as an efficient ecoindicator of seagrass health (e.g. Durako 2012).

Phytotoxic effect levels for sediment-bound chemicals, spiked or in a whole sediment matrix, are relatively unknown for seagrasses. The lack of this information, when coupled with the findings that concentrations of several anthropogenic chemicals in rooted sediments have exceeded sediment quality guidelines, indicates a need to better understand the phytotoxicities and bioavailability of sediment-adsorbed contaminants (Lewis and Devereux 2009).

3. The monitoring of the marine environment

3.1. "Biomonitoring"

In marine habitats, three measures of pollutant levels are usually available - namely concentrations in waters, sediments and biota (Rainbow 1995). Until the early 70^{ies}, the monitoring of terrestrial and marine environments mainly relied on the detection and quantification of pollutants in physical environments – air, water, soils and sediments (Burger 2006, Amiard 2011). During the years 80^{ies}, almost all of marine water (and freshwater) monitoring networks abandoned the use of the waters themselves to estimate the quality of aquatic ecosystems. The reasons of this abandon were diverse; among them:

- the measured concentrations of chemicals are generally low (concentrations of most dissolved TEs in the environment are in the order of a few ng.L^{-1}_{water}) and often close to the limits of detection of the analytical techniques;
- the risk of secondary accidental contamination during the sample collection and manipulation makes the measurements sensitive;

• the concentrations of dissolved substances may also vary considerably over time (e.g. with tidal cycles, water run-off, seasons etc), and episodic pollution events can be missed;

• the measurements of dissolved pollutants do not provide an assessment of the portion which is available for uptake and accumulation by marine organisms (Rainbow 1995, Amiard 2011).

The analysis of sediments overcomes some of these disadvantages. Pollutants accumulate in sediments, particularly in organically rich sediments, and sediment concentrations are therefore higher, more easily measurable and much less susceptible to accidental contaminations; sediments also offer a degree of time integration, overcoming the worst effects of temporal variability of pollutant availability. However, pollutant accumulation by sediments is much affected by sediment characteristics that vary temporally and geographically, not least particle size, mineralogical nature and organic carbon content. Yet again, pollutant concentrations measured do not correspond to that of bioavailable fractions (Rainbow 1995, Amiard 2011).

When compared with the conventional chemical analysis of aquatic environmental matrix, i.e. water and sediment, the monitoring relying upon the biota exhibits obvious predominance (Zhou et al. 2008): biomonitoring

• reveals the biological changes of organisms affected by exogenous chemicals;

• reveals the integrated effects of the complex pollutants on the organisms in the environment;

• has high sensitivity due to the rapid responses induced in the organisms exposed to pollutants, which helps to the declare of the precaution;

• realizes the monitoring of the pollutants at low levels which are below the detection limits of the instrumental analytical techniques due to the occurrence of the chronic toxicities of the pollutants in the organisms under long-term exposure;

• allows widely sampling even at remote areas;

• avoids the limits of the convention chemical analysis such as continuous sampling, needs of expensive instruments etc.

3.2. “Bioindicators”

Since the ultimate purpose of pollution monitoring is the protection of ecosystems and human beings, the main interest of the use of quantitative sentinel organisms with regard to water or sediment is their capacity to give information on the bioavailability of pollutants (Cossa 1989). Currently, the term "bioindicator" is a deeply ambiguous term which has different meanings in different contexts (Heink and Kowarik 2010). To prevent problems due to different interpretations of this term, we use the definition of Blandin (1986): “a biological indicator (or bioindicator) is an organism or a set of organisms that allows, by reference to biochemical, cytological, physiological, ecological or ethological variables, in a practical and safe way, to characterise the status of an ecosystem or an eco-complex and to highlight as early as possible their changes, natural or caused”. Bioindicators therefore allow to accurately assess the effects of anthropogenic activities on ecosystems.

To be considered as a good bioindicator of pollution, the selected species must meet a number of criteria, as listed by Cossa (Cossa 1989) or Rainbow (1993):

- the sentinel organism should be a net strong accumulators of contaminants and should not regulate the total concentration of a pollutant in its body tissues;
- it should be sedentary in order to be an authentic representative of the study area;
- it should be reasonably abundant at the sites of interest;
- it should have a sufficiently long life to permit sampling of more than one-year class;
- it should be large enough to provide sufficient tissue for chemical analyses;
- it should bioaccumulate sufficiently to allow direct measurement without preconcentration;
- it should be resistant to handling stress caused by laboratory studies or field transplantation;
- a correlation should exist between the level of contaminants in the organism and in the surrounding environment;
- it should be tolerant of exposure to environmental variations in physicochemical parameters and the effects on the organism of these variations should be known.

No single species combines all these qualities, and a compromise must be found (Cossa 1989). It was in this spirit that Goldberg suggested in 1975 the use of mussels of the

genus *Mytilus* as the first stage in an extensive monitoring program for the marine environment. Since then, numerous bioindicator species, from unicellular organisms to top predators, have been used worldwide to monitor marine ecosystems.

As shown in a review concerned with the use of bioindicators by Burger (2006), over 40 % of the bioindicator papers are about TE pollution, all field combined, wherein plants, invertebrates, fish and mammals are the dominant used bioindicator species. For marine pollution monitoring, the bioindicator species used belong to numerous taxonomic groupings of which micro- and macro-acroalgae, seagrasses, ascidians, sponges, bivalve and gastropods molluscs, polychetes, crustaceans, fishes, seabirds, marine reptiles and mammals (non-exhaustive list of reviews: Rainbow and Phillips 1993, Rainbow 1995, Boening 1999, Das et al. 2003, Markert et al. 2003, Giangrande et al. 2005, Burger 2006, Roberts et al. 2008, Zhou et al. 2008, Lewis and Devereux 2009, Gupta and Singh 2011, Tanabe and Ramu 2012 etc.).

Each bioindicator shows some special merits for the biomonitoring of pollution in marine ecosystem when compared to the others (Zhou et al. 2008). In the two next sections, we will present two bioindicator species widely used in the monitoring of the health status of the Mediterranean, from punctual surveys to international monitoring programs: the Neptune grass *Posidonia oceanica* and the Mediterranean mussel *Mytilus galloprovincialis*. These two bioindicators respond appreciably and quantitatively to the coastal pollution by trace elements, and complement one another: the two species accumulate pollutants dissolved in the water column; *P. oceanica*, deeply rooted in sediment, also reflects the contamination of this compartment; mussels, as a filter feeder, accumulate pollutants from their particle phase. Together, they give an estimate of the overall pollution (water, sediment, suspended matter) of the Mediterranean coastal environment.

4. *Posidonia oceanica*

4.1. A brief description of its biology

For more information on the subject, readers can refer to Boudouresque and Meinesz (Boudouresque and Meinesz 1982), Boudouresque et al. (Boudouresque et al. 2006), Gobert et al. (Gobert et al. 2006) and Pergent et al. (Pergent et al. 2012).

4.1.1. Biogeography

Posidonia oceanica (Linnaeus) Delile is a marine magnoliophyte endemic to the Mediterranean belonging to the order of the Najadales and to the family of the Posidoniaceae (den Hartog 1970, Phillips and Menez 1988, Den Hartog and Kuo 2006). *P. oceanica* grows on sandy and rocky bottoms and forms patchy and continuous meadows regarded as one of the climax communities of the Mediterranean (Procaccini et al. 2003). This seagrass supports temperatures ranging from 9 to 29°C, its optimum being between 17 and 20°C (Boudouresque and Meinesz 1982); as an eurytherm species, it colonizes large areas of the infralittoral floor from the surface to maximal depths of 45 m (Augier et al. 1980, Procaccini et al. 2003).

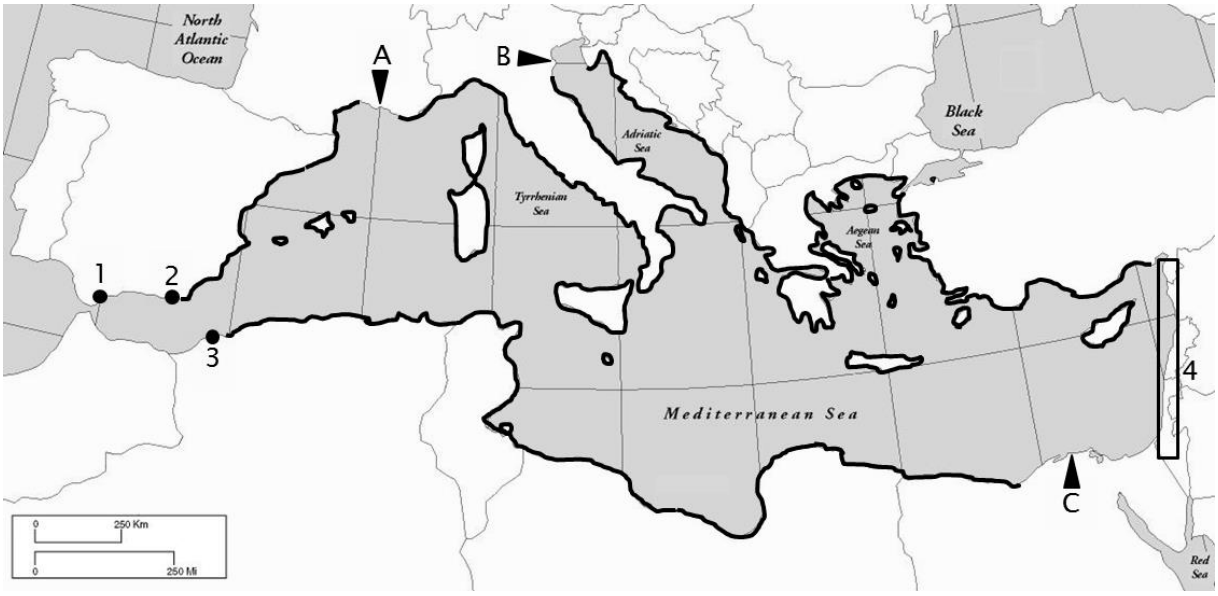

Fig. 9. Geographical distribution of *P. oceanica* (solid black line). 1: Gibraltar; 2: Almeria; 3: Oran; 4: Coasts of Syria, Israel and Lebanon; A: Rhone estuary; B: Po estuary; C: Nile estuary (Michel 2012).

Posidonia oceanica beds cover a surface estimated between 25 000 and 50 000 km^2, *i.e.* between 1 and 2 % of the Mediterranean (Pasqualini et al. 1998); it is the most abundant seagrass among the 5 species (*Zostera noltii*, *Zostera marina*, *Cymodocea nodosa*, *Halophila* stipulacea and P*osidonia oceanica*) encountered in the Mediterranean (Procaccini et al. 2003). *P. oceanica* colonizes all the European coasts and the major part of the North African coasts (Egypt, Libya, Tunisia, Algeria; Fig. 9); it is only missing in zones under the influence of large estuaries (Po, Rhone, Nile – diminution of salinity and increase of turbidity; Gobert et

al. 2006). The light and the transparency of the water are determining factors for its growth (in turbid waters, the lower limit of the beds is of the order of 9 m; Pergent et al. 1995). It is stenohaline, its optimum of salinity ranging between 36.5 and 39.5, but surveys carried out at the North-eastern distribution limits revealed large beds in the Dardanelles Strait and isolated beds in the Marmara Sea, where the salinity ranges between 21.5 and 28.0 (Meinesz et al. 2009).

4.1.2. Morphology

Posidonia oceanica has the same morphology as the other marine magnoliophyte (Fig. 10): below-ground parts consist of roots for anchoring and rhizomes for mechanical support; above-ground parts consist of shoots bearing several leaves (Kuo and den Hartog 2006). Rhizomes grow horizontally (competition for the space: plagiotropic rhizome) or vertically (competition for access to the light: orthotropic rhizome). The progressive silting and the two types of rhizome growth result in a typical terraced formation called "matte" consisting of the intertwining of various strata of rhizomes, roots, and sediment (Gobert et al. 2006).

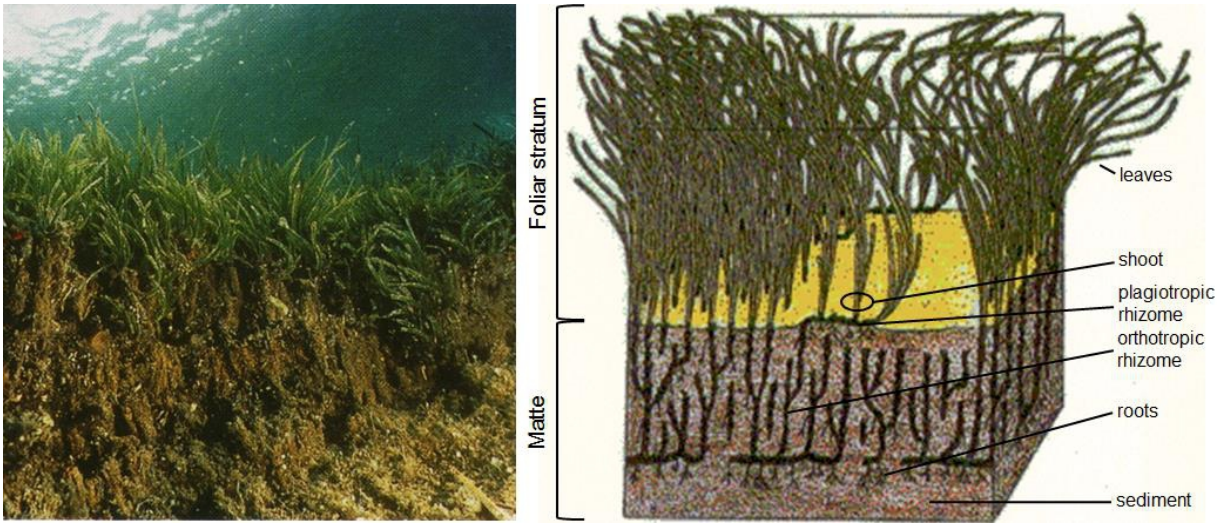

Fig. 10. Picture (left; © C. Petron) and schematic drawing (modified after Boudouresque and Meinesz 1982) of a piece of *P. oceanica* bed showing the matte structure and the foliar stratum.

Leaves are one cm wide in average, and 20 to 100 cm long (sometimes more); leaves are ribboned, alternate and sheathing (Augier 2010). Three types of leaves, different by their morphology, succeed one after the other from the inside toward the outside of the shoot:

• juvenile leaves (< 5 cm long), newly produced by the meristem, that will replace the intermediate leaves;

• intermediate actively growing leaves (> 5 cm long, with no distinct petiole or petiole < 2 mm) that will replace adult leaves;

• adult leaves, often epiphyted (i.e. colonized by other sessile organisms) compared to intermediate leaves, have completed their growth and break at the level of the ligule when they shed (Giraud 1979, Augier 2010).

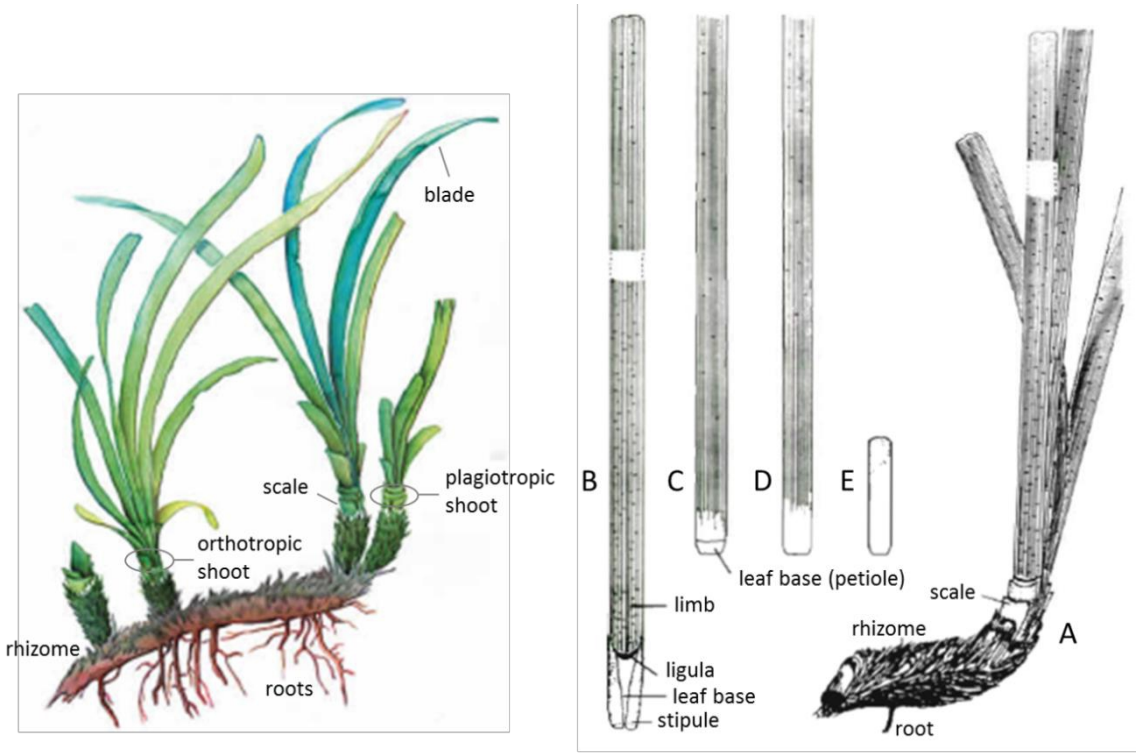

Fig. 11. Left: *P. oceanica* shoots fixed on a plagiotropic rhizome. Right: (A) shoot of leaves on a plagiotropic rhizome; (B, C) adult leaves; (D) intermediate leaf; (E) juvenile leaf (modified after Libes and Boudouresque 1987).

Posidonia oceanica is a perennial, deciduous plant. When leaf-limb shed, they leave scales on the shoots. These scales (i.e. petioles of old abscised blades) form a sheath around the new leaves, are resistant to degradation and remain on buried rhizomes (Pergent and Pergent-Martini 1991, Gobert et al. 2006).

4.1.3. Ecological roles

Posidonia oceanica plays various ecological and functional roles (reviews in Boudouresque et al. 2006, Larkum et al. 2006, Augier 2010). First of all, *P. oceanica* meadows are considered to be among the most productive ecosystems of our planet. This ecosystem is made up by the juxtaposition of two types of primary production: • the net primary production of *P. oceanica* which is on average of 420g_{DM}/m^2/year and can reach 1300g_{DM}/m^2/year; • the net primary production of the epiphytes which is between 100 and 500g_{DM}/m^2/year. On a global scale, only seagrass ecosytems display this specific feature.

Posidonia oceanica exhibits structural roles: • *P. oceanica* leaf canopy acts as a sediment trap, thereby reducing the water turbidity; • *P. oceanica* meadows reduce the coastal erosion and stabilize coastlines through direct effect on wave motion and through the formation of "banquettes" (wedge-shaped deposits of *P. oceanica* leaf litter). • *P. oceanica* shoots build up structurally complex ecosystems, providing adequate life conditions and ecological niches for an important number of organisms; as a result, *P. oceanica* meadows are Mediterranean biodiversity hot spots.

Posidonia oceanica plays a crucial role in the coastal biogeochemical cycles: • it modifies chemical properties (nutrient, oxygen, organic matter and dissolved inorganic carbon concentrations) of both water column and sediment; • the high diurnal rates of oxygen production support respiration of a significant amount of heterotroph organisms; • its presence enhances the nutrient recycling by the heterotroph bacteria; • the important formation of matte, and associated long-term burial of organic matter, is a significant carbon sink.

Posidonia oceanica is also an important food supplier: • direct herbivory, generally regarded as limited (only 10 % - up to 70 % locally - of living *P. oceanica* organic matter would enter the food webs); • detritivory (detritivores feeding on litter rely on micro-organisms colonizing detritus to achieve nutritional balance); • epiphyte consumers (epiphyte can represent more than 40 % of the total foliar biomass of *P. oceanica*).

4.2. seagrasses as bioindicator species

4.2.1. Posidonia oceanica descriptors

Posidonia oceanica is used since decades as a powerful integrator of the overall quality of Mediterranean marine coastal waters (e.g. Augier 1985, Boudouresque et al. 2000, Lopez y Royo et al. 2011). This large-size long-living species, sedentary and abundant, colonizes the major part of the Mediterranean coasts where it can be easily collected. It is sensitive to chemical pollution and mechanical disturbances (Augier 1985, Pergent-Martini et al. 2005, Boudouresque et al. 2006) and makes account, by its presence and its vitality (or its regression materialized by dead matte), of the quality of the Mediterranean coastal waters (Boudouresque et al. 2006).

The footprint of the quality of waters on *P. oceanica* is permanent. Consequently, many environmental parameters may be recorded by *P. oceanica* meadows (Boudouresque et al. 2006). In 2005, 39 scientists from as many as 23 Research Centres synthetized in a common publication the descriptors of *P. oceanica* in order to better define the respective advantages of each one to assess the good ecological status of coastal zones (Pergent-Martini et al. 2005). Descriptors covered all the levels of organization of *P. oceanica* meadows, from the biochemical composition of the seagrass to the structure of the entire ecosystem. *P. oceanica* descriptors, with the measured parameters and related information are detailed in Fig. 12.

In summary:

• chemical and biochemical composition of *P. oceanica* can provide information about the level of plant stress, and seems in adequacy with the level and impact of human activities;

• levels of contaminants provide information about the overall pollution of the meadow;

• leaf biometry is indicative of the environmental conditions (anthropisation, water motion, action of grazers etc.) and the dynamics and vegetative growth of the meadow;

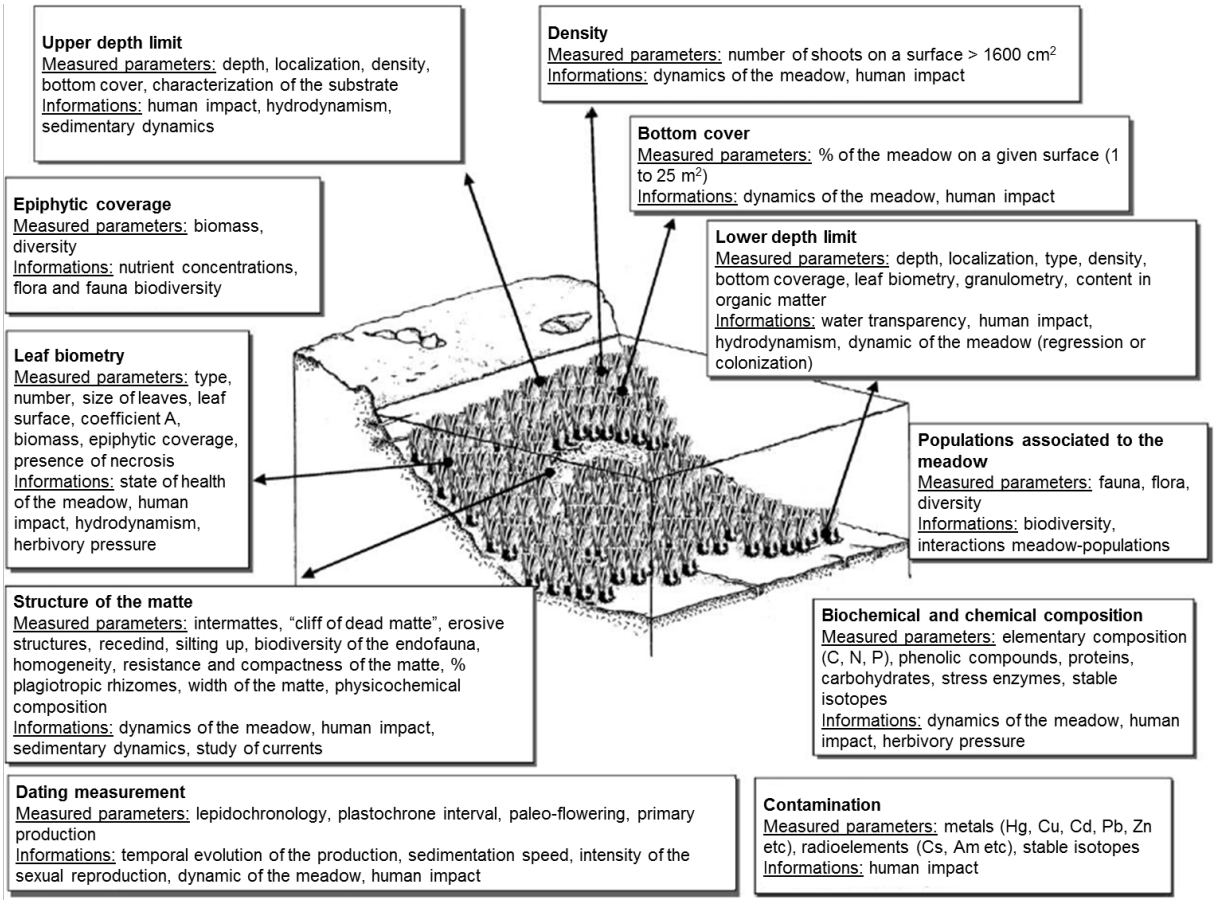

Fig. 12. Recapitulative plan of the main descriptors of *P. oceanica* meadows, with the measured parameters and related information (modified after Pergent-Martini et al. 2005).

• the lepidochronology (cyclic changes along rhizomes; Pergent et al. 1989, Pergent 1990) and the plastochrone interval index (an interpolation method used to estimate leaf age; Cebrian et al. 1994) provide information about the temporal evolution of primary production, sedimentation rates, sexual reproduction and dynamics of the meadow;

• structural characteristics of the meadow (shoot density, bottom cover, speed of growth of rhizomes and matte structure) provide information on the vitality, the macrostructure and dynamics of the meadow, as well as information on the sedimentation, hydrodynamism, currents or human impacts;

• lower and upper limits provide pertinent information about the quality of the meadow and environmental changes, both natural and human-induced (e.g. water transparency, hydrodynamics, sedimentary balance, coastal developments, anchorages etc.);

• disappearance of the seagrass bed is indicative of the freshening at the outlets of coastal rivers or groundwater;

• associated species (fauna and flora) supply relevant information concerning the biodiversity of the meadow and the interactions meadow-species; epiphytes also provide informations on water quality, especially data on nutrients inputs (Pergent-Martini et al. 2005, Boudouresque et al. 2006).

Some of the above descriptors provide data about disturbances in a specific way, allowing to identify direct and indirect causes of changes (e.g. the lower depth limit is directly linked to changes in water transparency; Duarte 1991); other descriptors supply less specific information and globally express the vitality of the meadow (e.g. leaf biometry; Augier 1985, Pergent et al. 1995). Natural and human-induced disturbances experimented by *P. oceanica* meadows (e.g. eutrophication, decrease of water transparency, water motion) and the time of answer of their various related descriptors (hours to decades) vary greatly; collaborators of this review thenceforth concluded that a global approach combining different descriptors should be recommended to allow a better understanding of the interactions and complexity of these disturbances (Pergent-Martini et al. 2005).

4.2.2. The choice of proper descriptors

Based on an exhaustive bibliographical review, Martínez-Crego et al. (2008) recently identified 59 seagrass descriptors sensitive to environmental changes at different levels of biological organisation; descriptors are for the most identical to the ones summarized in Pergent-Martini et al (2005) and will therefore not be more detailed. Authors validated these descriptors on deep (15 m) and shallow (5 m) *P. oceanica* meadows from the Catalan coast (Spain) covering a wide anthropogenic gradient ranging from undisturbed to severely disturbed sites. Numerous descriptors from the large list of candidate indicators were discarded:

• either because they failed to detect large scale (i.e. between-site) variability due to the masking effect of high spatial heterogeneity at smaller scales (i.e. variability between zones; e.g. number of leaves, amino acid contents in rhizomes etc.);

• either because they were influenced by natural sources of variability such as herbivory or physical settings and showed a low effect in ordering deep meadows (e.g. herbivore bite marks, leaf length and width etc.);

• or because, although they seemed to be linked to environmental status or to specific pollutants, responses of these indicators appeared to be influenced by interactions between different sources of pollution (e.g. interactions between metals and nutrients, interactions between different sources of anthropogenic nitrogen etc.).

Among the 59 seagrass descriptors assessed, only 16 were finally unequivocally related to the environmental status gradient under study (among them, 7 concerned TEs); they were representative of physiological (e.g. carbohydrates), biochemical (e.g. TEs), individual (e.g. shoot necrosis), and population (e.g. meadow cover) levels of biotic organisation. Their combination was necessary to cover the entire environmental gradient and to reflect the multiple anthropogenic disturbances causing the gradient (Martínez-Crego et al. 2008).

The choice of an adequate suite of indicators appears thenceforth decisive to ensure the consistency of multimetric indices and to provide an ecologically relevant interpretation of the response of biota to multiple stressors (Martínez-Crego et al. 2008). Such multimetric indices have been experimented to assess the ecological status of *P. oceanica* meadows, as for example: the POMI index (Posidonia oceanica multivariate index; Romero et al. 2007a, 2007b), the BiPo index (Biotic index using Posidonia oceanica ; Lopez y Royo et al. 2010), or the PREI index (Posidonia oceanica Rapid Easy Index ; Gobert et al. 2009) etc. These indices need the destructive sampling of shoots from the meadow in order to measure some of the indicators required for their calculation; however, *P. oceanica* is a protected species at a time by international conventions signed by most of the Mediterranean countries, but also protected at national or regional level (in France, Monaco, Italy, Algeria, Slovenia, Croatia, Malta, Spain, Libya, Turkey; Boudouresque et al. 2006). The responsible evolution in the monitoring of the state of conservation of the Mediterranean coastline using *P. oceanica* must therefore evolve toward the implementation of less invasive methods (Montefalcone 2009) and toward the development of non-destructive index, as proposed by Gobert et al. (2012).

4. 3. Trace elements bioaccumulation in seagrasses

4.3.1. Posidonia oceanica as model

Seagrasses are susceptible to the adverse effects of anthropogenic chemicals, including TEs, due to their restricted habitat in shallow, subtidal areas where exposure is greatest to phytotoxic chemicals originating from point and nonpoint sources in the watershed (Lewis and Devereux 2009). Within coastal seagrass beds, TE bioavailability is influenced by water and sediment pH, sediment particle size, redox potential, dissolved organic matter, sediment cation exchange capacity, water temperature, salinity, organic content, and concentrations of other TEs. These environmental physicochemical caracteristics influence processes such as chelation, complexing, precipitation, absorption and adsorption that in turn affect TE bioavailability (reviews in Batley et al. 2004, Burton 2010).

In the Mediterranean, *P. oceanica* is regarded as a powerful indicator of bioavailable TEs since it highly bioconcentrates these chemicals. For example, bioaccumulation factor values based on *P. oceanica* leaves – filtered seawater comparisons ranged between 2 000 and 36 000 for Cd, Cr, Cu, Pb and Zn in 2 Italian uncontaminated areas (Campanella et al. 2001, Conti et al. 2007). *P. oceanica* also accumulates TEs at levels reflecting the status of contamination of its environment (water and sediment), as shown from experiments and field studies (e.g. Warnau et al. 1996, Pergent-Martini and Pergent 2000, Lafabrie et al. 2007). This seagrass is resistant to pollution and persists in the vicinity of important contamination sources (Boudouresque et al. 2006). Furthermore, *P. oceanica* ability to record the past levels of pollutants, coupled to the dating possibilities offered by the lepidochronology, provide relevant biological archives about the temporal evolution of the pollution in the Mediterranean (e.g. Pergent-Martini and Pergent 1994, Ancora et al. 2004, Gosselin et al. 2006, Copat et al. 2012).

Seagrass meadows can be conceptualized as the juxtaposition of 5 separate components (seagrass shoots, epiphytes, associated algae and animals, detritus) exchanging flows of TEs between themselves and with their environment (water and sediment). This conceptualization can be modelled in energy circuit language (Fig. 13).

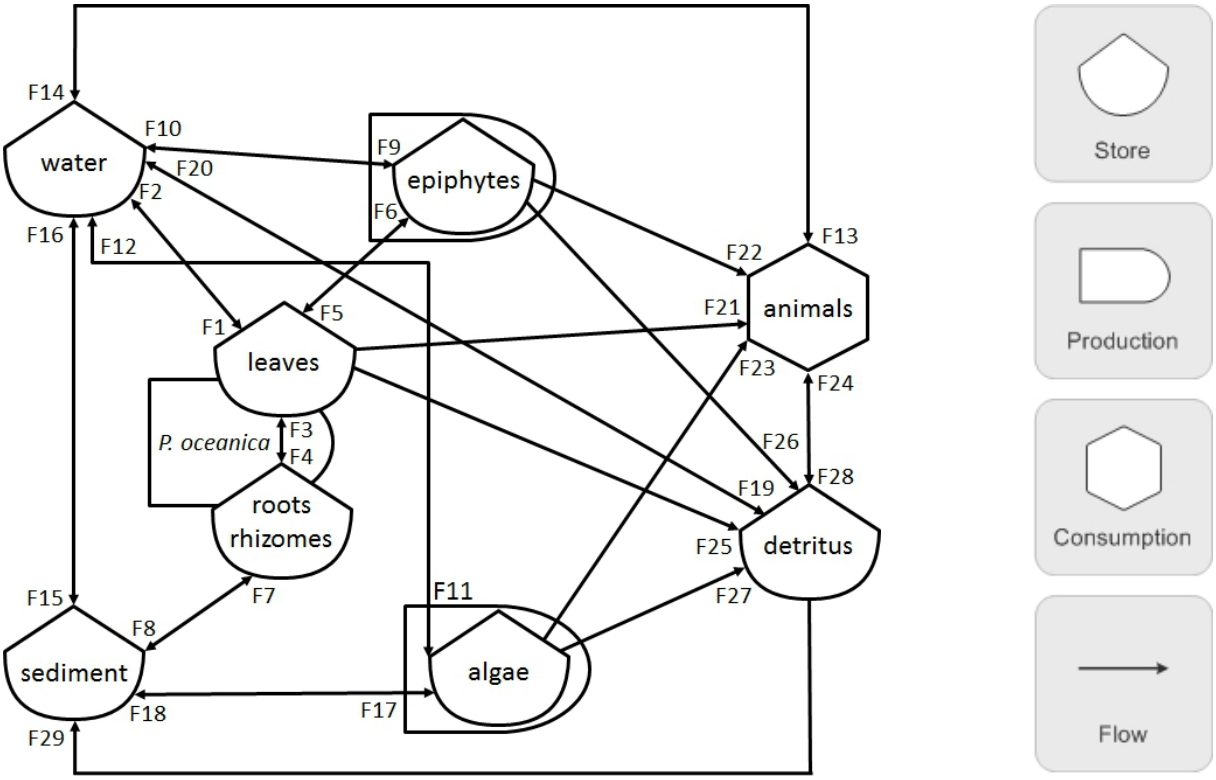

Fig. 13. Trace element cycling between the different components of a *P. oceanica* meadow. Model is drawn in energy circuit language (left) and symbol meaning is given (right; Odum and Odum 2000). In such schematic representations, varying the size of symbols indicates physical size of storages and/or their importance (Brown 2004); given that the quantification of flows and distribution of trace elements between *P. oceanica* bed components is poorly known, a similar size was given to each symbol. Trace element flows between *P. oceanica* bed components labelled with "F-numbered" are the following: F1 = uptake leaves from water; F2 = loss leaves to water; F3= translocation roots to leaves; F4 = translocation leaves to roots; F5 = translocation epiphytes to leaves; F6 = translocation leaves to epiphytes; F7 = uptake roots to sediment; F8 = loss roots to sediment; F9 = uptake epiphytes from water; F10 = loss epiphytes to water; F11 = uptake algae from water; F12 = loss algae to water; F13 = uptake animals from water; F14 = loss animals to water; F15 = uptake sediment from water; F16 = loss sediment to water; F17 = uptake algae from sediment; F18 = loss algae to sediment; F19 = uptake detritus from water; F20 = loss detritus to water; F21 = feeding animals on leaves; F22 = feeding animals on epiphytes; F23 = feeding animals on algae; F24 = feeding animals on detritus; F25 = conversion leaves to detritus; F26 = conversion epiphytes to detritus; F27 = conversion algae to detritus; F28 = conversion animals to detritus; F29 = mineralization detritus to sediment. Flow exchanges with adjacent ecosystems are not shown (modified after Schroeder and Thorhaug 1980).

Each symbol of the energy circuit language is rigorously and mathematically defined (see right side of Fig. 13). By writing a diagram, one, in essence, is writing equations describing a system. Varying the size of symbols further indicates physical size of storages and/or their importance (Odum and Odum 2000, Brown 2004).

Up to now, no detailed study does model the cycling of TEs within *P. oceanica* meadows; given that the quantification of flows and distribution of TEs between *P. oceanica* bed components is poorly known, a similar size was given to symbols modelling their cycling (Fig. 13). An ecological relevant size of the symbols could be gained by combining elemental analyses with TE uptake experiments, as did Schroeder and Thorhaug (1980) for a *Thalassia testudinum* community. The model given here for *P. oceanica* community is derived from their work, the sole known detailed study of global TE cycling within a seagrass ecosystem. TE flows within *P. oceanica* beds discussed in the next section will be referred to their corresponding "F-numbered" label given in Fig. 13 for clarity purpose. Providing details on all the components of *P. oceanica* meadows and their interconnecting flows exceed the frame of this introduction; particular attention will therefore be delivered to the processes directly related to the sole seagrass.

4.3.2. TE balance within Posidonia oceanica

Sanz-Lázaro et al. (2012) recently demonstrated the key role played by *P. oceanica* (as a species, not as an ecosystem) in the cycling of TEs in the Mediterranean coastal areas. These authors calculated the TE incorporation rates in *P. oceanica* rhizomes, roots and new leaves from mean tissue concentrations and tissue production rates, subtracted TE loss rates through leaf shedding, mechanical breakage (F25) and grazing (F21), and extrapolated the balances obtained for their reference meadow to the whole Mediterranean (Table 6, according to the estimates of the total cover of 50 000 km^2 of *P. oceanica* meadows; Pasqualini et al. 1998). Depending on the plant compartment where TEs were mainly accumulated and on their incorporation and loss dynamics, Sanz-Lázaro et al. (2012) calculated that *P. oceanica* could act either as a sink (positive balance) or as a source (negative balance; TEs given back accessible to others components of the system or exported) for these chemicals.

	TE	balance	world prod.	equivalence
sink	Fe	1 891	2 590 000	0.073 %
	Ni	175	1590	11 %
	Cr	30	7 290	0.41 %
	As	4.6	52.8	8.7 %
	Ag	3.6	23.1	16 %
source	Pb	-8	4 140	0.19 %
	Cd	-11	21	53 %
	Co	-21	90	24 %
	V	-38	58	67 %
	Cu	-45	16 000	0.28 %
	Mn	-587	43	1 375 %
	Zn	-1 459	12 000	12 %

Table 6. Annual balances of trace element amounts in *P. oceanica* (tons.y^{-1}) for the whole Mediterranean (Sanz-Lázaro et al. 2012). Positive or negative amounts indicate either incorporation or release by *P. oceanica*, respectively. These balances, expressed in equivalent % to the 2010 mean world production (tons.y^{-1}; see Table 1), reflect the quantitative importance of the role played by this species in the cycling of trace elements.

According to Sanz-Lázaro et al. (2012), *P. oceanica* compartment is the main driver of TE concentrations. Seagrass blades and associated epiphytes provide an expanded area to sorb and sequester chemicals (F1, F9), and their root-rhizome system facilitate the absorption and accumulation of sediment contaminants (F7; Ralph et al. 2006, Lewis and Devereux 2009). The extent to which these uptake processes are passive or subject to active physiological regulation will determine the final accumulation behaviour of seagrass compartments relative to the TE levels they are exposed to (Schlacher-Hoenlinger and Schlacher 1998). TEs accumulated by the leaf canopy and the root-rhizome system may afterward be translocated to below- (basipetal, F4) or above-ground (acropetal, F3) tissues, respectively (Ralph et al. 2006). In the case of *P. oceanica,* TE translocation seems to be low and acropetal in most cases (Sanz-Lázaro et al. 2012).

It is further interesting to notice that the concentrations of many TEs (e.g. V, Cr, Fe, As, Pb etc.) are highest in epiphytes when compared to the other compartments of *P. oceanica*; however, only two studies have focussed on that compartment (Schlacher-Hoenlinger and Schlacher 1998, Sanz-Lázaro et al. 2012). As epiphytes appear to be a key component of *P. oceanica* meadows and since they are ubiquitous on the leaves of seagrass species, they should be taken more into consideration when studying TE cycling in seagrass meadows (most of the studies focused on leaves and rhizomes; Sanz-Lázaro et al. 2012).

TE concentrations in both above- and below-ground compartments also follow an annual cycle (e.g. Malea et al. 1994, Schlacher-Hoenlinger and Schlacher 1998, Pergent-

Martini and Pergent 2000). This seasonality had been initially only attributed to variations in the plant growth dynamics that induced a dilution of the accumulated TEs (Lyngby and Brix 1982); but climatic patterns (seasonal rainfalls and storm frequency), leading to changes in chemical load in the water and sediment, show an equal or greater influence on this seasonality (Schlacher-Hoenlinger and Schlacher 1998, Prange and Dennison 2000).

Seagrass-accumulated chemicals and those associated with the epiphytic layer represent sources of potentially toxic chemicals to the grazer community (F21, F22) and can be lost to surrounding water in a dissolved form (F2, F10) or be exported bound to blade fragments (F25, F26) at senescence (Lewis and Devereux 2009). Some TEs like Zn, Cd, Sr or Rb show high release rate through decomposition of *P. oceanica* detritus and are expected to be released in the meadow (F20); *a contrario*, others like Cs, Tl or Bi show low release rate through decomposition and are more likely to be buried (F29) or exported to adjacent ecosystems (Sanz-Lázaro et al. 2012). Finally, the well-developed belowground system of *P. oceanica* roots and rhizomes forming mattes can persist for thousands of years (Mateo et al. 1997), thenceforth sequestrating a fraction of the accumulated and potentially toxic TEs and reducing the total amount bioavailable to other organisms (Pergent et al. 1997, Sanz-Lázaro et al. 2012). Seagrasses therefore act not only as biological filters, but also as storage compartments, thereby favouring the decrease of environmental toxic substances (Kaldy 2006).

5. *Mytilus galloprovincialis*

5.1. A brief description of its biology

5.1.1. Biogeography

The Mediterranean mussel *Mytilus galloprovincialis* Lamarck is a bivalve mollusc belonging to the order of Mytiloida, family of Mytilidae. It is considered that there exist 3 species of mussel within the genus *Mytilus – M. edulis*, *M. galloprovincialis* and *M. trossulus* – each inhabiting specifically parts of the European coasts (Fig. 14). *M. edulis* is distributed along the Atlantic coasts of Europe till high northern latitudes; *M. galloprovincialis* has a

more southern repartition along the French Atlantic coasts, from Britain to Morocco, and along various coastal regions of the British Isles. The third species, *M. trossulus*, is distributed in the Baltic Sea (Martinez-Lage et al. 1995).

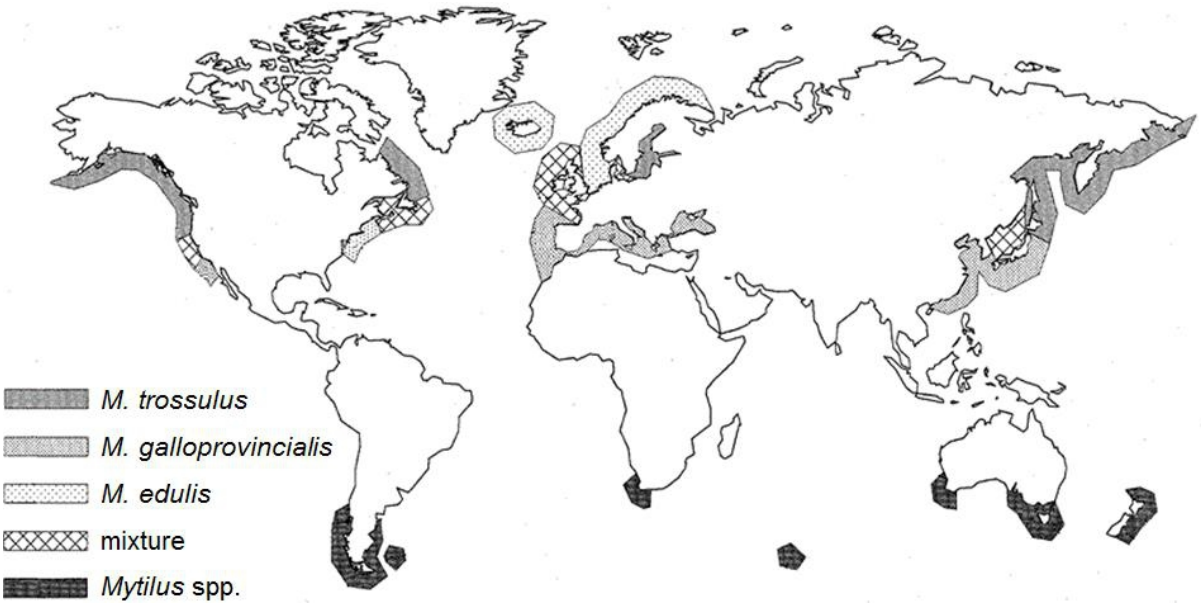

Fig. 14. Antitropical distribution of mussels of the genus *Mytilus* (medium grey areas: *M. trossulus*; light grey areas: *M. galloprovincialis*; dotted areas: *M. edulis*; hatched areas (mixture): regions of sympatry and hybridization between species pairs). For the northern hemisphere, identification and distribution of *Mytilus* taxa are well established. For the southern hemisphere, there had been no way until recently to differentiate between native southern and native northern hemisphere sibling species (Westfall and Gardner 2010, Westfall et al. 2010, Westfall 2011). There are therefore grouped as *Mytilus* spp. (dark grey ares; modified after Hilbish et al. 2000).

Mytilus edulis and *M. galloprovincialis* are sympatric in parts of Europe; in some areas of the United Kingdom, Ireland and France both hybridisation and introgression occur (López et al. 2002). *M. edulis* further locally intermixed with *M. trossulus* in the Danish straits (Martinez-Lage et al. 1995). There is very extensive mariculture of both *M. edulis* and *M. galloprovincialis* almost throughout their distribution areas (Beaumont et al. 2007). *M. galloprovincialis* has moreover become invasive in many parts of the world, including in Australia, Asia, California and the Puget Sound in the United States, and in South Africa (Stankovic et al. 2012).

5.1.2. General Characteristics

The objective of the present section is not to list and detail all of the characteristics of *Mytilus* spp., but well to raise certain aspects of their biology that facilitate their use as bioindicator species (shape, size, fixation, filtration etc.). The detailed list of specific features which characterises mussels as ideal bioindicators will be further given in section 5.2.

In mussels, the two shell valves are similar in size and are roughly triangular in shape (Fig. 15). Their foot secretes a byssus, a bundle of tough threads of tanned protein emerging through the ventral part of the shell and serving as mooring lines to strongly attach individuals to the substrate. When mussels are submerged and feeding, the shell valves are always open; when bivalves are emerged or threatened, two muscles, the anterior and posterior adductors, tightly close the shell valves. On a local scale, *Mytilus* spp. can dominate the intertidal to subtidal regions tightly fixed to rocky shores (Gosling 2003).

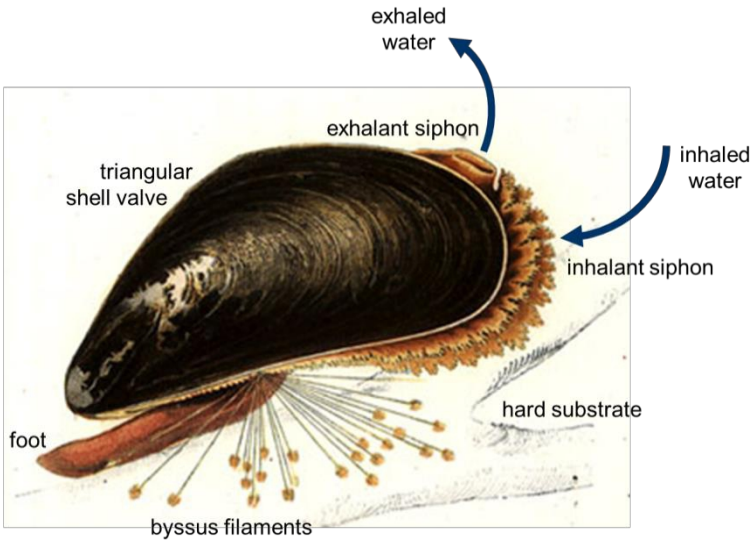

Fig. 15. *Mytilus* spp. schematic drawing. Arrows symbolize entering and outgoing water flows through mussel siphons (modified after http://www.vuvb.uniza.sk).

Under optimal conditions such as in the sublittoral zone, *M. edulis* and *M. galloprovincialis* attain a shell length of 100–130 mm, whereas in marginal conditions, e.g. the high intertidal zone on an exposed shore, mussels may measure as little as 20–30 mm even after 20 years. Feeding and respiration are carried out via currents of water directed across the gills. Water flows in through the inhalant siphon, through the gills, where filtering of suspended food particles takes place, and exits through the exhalant siphon. Food particles, trapped by cilia, are then conveyed towards the labial palps and mouth. Mussel gills function as one of the most efficient systems of ciliary feeding in the Animal Kingdom. Mussels can

also actively transport DOM across their gill membrane and utilise it as a nutritional supplement (Gosling 2003).

5.1.3. Reproduction

Reproduction in *Mytilus* spp. follows an annual seasonal cycle. Gametogenesis and energy storage occur in the mantle tissue, where a large shift in cell types (adipogranular cells and vesicular connective tissue vs. gametes) is evident throughout the annual cycle (Fearman et al. 2009). Sex of mature individuals can be told by the colour of their gonads: pink to orange for female and creamy-white to yellow for males (Fig. 16; Mikhailov et al. 1995). After spawning, this sex segregation based on mantle colour is not possible anymore, as mantle becomes thin and translucent (Mikhailov et al. 1995, Torrado and Mikhailov 1998).

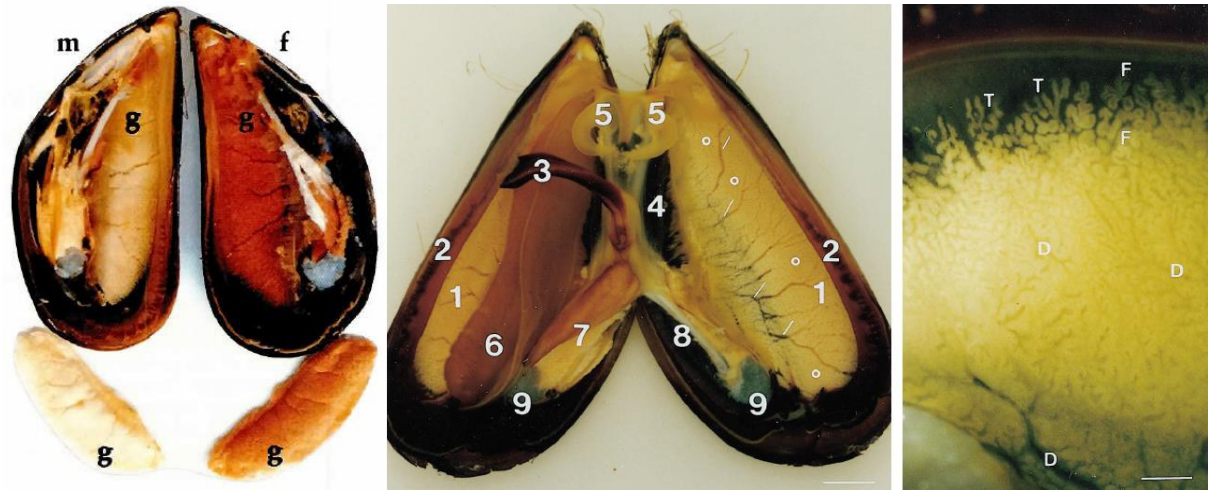

Fig. 16. Tissue morphology in *M. galloprovincialis*. **Left** - Sex-dependent differences in the coloration of the mantle (gonad) tissue: "opened" sexually matured male (m) and female (f) mussels (each valve contains the gonad and other tissues; the gills covering the gonads were removed) and isolated creamy-white and orange gonads (g) (Mikhailov et al. 1995). **Center** - "Opened" sexually matured male mussel. General view of right valve: the gills and portion of visceral mass were removed to demonstrate the foot retractor muscle, hepatopancreas, and gonad (mantle) lobe (1 = mantle (gonad), 2 = muscular mantle edge, 3 = foot, 4 = hepatopancreas, 5 = labial palps, 6 = gills, 7 = visceral mass, 8 = foot retractor muscle, 9 = posterior adductor muscle, *circles* = gonad follicle regions spread over the mantle, *small white lines* = indicate gonad duct). Scale bar 1 cm. **Right** - Fragment of the gonad representing large gonad ducts (D), tubules (T) and follicle-like (F) structures. Scale bar 2.5 mm (Torrado and Mikhailov 2000).

In *M. galloprovincialis* collected in Galicia (Spain), the development of the gonad tissue begins in autumn and gametogenesis then proceeds throughout the winter, culminating in spawning in spring and early summer. Spawning may occur throughout the summer until late August or September (Mancebo et al. 1992). Preponderant spawning in January/February and partial spawning in April/June is also observed along the French Mediterranean coasts (Lubet et al. 1986, Bodin et al. 2004), and is in agreement with descriptions of the reproductive cycle of mussels in the Estuary of Vigo (Spain; Suárez et al. 2005). As most studies regarding the uptake and elimination kinetics of TEs implicitly assume steady-state conditions for physiological processes, *i.e.* without a reproductive period (Casas et al. 2008), the knowledge of mussel reproductive cycle in the area under study is a necessary prerequisite to their use.

5.2. *Mytilus* spp. as bioindicator species

5.2.1. Quality indicators

The aspects that make mussels well suited as bioindicator species are also shared with other systematic groups. However, it is the unique combination of these different features which characterises mussels as ideal bioindicators (Cossa 1989, Oehlmann and Schulte-Oehlmann 2003).

- Mussels exhibit a broad geographical distribution, ranging from temperate to subarctic regions.
- Mussels, as euryhalin, colonize from estuarine to fully marine environments.
- Mussels are key species for the functioning of ecosystems; it is likely that a pollutant that affects a mussel population will also exhibit a negative impact for the entire ecosystem.
- Mussels, completely sessile as adults, represent the contamination of their habitat ideally. Their planktonic larval stage however guarantees a high dispersal potential and allows a recruitment of populations even in habitats where sexually mature adults might have become extinct due to the high level of contamination.

• Mussels are long-living so that they can integrate contaminations of their environment over long time periods and they permit sampling of more than one year-class.

• Mussels are relatively large, easy to handle, and can be used both under laboratory and field conditions, for active and passive biomonitoring.

• Due to the lack of an exoskeleton, mussels are in direct contact with the ambient medium. Therefore, chemicals can be taken up not only from the diet (via the gastro-intestinal tract) but also additionally from ambient water, including the respiratory organs, resulting in a greater accumulation potency for contaminants.

• Mussels exhibit only a limited ability to eliminate pollutants and attain higher bioconcentration factors (10^3 to 10^5 when compared to the surrounding water) for many toxicants than other systematic groups.

• Consequently, pollutants might exhibit negative impacts on mussels at lower environmental concentrations, facilitating their use as an ecological early warning system.

• The internal organisation, especially the normal morphological and histological structure of the different organs and tissues, and the physiology of mussel species used for biomonitoring is characterised quite well.

• Consequently, biological effects of environmental stress in general and of contaminant exposure in particular are measurable at various levels of biological organisation (from molecules to communities).

• Mussels are non-controversial as organisms for ecotoxicological research, especially as test animals and for environmental monitoring.

• Finally, mussels used for human consumption are therefore a potential source of contaminants for humans.

For all these reasons, mussels are very widely used in programs monitoring the chemical pollution of the marine coastal environment (e.g. Goldberg 1975, Amiard et al. 1986, Kljakovic-Gaspic et al. 2006).

5.2.2. Pollution monitoring

After a stay of several months in water, the content of contaminants in the flesh of mussels reflects the concentration of bioavailable contaminants in the water (Casas and Bacher 2006). This content results from a balance between the concentration in the organism and its environment, dependent on the process of absorption, excretion and accumulation (Cossa 1989). Based on the use these molluscs for monitoring purposes, two types of strategies have been adopted: some scientists use of the native populations of wild or cultivated mussels (passive biomonitoring – e.g.: the Mussel Watch Program in the USA, Goldberg 1975, the RNO program in France, Chiffoleau et al. 2005) while others resort to transplants of individuals (active biomonitoring – e.g. : RINBIO and MYTILOS programs in the Mediterranean, Andral et al. 2004, Benedicto et al. 2011). In the latter case, caged mussels are placed several months during their sexual dormancy in the water body of interest in order to accumulate contaminants up to achieve a balance with their transplantation environment.

The biomonitoring of chemical contaminants in coastal waters is mostly carried out by direct quantification of the accumulated pollutants within individuals (e.g. Lobel and Wright 1983, Blackmore and Wang 2003, Andral et al. 2004, Andral et al. 2011, Galgani et al. 2011). However, the use of biological responses to pollutants (or biomarkers), based on responses at the molecular and cellular level, has been showed to be a useful complementary tool in environmental quality evaluation and risk assessment (Bocchetti et al. 2008, Serafim et al. 2011). Alteration in specific biomarkers reflect the type of pollution which are facing organisms; for example, metallothioneins and the enzyme d-aminolevulinic acid dehydratase are indicative of metal contamination, the mixed function oxidase system, glutathione-Stransferase and acetylcholinesterase are indicative of organic contamination and superoxide dismutase, catalase, glutathione peroxidase and lipid peroxidation are biomarkers of oxidative stress. A multibiomarker approach therefore turns out to be a useful approach as complex mixtures of contaminants usually occur in the environment (Serafim et al. 2011).

The use of mussels as bioindicator species is not limited to the sole chemical contaminants. So, Kaçar et al. (2011) have shown that *M. galloprovincialis* bioconcentates microbial pollution indicators (heterotrophic bacteria and faecal coliforms) and pathogens (*Salmonella* spp.), and could therefore be used to prevent potentially harmful adverse effects

of microorganisms from polluted waters and shellfish. Browne et al. (2008) experimentaly demonstrated that *Mytilus edulis* bioaccumulated microplastic in its flesh, and Lassauque et al. (2010) successfully traced sewage and natural freshwater input in a Northwest Mediterranean bay from carbon and nitrogen isotopic ratio measurements in *M. galloprovincialis*.

5.2.3. Seafood and human health

Mussels are commercially important seafood species; the determination of accumulated concentrations of contaminants in their flesh is essential because of the potential adverse effects of their consumption on human health (Guéguen et al. 2011, Stankovic and Jovic 2012). In the particular case of metals, the maximum permissible limits fixed by the European Commission (EC 2001) in edible tissues of mussels are 0.5 $mg.kg^{-1}$ for Hg and 1 $mg.kg^{-1}$ for Cd and Pb (related to fresh weight).

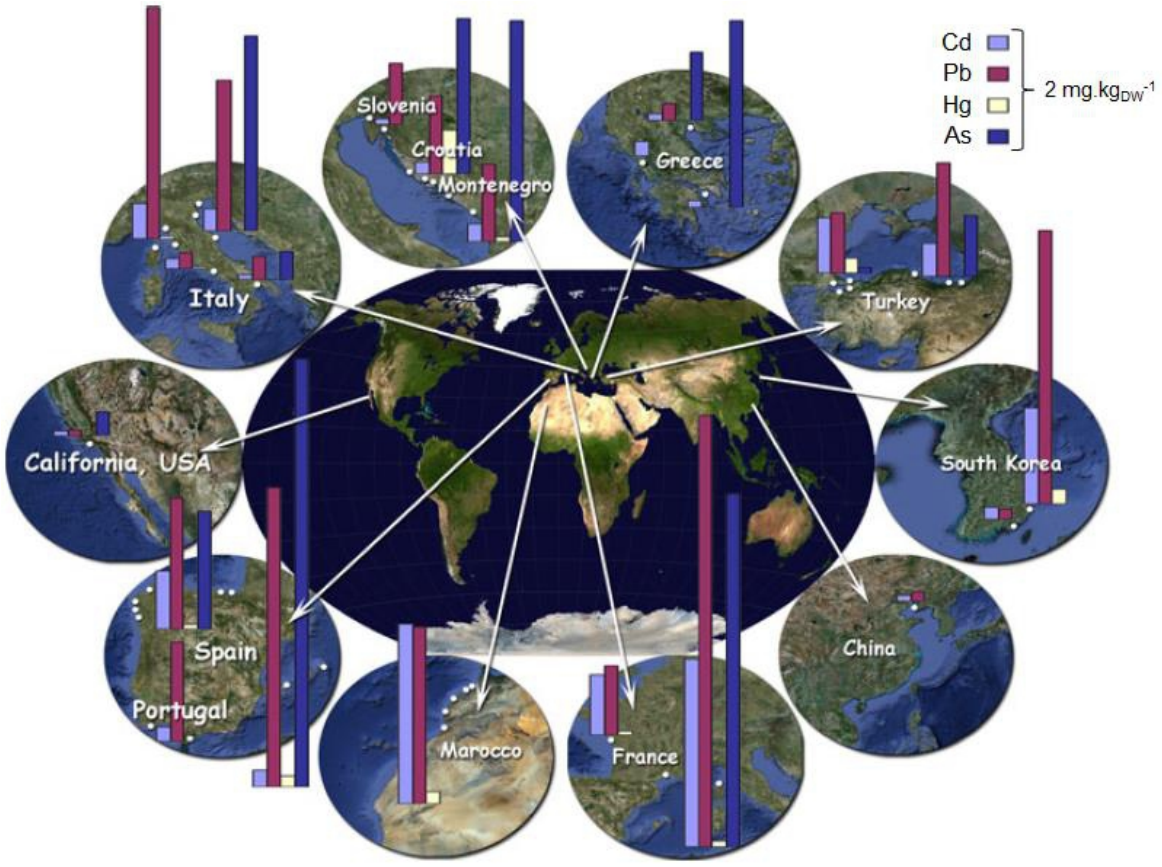

Fig. 17. Studied areas for Cd, Pb, As, and Hg levels in *M. galloprovincialis* collected worldwide (full white circles), with a focus on regional hot spots (bar histograms; Stankovic and Jovic 2012).

Comparison of the concentrations determined in the soft tissues of *M. galloprovincialis* sampled worldwide with the European legislation show that the levels of these metals generally do not exceed the existing limits (detailed dataset in Stankovic et al. 2012). Nevertheless, these toxic elements may pose sanitary risks to consumers of contaminated shellfish, especially related to the levels of Pb and Cd recorded in *M. galloprovincialis* from hot spots (Fig. 17) in all the investigated seas (Stankovic and Jovic 2012, Stankovic et al. 2012).

5.3. Trace element bioaccumulation in *Mytilus galloprovincialis*

5.3.1. Driving factors

Since the mid-70s, *Mytilus* spp. have been widely used to monitor the chemical contamination of coastal and estuarine ecosystems (e.g. Goldberg 1975, Cossa and Bourget 1980, Cossa 1989, Odzak et al. 1994, Andral et al. 2004, Benedicto et al. 2011). It is well known that TE levels in aquatic organisms represent a time-integrated response to bioavailable pollutants in food and water (Wang and Fisher 1999, Croteau and Luoma 2005) But these levels are not only the result of their bioavailability in the environment, and biotic and abiotic factors are further acting (Cossa 1989).

In *Mytilus* spp., biotic factors implied in the establishment of body TE concentrations can be considered in two categories, respectively growth (age, size, soft tissue weight) and reproduction (sex and gametogenesis). Environmental factors essentially revolve around seasonal cycles (temperature, primary production, salinity etc.), although other parameters may also be involved (e.g. properties of TEs and their interactions, the position of mussels in the intertidal etc.). The influence of these factors must be identified and measured so that they may be taken into account during sampling, validation of results, or in the interpretation of monitoring data (Cossa 1989). The study of the pollution is thenceforth made extremely complex due to the diversity of contaminant characteristics (nature, speciation, concentrations and interactions), the diversity of ecological factors (abiotic and biotic) and their variations and interactions in space and time (Casas 2005).

5.3.2. Physiological correction

According to Amiard et al. (1986), the seasonal variations of the concentrations of TEs can be explained primarily by the fluctuations of the mussel body mass. These variations of the flesh weight are related to the availability of food in the environment but also to the physiology of these organisms, which includes the gametogenesis and the constitution of substances of reserve. The gonad development leads to an increase in the body mass and, during this period, TEs present in their flesh are in some way "diluted", while they are "concentrated" when their body mass decreases at the release of the breeding material. The same goes for an increase/decrease in body mass linked to a high/low availability of food (e.g. Cossa et al. 1980, Cossa and Rondeau 1985, Rainbow 1995).

A comparison of the raw concentrations between mussels from sites with different trophic and physicochemical characteristics is thenceforth not possible and an adjustment of the data is requested (Andral et al. 2004). For this reason, the IFREMER adjusts the concentrations of pollutants measured in caged mussels in the frame of their Mussel-Watch programs with a condition index corresponding to the ratio of the flesh dry weight on the shell dry weight. This condition index has the advantage of being easy to measure, of being a global index comprising several physiological factors (nutrition, reproduction etc) (Kantin and Pergent-Martini 2007), and seems to be the biometric variable which is the more closely related to tissue concentrations for a large panel of contaminants (Andral et al. 2004). For TEs, the tissue concentration is inversely proportional to the condition index. This relationship has allowed correcting the influence of the trophic characteristic of the monitored area by adjusting each measured concentration regarding to the same index of condition said of reference. These "adjusted" concentrations then allow the intercomparison of the different sites (Andral et al. 2004).

6. Objectives of the study

As reported earlier, the Mediterranean Sea is submitted to various growing anthropogenic pressures. One of the most pernicious threats is the invisible chemical contamination of coastal ecosystems, notably by trace elements. The biological relevance of this pollution may be monitored with bioindicator species, *i.e.* organisms allowing to characterise the health status of an ecosystem (Blandin 1986).

Two of the most widely species used in monitoring surveys in the Mediterranean are the marine magnoliophyte *Posidonia oceanica* and the Mediterranean mussel *Mytilus galloprovincialis*. These bioindicators have shown to be relevant species to monitor coastal pollution by numerous trace element (Cr, Fe, Ni, Cu, Zn, Cd, Pb, V, As and Ag). However, trace element pollution is not a static problem, and it continuously evolves both spatially and temporally. Furthermore, some trace elements of previous little concern (Be, Al, Mn, Co, Se, Mo, Sn, Sb and Bi) can also be considered as potential stressors of coastal environments. The biomonitoring of trace element pollution is henceforth always a topical subject.

The global objective of the present study was therefore (i) to monitor the present status of trace element pollution along the French Mediterranean coasts, (ii) to investigate the potential use of *P. oceanica* and *M. galloprovincialis* to biomonitor trace elements of environmental "emerging concern", and (ii) to study the underlying physiological mechanisms determining trace element accumulation in both species, under reference conditions or when exposed to environmental changes of pollutant loads.

More precisely, we measured levels of trace elements of emerging concern in *P. oceanica* shoots sampled along the French littoral, as we firstly wanted to know whether some spatial variability of their concentrations could be linked to polluting human activities. In the framework of a continuous monitoring of the chemical pollution of the Mediterranean, we have also measured levels of trace elements classically surveyed with this bioindicator.

All biomonitoring surveys compared sites between them or to references. Previous studies have notably suggested electing the Calvi Bay, Northwestern Corsica (France), as a reference site for the monitoring of the pollution by trace elements. However, the number of studied chemicals was limited, long-time records were missing, or environmental levels

(water, sediments) were unknown. To validate (or invalidate) the election of this specific site as a reference for the Northwestern Mediterranean, we measured trace element levels in *P. oceanica* shoots sampled seasonally during three years, as well as in their environment (water and superficial sediments).

Mytilus galloprovincialis and *Posidonia oceanica* complement each other in the monitoring of chemical pollution. *P. oceanica*, as a sessile primary producer strongly anchored in sediments, accumulate trace elements from the water column and from sediments; *M. galloprovincialis*, as a filter feeder, accumulate dissolved chemicals and the ones associated to their feeding particles. Their combined use thenceforth allows to globally describe the health status of the environment they were collected from. Thus, we attempted to compare their trace element bioaccumulation behaviours according to their specific lifestyle.

If *P. oceanica* has been widely used in monitoring surveys, very little is *a contrario* known about the trace element uptake and loss kinetics by that species. Yet, this aspect appears essential, notably to quantify the efficiency of the species to respond to chemical environmental relevant changes of its surrounding habitat. In order to study these kinetics, we *in situ* experimentally contaminated *P. oceanica* shoots. The physiological status of organisms further strongly influences their response when exposed to pollutants. The inner pollutant levels may be more or less regulated, depending on the species considered and the environmental chemical load, and their biological cycle, mainly through growth, aging and reproduction can further concentrate or dilute trace elements. We have therefore evaluated the influence of the physiology and the biological cycles of *P. oceanica* and *M. galloprovincialis* on their bioaccumulation behaviours of trace elements.

The body compartmentalization of trace elements in bioindicator species also play a decisive role when monitoring chemicals, but this particular consideration is too often little taken into account. So, for *P. oceanica*, authors have studied integral shoots, adult leaves, rhizome etc. If numerous scientists suggest using mussel hepatopancreas in monitoring surveys, others used gills while large spatial-scale programs (e.g. RINBIO mussel watch program in the Mediterranean) use entire individuals. We thenceforth studied this compartmentalization aspect in order to propose common protocol of use of the 2 species in the monitoring of the health status of the Mediterranean.

Chapters 2 to 4 of this manuscript present, in the form of scientific publications, many of the aspects stated above as follows:

• **Chapter 2** (published in the peer reviewed journal Ecological Indicators - 2012, 18(1): 269-277) investigates the spatial variability of levels of trace elements of "emerging concern" in *P. oceanica* sampled along the French Mediterranean coasts. A time-integrated efficient monitoring of trace elements requires the continuous survey of their environmental levels; we therefore report levels of trace elements classically monitored with this species. We also study the compartmentalization of trace elements between *P. oceanica* above-ground tissues at the scale of the French Mediterranean.

• **Chapter 3** (article in press) investigates the uptake and loss kinetics of trace elements by *P. oceanica* experimentally *in situ* exposed to pollutants. Distribution of chemicals between above- and below-ground tissues is discussed. Moreover, we attempt to confirm (or invalidate) the reference clean status of the Calvi Bay via the analysis of trace element levels in seasonally sampled *P. oceanica* shoots, like in their environment (water and superficial sediments).

• **Chapter 4** (article in press) investigates the potential use of *M. galloprovincialis* in the biomonitoring of trace elements of "emerging concern". It further gives some insight into the decisive role played by some relevant biological parameters (size, body compartmentalization, sex, gametogenesis) in the bioaccumulation process of trace elements by rope-grown mussels.

Finally, C**hapter 5** globally discusses results presented in Chapters 2 to 4. In order to look further into certain aspects of this general discussion, and to gives relevant answer to the many objectives listed above, results of complementary studies (master thesis co-directed with Dr. Sylvie Gobert, in collaboration with STARESO (STARE-CAPMED contracts), IFREMER and the French Water Agency) related to the present work will be presented each time that is sensible. Throughout the discussion, in the light of the results presented, we propose rules of use of the two studied species in order to improve their bioindicators potential in the monitoring of the pollution by trace elements.

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Chapter 2

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Spatial variation of TE levels in *Posidonia oceanica*

Chemical contamination along the Mediterranean French coast using *Posidonia oceanica* (L.) Delile above-ground tissues: a multiple trace element study

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Abstract

Levels of Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb, Bi as well as of Cr, Fe, Ni, Cu, Zn, Cd and Pb in *Posidonia oceanica* (L.) Delile from the Mediterranean French coast were analysed using DRC ICP-MS. The first twelve elements have not been well studied and can be considered to be potential pollutants as a result of potentially increased levels resulting from anthropogenic activities. Spatial variation and/or compartmentalization were found for all trace elements. Except for Al, Cr, Fe, Cu and Ag, most trace elements were preferentially accumulated in photosynthetic tissues, suggesting uptake from the water column. Moreover, for Be, V, Mn, Co, Ni, As, Mo, Sb, Sn and Pb, adult leaves had higher levels than intermediate leaves, suggesting low kinetics of accumulation. Levels in the third intermediate leaf were representative of the average levels of the integral shoot, and thus can be used alone in chemical biomonitoring. For most of the twelve little-studied trace elements, the background levels of the northwestern Mediterranean Sea can be measured, and their spatial variation can be related to anthropogenic activities. Levels of the seven widely studied trace elements seem to decrease or stabilize over time, probably due to their reduced anthropogenic use. These observations show that *P. oceanica* is a sensitive bioindicator for the monitoring of chemical contamination of a large number of trace elements.

Keywords: trace element, seagrass, *Posidonia oceanica*, Mediterranean, pollution, ICP-MS

1. Introduction

Posidonia oceanica (L.) Delile, the endemic seagrass of the Mediterranean, forms monospecific meadows from the surface to depths of 40 m (Boudouresque and Meinesz, 1982). They cover large areas in clear coastal regions estimated to be between 2.5 and 5 million hectares (Pasqualini et al., 1998). They constitute an engineering ecosystem playing major ecological, geological and economic roles, but are sensitive to human disturbances such as coastal development, pollution, high water turbidity and trawling (Boudouresque et al., 2006). In 2000, *P. oceanica* was selected as a Biological Quality Element (BQE; Med-GIG, 2009) representative of aquatic Mediterranean angiosperms for monitoring the ecological status of coastal waters under the Water Framework Directive (EC, 2000). Therefore, many indices based on physiological, morphological and/or structural descriptors of *P. oceanica* have been developed, e.g. POMI (Romero et al., 2007a,b), PREI (Gobert et al., 2009), BiPo (Lopez y Royo et al., 2010), etc.

Metals are regarded as serious pollutants of the aquatic environment because of their toxicity, their persistence, their difficulty in biodegrading and their tendency to concentrate in aquatic organisms (Ikem and Egiebor, 2005). Many metalloids (As, Sb, etc.) and some non-metals (Se, etc.) can also be considered as serious pollutants of the aquatic environment. However, some of these are needed for seagrass subsistence (micronutrients) such as Fe, Mn, Co, Ni, Cu and Zn (Babula et al., 2008). In agreement with Duffus (2002), we use the term "trace element" (TE) for those elements that are present in trace amounts in the environment.

Levels of some TEs (Cd, Hg, Cu, Cr, Pb, Zn, Fe and Ni) have been largely studied in *P. oceanica* tissues (see Annex A) and both below- and above-ground tissues have been analysed. In particular, *P. oceanica* leaves can give an indication of TE levels in seawater over a short period with accuracy (Pergent-Martini et al., 2005). Furthermore, Romero et al. (2007b) suggested that the third intermediate leaf alone (bearing few epiphytes and between 100 and 150 days old) can be used for TE monitoring instead of the integral shoot.

The development of new equipment and techniques now allows us to measure many TEs found at very low levels (Wieser and Schwieters, 2005); moreover, recent technological and industrial developments still modify levels of TEs naturally present in the environment (Ravindra et al., 2004). Environmental quantification of other previously unstudied potential pollutants is henceforth now possible and relevant: (i) V is used in many fields: railway,

aerospace, catalysis, etc. It is found in large amounts in hydrocarbon fuels, the main source of this pollutant, and it can be used as a tracer for petrol pollutions such as oil spills (Amiard et al., 2003); (ii) Sb is a component of many alloys, and is often used as fireproofing additive. This TE is released in large amounts during its extraction, smelting, as well as during waste incineration and fossil energy combustion (Filella et al., 2002); (iii) Bi is mainly used in specialized industry; Karlsson et al. (2007) determined that the major worldwide atmospheric deposition of Bi took place after the Second World War.

The objectives of this study were firstly to measure levels of TEs that were little or never studied in *P. oceanica* tissues (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi) along the Mediterranean French coast, to examine compartmentalization and spatial variation, and to evaluate the potential use of *P. oceanica* above-ground tissues as bioindicators. Secondly, we investigated spatial and temporal variations of TEs classically investigated (Cd, Cu, Cr, Pb, Zn, Fe and Ni), as well as their compartmentalization. Finally, the ability to use the *P. oceanica* third intermediate leaf instead of the integral shoot for the biomonitoring of these nineteen TEs was investigated.

2. Materials and methods

2.1. Sampling location and design

In April 2007, 15 shoots of *P. oceanica*, each spaced apart 5 m, were sampled at a depth of 15 ± 1 m at 18 sites located along the Provence-Alpes-Côte d'Azur (PACA) and Corsican coasts (Fig. 1). Collected samples were placed in acid-rinsed sample bags and stored at -28 °C.

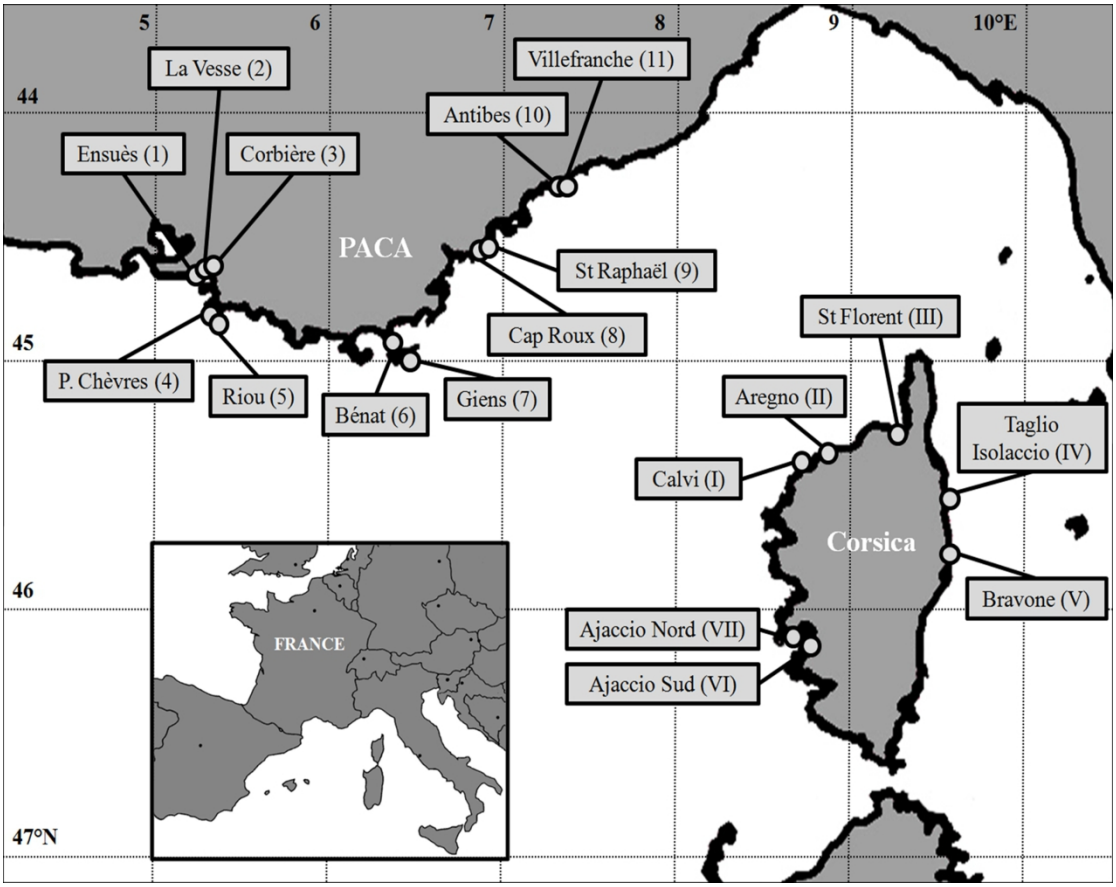

Fig. 1. Map showing the locations of the study sites along the Provence-Alpes-Côte d'Azur (PACA) and Corsican coasts.

2.2. Sample treatment

For each site, *P. oceanica* shoots were dissected according to the method proposed by Giraud (1979). Epiphytes were scraped from leaves using a ceramic scalpel blade. Furthermore, each shoot was sorted and recorded as follows: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL). Sorted tissues were lyophilized (BenchTop 3L, VirTis Company Inc.), weighed and then grouped together (by site) to constitute pools of dried tissues weighing a minimum of 300 mg (mostly 5 pools, sometimes down to 3 for SAL). These pools were ground with liquid nitrogen in an agate mortar and then re-lyophilized to eliminate condensed ambient water vapour. Dried powders were finally mineralized in Teflon bombs using a closed microwave digestion labstation (Ethos D, Milestone Inc.). Two digestion procedures were performed: a nitric acid mineralization (HNO_3/H_2O_2; all TEs except Sn and Sb) and an *aqua regia*

mineralization (HNO_3/HCl; Sn and Sb). Acids and hydrogen peroxide (suprapure grade) were purchased from Merck. Finally, mineralisats were diluted to an appropriate volume of 50 cm^3.

2.3. Trace element analysis

Trace element levels were determined by inductively coupled plasma mass spectrometry (ICP-MS) using dynamic reaction cell (DRC) technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). This instrument uses ion-molecule reactions to overcome spectral overlaps and requires selection of the appropriate reaction gas (Olesik and Jones, 2006): no reaction gas in standard mode (for ^{9}Be, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{208}Pb and ^{209}Bi), NH_3 (for ^{27}Al, ^{51}V, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu and ^{66}Zn) or H_2 (for ^{75}As and ^{78}Se) in DRC modes. Analytical accuracy was checked by analysing certified reference materials (Table 1A): BCR 60 (*Lagarosiphon major*), BCR 62 (*Olea europaea*), GBW 07603 (brush branch and leaves) and V463 (maize).

2.4. Statistical analysis

General trends of TE levels (compartmentalization and shoot average levels, Table 1B) were calculated for all sites together, excluding extreme values: only values between the 10^{th} and 90^{th} percentiles were included. Each replicate (Fig. 2 *Posidonia oceanica* compartmentalization and Table 1B tissue trends; Annex B, C) represents the TE level of an individual pooled subsample, obtained by pooling the same tissues from 3 shoots (up to 5 for SAL) from the same site. TE levels measured in the 4 compartments of *P. oceanica* were balanced by their respective dry weight to calculate shoot TE levels (Table 1B shoot trends, Table 1C). For each element, detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated according to Currie (IUPAC et al., 1998; Currie, 1999) or Grinzaid et al. (1977), depending on their specific blank distribution. One-way analysis of variance (ANOVA) followed by Tukey HSD pairwise comparison test of means with unequal n's ($p < 0.05$) were performed (Table 1B tissue trends), after testing for homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric analysis of variance (Kruskal–Wallis test) was only performed for V, Sb and Pb (Table 1B; assumptions prior to unbalanced ANOVAs not encountered), followed by Dunn pairwise comparison test of means ($p < 0.05$) (STATISTICA 8.0, StatSoft Inc.).

	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
A: CRMs										
CRM values										
BCR 60		4180 ± 120	*6*	*26*	1760 ± 60	*2380*	*4*	*40*	51,2 ± 1,9	313 ± 8
BCR 62		448 ± 18	*1*	*2*	57,0 ± 2,4	*280*	*0,2*	*8*	46,6 ± 1,8	16,0 ± 0,7
GBW 07603	0,051 ± 0,004	2000 ± 300	2,40 ± 0,40	2,6 ± 0,2	61 ± 5	1070 ± 57	0,41 ± 0,05	1,7 ± 0,3	6,6 ± 0,8	55 ± 4
V463		*172 ± 13*		*3,37 ± 0,61*	*24,87 ± 0,78*	*366 ± 25*	*0,18 ± 0,06*	*3,37 ± 0,18*	*4,72 ± 0,54*	*61,19 ± 1,39*
Our values										
BCR 60 (n = 1-11)	0,069 ± 0,015	1823 ± 443	4 ± 0,3	17 ± 2,5	1571 ± 126	1913 ± 88	4 ± 0,1	45 ± 3	52,1 ± 5,5	299 ± 13
BCR 62 (n = 1-11)	0,014 ± 0,004	370 ± 29	1 ± 0,05	2 ± 0,5	54,9 ± 3,1	302 ± 21	0,3 ± 0,07	8 ± 2	44,6 ± 3,6	15,5 ± 0,9
GBW 07603 (n = 3-14)	0,035 ± 0,012	1189 ± 224	1,98 ± 0,15	1,8 ± 0,1	65 ± 2	963 ± 34	0,59 ± 0,09	5,7 ± 2,1	9,3 ± 3,9	56 ± 2
V463 (n = 1-13)	0,006 ± 0,004	129 ± 21	0,27 ± 0,03	1,63 ± 0,34	24,45 ± 0,99	348 ± 23	0,14 ± 0,02	3,13 ± 0,98	4,92 ± 0,93	54,75 ± 3,34

		Be		Al		V		Cr		Mn		Fe		Co		Ni		Cu		Zn
B: Plant tissues																				
3IL (n = 72-74)	**ab	0,0063 ± 0,0026	a	84 ± 37	a	6,5 ± 4,5	ab	0,29 ± 0,10	a	61 ± 10	a	98 ± 32	a	2,59 ± 0,45	a	31 ± 4	a	12,8 ± 3,3	a	107 ± 22
OIL (n = 73)	**ab	0,0065 ± 0,0024	ab	81 ± 28	a	5,6 ± 4,2	ab	0,23 ± 0,08	a	54 ± 9	a	92 ± 26	a	2,21 ± 0,42	ab	24 ± 2	ab	13,2 ± 3,6	a	98 ± 22
BAL (n = 71-73)	*a	0,0097 ± 0,0027	a	90 ± 38	b	9,9 ± 7,1	a	0,35 ± 0,09	a	76 ± 14	a	99 ± 27	a	3,42 ± 0,71	b	39 ± 5	a	11,4 ± 3,5	a	107 ± 22
SAL (n = 54-55)	b	0,0058 ± 0,0026	b	151 ± 66	c	1,2 ± 0,6	b	0,32 ± 0,24	b	14 ± 3	a	126 ± 54	b	0,28 ± 0,04	c	13 ± 2	b	16,8 ± 4,4	b	34 ± 8
Shoot (n = 217)	*	0,0076 ± 0,0018		96 ± 33		7,2 ± 4,9		0,30 ± 0,09		60 ± 10		102 ± 29		2,56 ± 0,47		30 ± 3		12,8 ± 3,4		98 ± 20

		Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
C. Sites											
PACA											
Ensuès (1)	*	0,0094 ± 0,0015	118 ± 18	9,6 ± 2,6	0,37 ± 0,04	64 ± 2	139 ± 14	2,38 ± 0,12	30 ± 2	13,0 ± 0,4	105 ± 5
La Vesse (2)	*	0,0079 ± 0,0022	114 ± 29	11,8 ± 3,2	0,33 ± 0,05	65 ± 4	122 ± 14	2,81 ± 0,15	32 ± 2	19,8 ± 0,6	157 ± 7
Corbière (3)	*	0,0095 ± 0,0011	143 ± 16	13,1 ± 3,2	0,38 ± 0,03	59 ± 7	138 ± 13	2,32 ± 0,18	27 ± 2	18,1 ± 1,3	136 ± 6
P. Chèvres (4)	**	0,0060 ± 0,0019	63 ± 6	17,3 ± 2,7	0,34 ± 0,02	45 ± 2	82 ± 4	2,07 ± 0,12	29 ± 2	12,0 ± 0,4	91 ± 5
Riou (5)	**	0,0037 ± 0,0016	27 ± 4	1,6 ± 0,1	0,18 ± 0,02	42 ± 3	57 ± 4	2,30 ± 0,15	34 ± 2	10,9 ± 0,7	94 ± 6
Bénat (6)	*	0,0080 ± 0,0022	90 ± 18	1,8 ± 0,2	0,22 ± 0,02	63 ± 4	95 ± 9	2,89 ± 0,31	35 ± 3	12,5 ± 1,5	90 ± 7
Giens (7)	*	0,0088 ± 0,0018	144 ± 19	1,8 ± 0,4	0,25 ± 0,02	69 ± 4	134 ± 11	3,42 ± 0,31	35 ± 1	12,8 ± 1,4	122 ± 5
Cap Roux (8)	*	0,0095 ± 0,0015	120 ± 15	8,7 ± 1,7	0,26 ± 0,01	74 ± 7	78 ± 5	3,23 ± 0,27	35 ± 3	9,9 ± 0,7	85 ± 5
St Raphaël (9)	**	0,0066 ± 0,0010	41 ± 8	3,4 ± 0,8	0,21 ± 0,02	93 ± 14	56 ± 6	4,49 ± 0,72	48 ± 4	12,6 ± 1,6	123 ± 13
Antibes (10)	*	0,0072 ± 0,0015	95 ± 13	22,9 ± 6,5	0,27 ± 0,03	52 ± 5	91 ± 6	2,40 ± 0,17	29 ± 2	14,2 ± 1,2	65 ± 5
Villefranche (11)	*	0,0076 ± 0,0021	60 ± 6	12,3 ± 4,1	0,31 ± 0,05	57 ± 12	85 ± 3	1,97 ± 0,21	20 ± 2	22,9 ± 1,4	83 ± 5
Corsica											
Calvi (I)	**	0,0060 ± 0,0013	20 ± 3	8,9 ± 1,3	0,16 ± 0,02	48 ± 4	41 ± 2	1,90 ± 0,09	29 ± 2	6,8 ± 0,7	69 ± 6
Aregno (II)	**	0,0050 ± 0,0018	66 ± 11	1,6 ± 0,1	0,22 ± 0,05	56 ± 2	69 ± 15	3,16 ± 0,11	32 ± 1	15,0 ± 0,9	109 ± 7
Saint Florent (III)	**	0,0042 ± 0,0015	102 ± 18	2,8 ± 0,4	0,98 ± 0,12	45 ± 4	135 ± 16	1,97 ± 0,20	24 ± 1	9,2 ± 0,9	73 ± 8
Taglio Isolaccio (IV)	*	0,0090 ± 0,0015	120 ± 13	18,6 ± 5,6	0,51 ± 0,04	89 ± 9	140 ± 9	2,32 ± 0,15	28 ± 1	10,7 ± 0,7	88 ± 9
Bravone (V)	*	0,0090 ± 0,0009	147 ± 32	2,3 ± 0,3	0,60 ± 0,08	77 ± 8	179 ± 29	3,62 ± 0,25	29 ± 2	22,6 ± 1,8	1282 ± 241
Ajaccio Sud (VI)	*	0,0077 ± 0,0012	112 ± 24	3,5 ± 0,9	0,21 ± 0,03	56 ± 2	80 ± 9	2,65 ± 0,09	34 ± 1	8,4 ± 0,3	89 ± 6
Ajaccio Nord (VII)		0,0113 ± 0,0014	151 ± 29	6,8 ± 2,8	0,24 ± 0,02	55 ± 2	143 ± 7	1,56 ± 0,06	22 ± 1	7,5 ± 0,4	72 ± 7

Table 1. (A) Evaluation of analytical accuracy through certified reference materials (CRMs). (B) General trends of *Posidonia oceanica* trace element levels (tissue compartmentalization [n = 54–74] and shoot concentrations [n = 217]). (C) Spatial variation of trace element levels in *Posidonia oceanica* shoots [n = 15 for each site]. Levels are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$; letters represent significant differences between tissues ($p < 0.05$); *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively; italic values represent indicative values of CRMs; n represents number of replicates.

	As	Se	Mo	Ag	Cd	Sn	Sb	Pb	Bi
A: CRMs									
CRM values									
BCR 60	*8*	*0,7*	*2*	*0,2*	2,20 ± 0,10	*6*	*0,4*	64 ± 4	
BCR 62	*0,2*	*0,1*	*0,2*	*0,2*	0,10 ± 0,02	*1*	*3*	25,0 ± 1,5	
GBW 07603	1,25 ± 0,15	0,12 ± 0,02	0,28 ± 0,05	0,049 ± 0,007	*0,38*	*0,27*	0,10 ± 0,01	47 ± 3	0,023 ± 0,005
V463			*0,83 ± 0,08*		*1,66 ± 0,32*				
Our values									
BCR 60 (n = 1-11)	6 ± 0,2	1,6 ± 0,5	0,9 ± 0,04	0,3 ± 0,03	2,10 ± 0,06		0,3	62 ± 1	0,440 ± 0,046
BCR 62 (n = 1-11)	0,2 ± 0,02	0,3 ± 0,2	0,2 ± 0,04	0,03 ± 0,01	0,09 ± 0,01		0,1	25,0 ± 1,5	0,046 ± 0,013
GBW 07603 (n = 3-14)	1,33 ± 0,10	0,32 ± 0,23	0,35 ± 0,04	0,057 ± 0,004	0,80 ± 0,08	0,84 ± 0,75	0,09 ± 0,02	50 ± 1	0,031 ± 0,007
V463 (n = 1-13)	0,11 ± 0,02	0,04 ± 0,05	0,48 ± 0,06	0,029 ± 0,003	1,50 ± 0,14		0,07	13,1 ± 9,2	0,123 ± 0,034

	As		Se		Mo		Ag		Cd		Sn		Sb		Pb		Bi
B: Plant tissues																	
3IL (n = 72-74)	a	1,73 ± 0,64	*a	0,25 ± 0,05	a	2,25 ± 1,15	a	0,93 ± 0,20	a	2,60 ± 0,53		nd	a	0,196 ± 0,029	a	1,96 ± 0,93	a	0,0105 ± 0,0061
OIL (n = 73)	ab	1,62 ± 0,58	ab	0,26 ± 0,04	ab	2,04 ± 0,74	abc	1,03 ± 0,21	ab	2,46 ± 0,43	**a	0,022 ± 0,021	a	0,178 ± 0,025	a	1,73 ± 0,87	ab	0,0088 ± 0,0054
BAL (n = 71-73)	b	2,12 ± 0,80	a	~~0,26~~ ± 0,05	a	2,54 ± 1,27	b	0,68 ± 0,17	a	2,53 ± 0,60	*a	0,041 ± 0,025	b	0,243 ± 0,025	b	3,02 ± 1,44	a	0,0152 ± 0,0100
SAL (n = 54-55)	a	1,26 ± 0,43	b	~~0,12~~ ± 0,05	b	0,82 ± 0,23	c	1,21 ± 0,27	b	1,18 ± 0,24		nd	*c	0,014 ± 0,008	c	0,61 ± 0,22	**b	0,0035 ± 0,0021
Shoot (n = 217)		1,81 ± 0,64	**	0,24 ± 0,04		2,20 ± 0,95		0,90 ± 0,16		2,40 ± 0,45	**	0,024 ± 0,020		0,191 ± 0,020		2,14 ± 0,96		0,0109 ± 0,0064

	As		Se		Mo	Ag	Cd		Sn (BAL)	Sb	Pb		Bi
C. Sites													
PACA													
Ensuès (1)	3,51 ± 0,41	*	0,30 ± 0,02		1,64 ± 0,22	0,91 ± 0,06	2,13 ± 0,08	*	0,041 ± 0,012	0,192 ± 0,009	2,58 ± 0,16		0,0169 ± 0,0011
La Vesse (2)	2,15 ± 0,22	*	0,27 ± 0,02		1,19 ± 0,08	1,63 ± 0,11	2,40 ± 0,11		0,105 ± 0,017	0,159 ± 0,007	4,09 ± 0,44		0,0269 ± 0,0034
Corbière (3)	2,54 ± 0,15	**	0,24 ± 0,02		1,62 ± 0,19	0,94 ± 0,09	1,78 ± 0,07		0,115 ± 0,028	0,222 ± 0,020	4,22 ± 0,49		0,0227 ± 0,0021
P. Chèvres (4)	9,44 ± 0,92	*	0,29 ± 0,03		2,27 ± 0,91	0,97 ± 0,04	2,18 ± 0,10		0,055 ± 0,013	0,184 ± 0,008	3,26 ± 0,33		0,0493 ± 0,0037
Riou (5)	1,73 ± 0,10	**	0,22 ± 0,02		1,21 ± 0,11	1,02 ± 0,07	2,95 ± 0,12	*	0,017 ± 0,007	0,169 ± 0,011	1,33 ± 0,13		0,0135 ± 0,0015
Bénat (6)	1,28 ± 0,11	**	0,22 ± 0,02		1,68 ± 0,23	1,03 ± 0,12	2,86 ± 0,16	*	0,024 ± 0,003	0,189 ± 0,025	1,30 ± 0,11		0,0051 ± 0,0005
Giens (7)	0,98 ± 0,05	**	0,22 ± 0,01		1,28 ± 0,11	0,97 ± 0,05	2,99 ± 0,13	*	0,026 ± 0,004	0,182 ± 0,014	1,62 ± 0,13		0,0051 ± 0,0005
Cap Roux (8)	1,71 ± 0,08	**	0,19 ± 0,02		1,75 ± 0,13	0,80 ± 0,07	2,87 ± 0,22	*	0,038 ± 0,012	0,218 ± 0,013	1,34 ± 0,17		0,0068 ± 0,0003
St Raphaël (9)	1,17 ± 0,09	*	0,29 ± 0,04		1,64 ± 0,24	1,05 ± 0,09	4,21 ± 0,41	*	0,020 ± 0,003	0,185 ± 0,032	1,11 ± 0,13		0,0053 ± 0,0005
Antibes (10)	2,80 ± 0,32	*	0,27 ± 0,02		3,29 ± 0,96	0,83 ± 0,07	2,12 ± 0,08	*	0,027 ± 0,004	0,190 ± 0,017	1,22 ± 0,09		0,0087 ± 0,0012
Villefranche (11)	1,79 ± 0,11	**	0,23 ± 0,03		3,89 ± 1,35	0,71 ± 0,05	1,08 ± 0,06		0,098 ± 0,008	0,243 ± 0,023	3,82 ± 0,67		0,0240 ± 0,0024
Corsica													
Calvi (I)	2,87 ± 0,37	*	0,31 ± 0,02		5,23 ± 1,08	0,55 ± 0,04	2,45 ± 0,10	*	0,019 ± 0,002	0,175 ± 0,006	1,52 ± 0,23		0,0056 ± 0,0003
Aregno (II)	0,97 ± 0,02	*	0,29 ± 0,03		27,03 ± 13,88	1,01 ± 0,08	3,74 ± 0,11	*	0,023 ± 0,027	0,182 ± 0,007	1,10 ± 0,07	*	0,0036 ± 0,0004
Saint Florent (III)	0,89 ± 0,06	**	0,20 ± 0,03		1,44 ± 0,13	0,56 ± 0,04	1,65 ± 0,07	*	0,026 ± 0,010	0,179 ± 0,047	1,98 ± 0,40		0,0063 ± 0,0009
Taglio Isolaccio (IV)	1,20 ± 0,17	**	0,20 ± 0,05		3,17 ± 1,09	0,75 ± 0,05	2,17 ± 0,10	*	0,025 ± 0,010	0,173 ± 0,012	1,17 ± 0,10		0,0066 ± 0,0008
Bravone (V)	1,19 ± 0,18	**	0,18 ± 0,02		1,58 ± 0,19	1,14 ± 0,10	2,49 ± 0,12		0,059 ± 0,037	0,699 ± 0,130	2,55 ± 0,44		0,0055 ± 0,0003
Ajaccio Sud (VI)	2,21 ± 0,16	*	0,28 ± 0,03		3,37 ± 0,30	1,13 ± 0,12	2,45 ± 0,14		0,072 ± 0,054	0,201 ± 0,021	2,32 ± 0,49		0,0105 ± 0,0013
Ajaccio Nord (VII)	1,70 ± 0,09	**	0,20 ± 0,02		2,38 ± 0,21	0,52 ± 0,02	1,56 ± 0,08		0,080 ± 0,029	0,188 ± 0,015	4,85 ± 0,92		0,0134 ± 0,0012

Table 1 (*Continued*). (A) Evaluation of analytical accuracy through certified reference materials (CRMs). (B) General trends of *Posidonia oceanica* trace element levels (tissue compartmentalization [n = 54–74] and shoot concentrations [n = 217]). (C) Spatial variation of trace element levels in *Posidonia oceanica* shoots [n = 15 for each site]. Levels are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$; letters represent significant differences between tissues ($p < 0.05$); *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively; italic values represent indicative values of CRMs; n represents number of replicates.

3. Results and discussion

Based on general trends of average levels found in shoots (Table 1B), TE levels decreased in the following order: Zn, Fe, Al > Mn > Ni > Cu > V > Co, Cd, Pb, Mo, As > Ag > Cr, Se, Sb > Sn > Bi, Be. This sequence completes and confirms the sequence commonly observed in *P. oceanica*: Zn > Ni > Cu > Cd, Pb > Cr (Pergent-Martini, 1994; Warnau et al., 1995; Schlacher-Hoenlinger and Schlacher, 1998; Campanella et al., 2001; Conti et al., 2010).

3.1. *Posidonia oceanica* compartmentalization

General trends of compartmentalization are reported in Table 1B (detailed data by site in Annex B). Compartmentalization of V, Zn, Cr and Cu are shown for the four contrasted sites being compared, with respect to their TE levels (Fig. 2). Compartmentalization profiles of the other TEs are similar to one of these four characteristic profiles (Annex C).

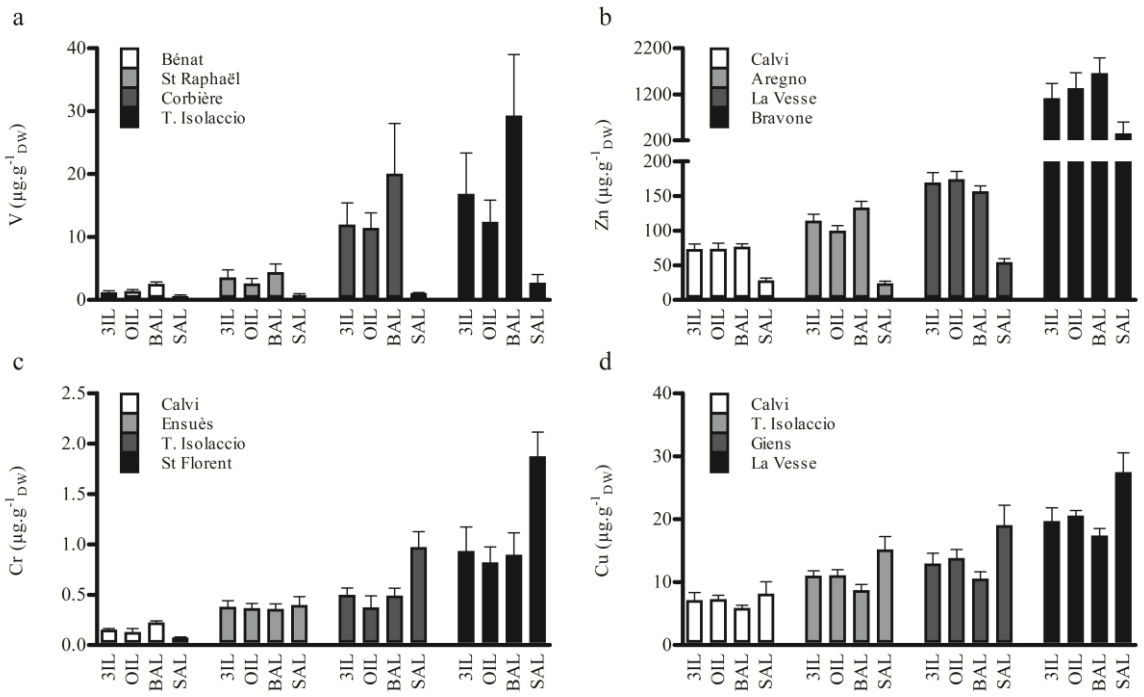

Fig. 2. *Posidonia oceanica* compartmentalization of V, Zn, Cr and Cu (a–d) at 4 of the 18 sites. Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. X-labels indicate the different compartments: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL). Number of replicates varies from 3 to 5, depending on site and compartment.

Most TEs were preferentially concentrated in photosynthetic tissues (i.e. 3IL, OIL and BAL) rather than in non-photosynthetic tissue (SAL): V, Mn, Co, Ni, Zn, As, Se, Mo, Cd, Sb, Pb and Bi (levels twice as high, except for As and Se). Furthermore, Mn, Co, Ni, As, Mo, Sb, Pb, Bi, and particularly V, were present in higher levels in BAL (e.g. V in Fig. 2a), contrary to Zn, Se and Cd, present in similar levels in all photosynthetic tissues (e.g. Zn in Fig. 2b), regardless of site. In the particular case of Be, the general trend of compartmentalization was not confirmed when observing sites independently from each other. For Sn, a signal could be clearly measured only in BAL. On the other hand, Al, Fe, Ag and Cu were preferentially accumulated in non-photosynthetic tissues. More precisely, Al, Fe and Ag, as well as Cr levels were systematically higher in SAL only in sites where the highest levels were recorded (e.g. Cr in Fig. 2c). Concerning Cu, levels in SAL were systematically higher than in other tissues, but differences were quite small (Fig. 2d).

These findings are in agreement with previous studies: Pergent- Martini (1994) for Cr, Fe, Cu, Zn, Cd and Pb; Campanella et al. (2001) for Cr, Zn, Cd and Pb, but not for Cu; Kljakovic-Gaspic et al. (2004) for Cd and Pb; Lafabrie et al. (2008) for Cr, Co, Ni, Cd and Pb; Conti et al. (2010) for Cr, Cu, Zn, Cd and Pb.

Assimilation of TEs among rooted aquatic plants occurs through two pathways: uptake by leaves from the water column or by roots from interstitial water. A preferential assimilation from the water column to photosynthetic tissues can be assumed for TEs which show higher levels in these tissues. This is particularly true for V, Mn, Co, Ni, Zn, Mo, Cd, Sb, Pb and Bi, which was also suggested by Lafabrie et al. (2008) for Co, Ni, Cd, Hg and Pb.

At least two hypotheses can be used to explain the differences between photosynthetic tissues (upper values in BAL): (i) longer exposure to TEs loaded in the ambient habitat, as suggested by Warnau et al. (1996) for adult leaves compared to intermediate leaves, and by Campanella et al. (2001) for the tip of the leaf compared to its younger basal part; (ii) dilution effect due to the higher growth rate of intermediate leaves. These hypotheses can only play a major role for TEs characterized by low kinetics of accumulation and little regulation such as Mn, Co, Ni, As, Mo, Sb, Pb, Bi and V.

On the other hand, the different behaviour of TEs preferentially accumulated in SAL suggests different uptake and distribution routes. For the particular case of Cu, the systematic but quite limited upper level in SAL (as opposed to BAL) could be explained by an increase

in metabolic activity during growth, when Cu (an essential micronutrient) is needed, as mentioned by Conti et al. (2010).

Physiological metrics vary with the tissue age and are influenced by the presence of epiphytes. However, this work supports the hypothesis of Romero et al. (2007b) concerning 3IL, except for sites widely contaminated by TEs which are characterized by low kinetics of accumulation from the water column, such as V. For most of the TEs analysed, future analyses of TE levels could be done using this single 3IL sample. This will simplify the laboratory pretreatment process and also permit a non-destructive sampling strategy: only *P. oceanica* leaves will have to be cut *in situ* close to their basal part, which avoids uprooting the shoots and permits the subsequent regrowing of leaves (Gobert et al., 2012).

3.2. Spatial (temporal) variation of trace element levels

Some TEs exhibit spatial variation of their levels in *P. oceanica* (higher than a factor 10 for Mo, Zn, V, Bi, Sn and As), while others show moderate (by a factor of between 4 and 10 for Al, Cr, Fe, Pb and Sb) and several exhibit small (lower than a factor 4 for Cd, Cu, Ag, Be, Co, Ni, Mn and Se) spatial variation (Table 1C and Fig. 3; Annex D).

3.2.1. Be – Se

In all sites and tissues, Be and Se signals could be detected and measured, but the differences between sites were not really quantifiable as levels were close to L_D (0.007 and 0.27µg.g$^{-1}_{DW}$, respectively). Barwick and Maher (2003) determined upper Se levels in one seagrass (*Zostera capricornii*; 0.38 ± 0.08 µg.g$^{-1}_{DW}$) and in one green macroalga (*Enteromorpha* sp.; 0.34 ± 0.09 µg.g$^{-1}_{DW}$) in Lake Macquarie (New South Wales, Australia), an estuary considered to be contaminated, whereas Baldwin et al. (1996) determined lower Se levels in *Posidonia australis* (0.064 ± 0.009 µg.g$^{-1}_{DW}$) in Jervis Bay (Australia), an estuary considered to be unpolluted. Levels recorded in the present study, which do not differ much between different sites, appear to reflect the background level of the northwestern Mediterranean Sea.

3.2.2. Sn (Fig. 3a) – Bi (Fig. 3b)

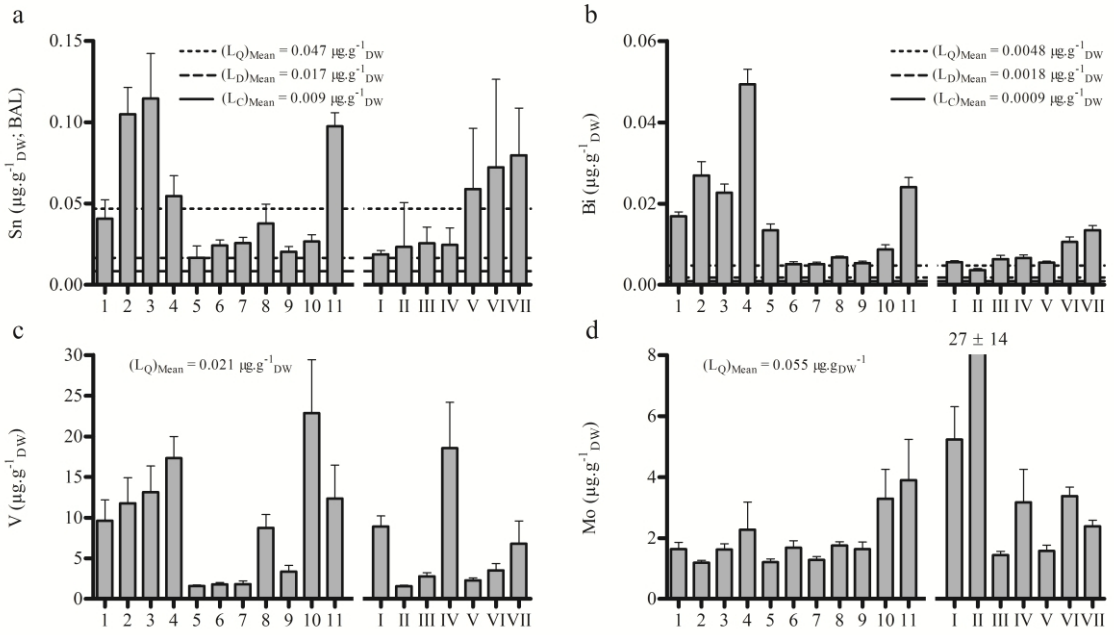

Fig. 3. Spatial variation of a) Sn (in BAL), b) Bi, c) V and d) Mo concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.

Sn and Bi had levels close to L_Q (0.07 and 0.005 $\mu g.g^{-1}_{DW}$, respectively); contrary to levels of Be and Se, a significant spatial variation was perceptible and quantifiable. Moreover, elevated levels were recorded for both elements at the same sites: Marseilles Bay (Ensuès, La Vesse, Corbière and Plateau des Chèvres), Villefranche and Ajaccio. Since they are principally used in specialized industry (Bi is also used in medicine and Sn was largely used in antifouling paints as tributyltin or TBT), it is not surprising to record elevated values in industrialized areas and harbours. Little data are available concerning Bi levels in aquatic macrophytes. Bertine et al. (1996) determined a similar range of levels in some brown and red algae (from 0.0046 to 0.0480 $\mu g_{Bi}.g^{-1}_{DW}$). Sn and its compounds (mainly TBT) are largely studied in molluscs, since TBT is an endocrine disruptor in gastropods (e.g. *Nucella lapillus*) and is particularly persistent in sediment (Santos et al., 2002). However, macrophytes, including the seagrass *Ruppia maritima*, are also affected by TBT stored in sediment (Jensen et al., 2004) in realistic environmental levels (10 $\mu g_{TBT\text{-}Sn}.kg^{-1}_{DW}$).

3.2.3. V (Fig. 3c)

Since V is a tracer of oil spill (hydrocarbon) pollutants (Amiard et al., 2003; Alfonso et al., 2008), the presence of four oil refineries in the 13th French district (Bouches-du-Rhône), an old oil-exporting harbour at Antibes and the petroleum depot at Lucciana (Taglio Isolaccio) can explain the high V levels in Marseilles Bay, Antibes and Taglio Isolaccio. V levels varied from 1.6 ± 0.1 µg.g$^{-1}_{DW}$ at Riou and Aregno, to 22.9 ± 6.5 µg.g$^{-1}_{DW}$ at Antibes. Alfonso et al. (2008) found higher V levels in below-ground tissues (4.49–15.14 µg.g$^{-1}_{DW}$) than in above-ground tissues (1.09–2.20 µg.g$^{-1}_{DW}$) of *Thalassia testudinum*. They attributed the highest levels in root-rhizomes to petroleum refining and transportation activities. Furthermore, the highest levels determined by Amiard et al. (2003) in three mollusc species: *Mytilus edulis* (0.98 µg.g$^{-1}_{DW}$), *Nucella lapillus* (0.57 µg.g$^{-1}_{DW}$) and *Littorina littorea* (1.42 µg.g$^{-1}_{DW}$) along the south coast of Brittany (France), after the sinking of the tanker Erika, are in the lower range of the levels determined in this study. These observations confirm the use of *P. oceanica* as a good biological indicator of hydrocarbon pollution.

3.2.4. Mn – Mo (Fig. 3d)

The spatial variation of Mn levels was small. Furthermore, our levels (42–93 µg.g$^{-1}_{DW}$), and particularly our SAL levels (8–28 µg.g$^{-1}_{DW}$), were in the lower range of the values determined by Ancora et al. (2004) in *P. oceanica* scales in the Gulf of Naples (5–200 µg.g$^{-1}_{DW}$). Mo is an essential micronutrient for citrus culture (Srivastava and Shyam, 2007). Aregno, known for its culture of oranges and almonds, is located in the small catchment basin of Nonza, an agricultural valley that opens onto the sea. The particularly high Mo levels measured at Aregno are probably due to this specific agricultural activity. However, the levels at the 17 other sites are lower than those determined by Augier et al. (1991) in *P. oceanica* leaves (5.2 ± 0.6 µg.g$^{-1}_{DW}$) at the uncontaminated site of La Palu (Port Cros Island, France). So, measured levels of these two TEs (Mn: 60 ± 10 µg.g$^{-1}_{DW}$; Mo: 2.20 ± 0.95 µg.g$^{-1}_{DW}$) should reflect the background levels of the northwestern Mediterranean Sea.

3.2.5. Ag (Fig. 4a)

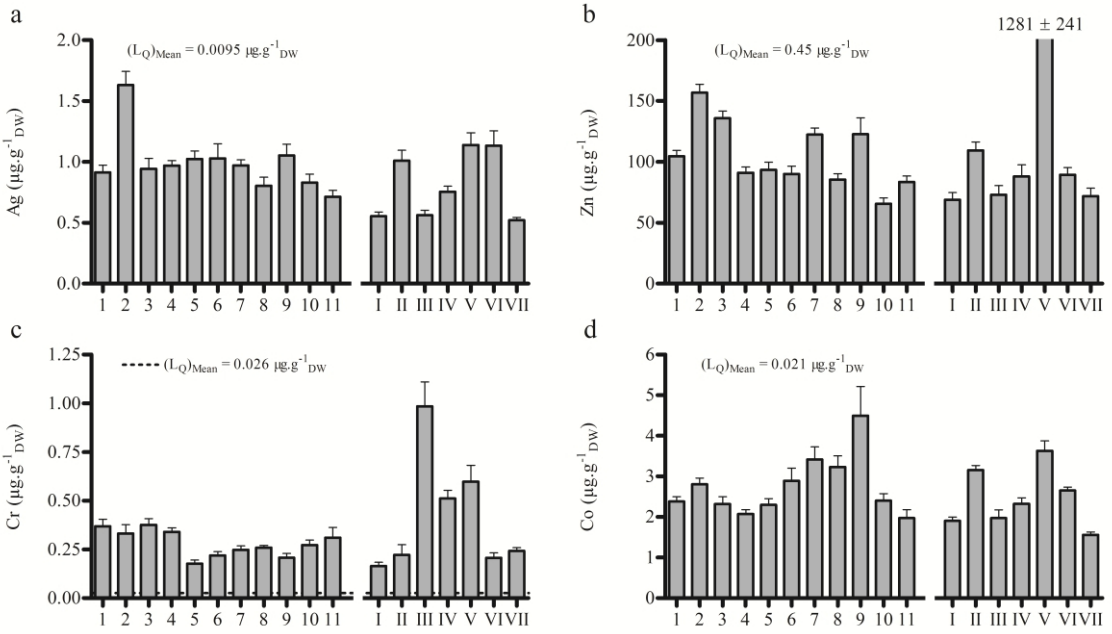

Fig. 4. Spatial variation of a) Ag, b) Zn, c) Cr and d) Co concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.

Chiffoleau et al. (2005) used wide mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) and determined contamination by Ag along the French Mediterranean coast as homogeneous and limited compared to the Atlantic coast. However, the lowest values recorded from this study (~0.5 $\mu g.g^{-1}_{DW}$) were found in Corsica, and corroborate the low values measured by Lopez y Royo et al. (2009) in the northwestern Mediterranean Sea. Hence, these low levels should reflect the Ag background level of this area. Higher values in Corsica (~1 $\mu g.g^{-1}_{DW}$) correspond to the lower ones of the PACA region. This could stem from diffuse urban or industrialized contamination, with the exception of La Vesse (1.63 ± 0.11 $\mu g.g^{-1}_{DW}$) which seems to be more locally contaminated, as observed by Lopez y Royo et al. (2009) at Ile Rousse (1.55 ± 0.20 $\mu g.g^{-1}_{DW}$).

3.2.6. Sb – As – Zn (Fig. 4b)

With the exception of Bravone (Sb and Zn) and Plateau des Chèvres (As), these TEs have relatively small spatial variation. Levels of Zn and As found in this study correspond to unpolluted sites when compared to the literature (Tranchina et al., 2004; Fourqurean et al., 2007). The high Sb levels detected only at Bravone could be attributed to the As/Sb deposit at Matra. This mine, which closed in 1946, is crossed by the Presa River, a tributary of the Bravone River which flows into the open sea close to the city of the same name (Bravone). However, if the high Sb levels are associated with high Zn levels (which could also be attributed to the Matra deposit), it is not the case with As, as shown in mussels by Andral et al. (2004). On the other hand, the high As levels detected only at Plateau des Chèvres corroborate the observation made by Andral et al. (2004), and could be attributed to the industrial history of the south of Marseilles, chronically contaminated by As and Pb, as suggested by Lassalle (2007).

3.2.7. Cr (Fig. 4c) – Co (Fig. 4d) – Ni – Cd

The high Cr levels determined at Saint Florent (0.98 ± 0.12 $\mu g.g^{-1}_{DW}$) corroborate similar high values determined west of Cap Corse (1.07 ± 0.07 $\mu g.g^{-1}_{DW}$) by Lafabrie et al. (2008) using *P. oceanica* leaves and by Andral et al. (2004) using the RINBIO biointegrator network based on mussel caging. These values have been linked to waste from the disused Canari asbestos mine, closed in 1965, located at the northwestern area of the Gulf of Saint Florent. However, contrary to Lafabrie et al. (2008) and Andral et al. (2004), the high Cr levels at Saint Florent were not associated with high levels of Co or Ni, which were presumed to be linked to waste from the disused Canari asbestos mine. Furthermore, the spatial distribution of Co and Ni appeared to be more similar to that of Cd than to that of Cr. However, Co, Ni and Cd had low spatial variation and the ranges of levels for these three TEs were quite narrow, which reflect similar contamination levels across sites. Moreover, a decrease in Cd levels is observed at Calvi, St Florent and Ajaccio, when compared to Lafabrie et al. (2008).

3.2.8. Al (Fig. 5a) – Fe

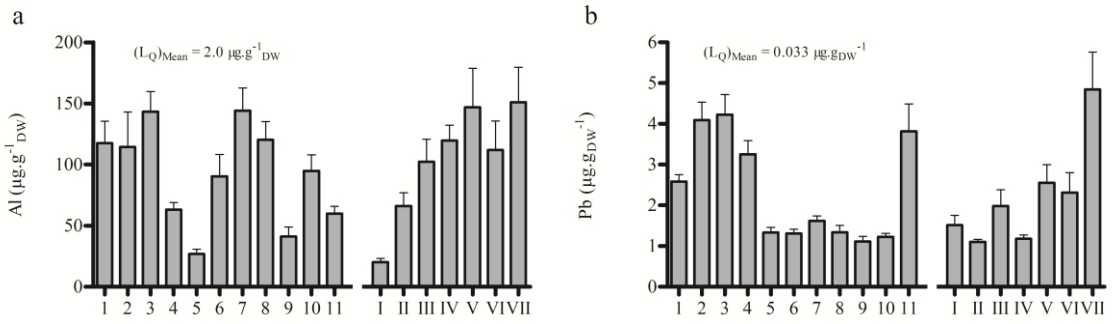

Fig. 5. Spatial variation of a) Al and b) Pb concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.

Al and Fe have similar profiles; this is consistent with the observations of Barabasz et al. (2002): one effect of Al on plants is the stimulation of Fe absorption by the root system. Our range of levels for Fe (41–179 $\mu g.g^{-1}_{DW}$) corroborates the low range of values determined by Fourqurean et al. (2007) in the Balearic Islands (Spain), and by Warnau et al. (1995) and Pergent-Martini (1994) at Calvi and Marseilles. However, no extreme level was determined at the 18 selected sites, as measured by Warnau et al. (1995) at Lacco Ameno (419 ± 431 $\mu g.g^{-1}_{DW}$; Ischia Island, Italy). Hence, these observations allow us to hypothesize that the spatial distribution results from a natural heterogeneity rather than anthropogenic activities. Fe deficiencies (<100 $\mu g_{Fe}.g^{-1}_{DW}$) have been found in seagrasses growing above carbonate sediment (Duarte et al., 1995), but the low Fe levels determined at Calvi (48 ± 4 $\mu g.g^{-1}_{DW}$) do not negatively affect *P. oceanica* growth. Indeed, *P. oceanica* shoot biomass and leaf chlorophyll levels were high at Calvi (Gobert, pers. comm.), and the state of the *P. oceanica* meadow was determined as "good" according to PREI (Gobert et al., 2009). Furthermore, the low levels of other micronutrients (Mn, Co, Ni, Cu and Zn) determined at Calvi seem also not to be deficient for *P. oceanica*.

3.2.9. Cu

Cu has several uses, including use as conductor, a constituent of alloys, as a copolymer, in antifouling paint, in ship rustproof enamel, and as a fungicide, an algaecide, fertilizer, etc. Hence, there may be multiple sources of contamination which are yet to be specified in the more contaminated sites of Bravone, Aregno, Villefranche, La Vesse and Corbière. However, levels determined in this study (7.5–22.9 $\mu g.g^{-1}_{DW}$) were clearly lower than those determined by Conti et al. (2007) at Ustica Island (31.9 ± 15.8 $\mu g.g^{-1}_{DW}$; Sicily, Italy) where TE contamination was estimated at a medium-low level according to the literature. All sites sampled can thus be considered as having a low level of Cu contamination.

3.2.10. Pb (Fig. 5b)

Pb, not highly soluble in sea water, accumulates in sediments. Nevertheless, Mayes et al. (1977) suggested for another rooted aquatic plant (*Elodea canadensis*) that movement of Pb from contaminated sediment to leaves probably occurs through the water (released from the sediment by turbulence), and not through internal transport (acropetal translocation). Since its prohibited use as an anti-knock additive in gasoline (lead alkyls) and as the principal component of water pipes, contamination levels of Pb have been in decline, as determined by Ancora et al. (2004) between 1989 and 1999 in the Gulf of Naples. Moreover, Tranchina et al. (2005) determined that Pb levels measured in *P. oceanica* scales were statistically correlated to Pb emissions in the air and reflected the level of Pb pollution in the coastal marine environment. However, its significant environmental persistence in the sediment explains the relatively higher values determined in the highest industrialized sites (Marseilles Bay, Villefranche and Ajaccio).

4. Conclusion

The general trends of compartmentalization in *P. oceanica* allow us to propose hypotheses concerning the uptake and distribution routes of these nineteen TEs. However, such hypotheses have yet to be confirmed either by means of analysis of different parts of the same leaf (whole leaf, leaf tip and leaf basal tissue), or by means of analysis of both above- (leaves) and below-ground (rhizomes, scales and roots) tissues. Alternatively, experimental contamination in controlled mesocosms would allow us to clarify the uptake and distribution

routes of TEs and to quantify the kinetics of accumulation and excretion. Furthermore, this study supports the hypothesis that future analyses of most TEs in *P. oceanica* in the spring could be undertaken only on the single third intermediate leaf, except for sites widely contaminated by TEs, such as V, characterized by very low kinetics of accumulation from the water column. On the scale of the Mediterranean French coast, the natural levels of most of the twelve little-studied TEs measured in *P. oceanica* could be determined, and their spatial variation as well as their contamination sources could be explained. *P. oceanica* effectively concentrates Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi from its surrounding environment, and might be used de facto as a sentinel species for their coastal monitoring. Special attention should be given to these twelve potentially toxic TEs due to their local (Mo), diffuse (Ag) and/or chronic (As) observed contamination. On the other hand, the levels of the seven widely studied TEs (Cr, Fe, Ni, Cu, Zn, Cd and Pb) seem to decrease or stabilize over time, probably due to their reduced anthropogenic inflows. These observations taken together suggest that *P. oceanica* is a sensitive bioindicator for the monitoring of chemical contamination for the nineteen TEs studied.

Acknowledgements

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Ferrara et al. (1989)						1	1		1													
Augier et al. (1980)	1	1	1	1	1			1														
Vincente et Chabert (1981)	1		1	1																		
Chabert et al. (1983)	1		1	1	1			1														
Catsiki et Florou (1984)	1			1		1		1	1													
Catsiki et al. (1987); Panayotidis (1988)	2	2				2		2	2													
Giaccone et al. (1988)		1		1		1																
Malea et Haritodinis (1989)	1		1	1	1																	
Panayotidis et al. (1990)	1			1		1																
Sanchiz et al. (1990)	1	1	1		1																	
Augier et al. (1991)					1	1	1		1	1	1					1		1				
Costantini et al. (1991)	1	1	1																			
Grauby et al. (1991)					1	1	1		1	1	1	1	1			1		1				
Taramelli et al. (1991); Carlotti et al. (1992)	2																					
Catsiki et Bei (1992)	1			1		1	1	1											1			
Catsiki et Panayotidis (1993)				1		1		1														
Ledent et al. (1993)	1		1	1	1	1	1													1		
Malea (1993)																					1	
Pergent-Martini et al. (1993)	1		1	1	1		1															
Schlacher-Hoenlinger and Schlacher (1998a, 1998b)	2		2	2	2																	
Augier et al. (1994)	1			1	1			1							1							
Catsiki et al. (1994)						1																
Malea et al. (1994)	1		1	1	1		1			1	1		1		1							
Duarte et al. (1995)							1															
Ledent et al. (1995)	1		1	1	1	1	1			1	1		1		1							
Romeo et al. (1995)	1		1	1	1		1												1			
Warnau et al. (1995)	1		1	1	1	1	1													1		
Warnau et al. (1996)	1				1				1								1					1
Sanchiz et al. (2000; 2001)	2	2	2		2																	
Campanella et al. (2001)	1		1	1	1	1																
Baroli et al. (2001)	1		1			1	1															
Ancora et al. (2004)	1	1	1	1	1		1															
Kljakovic-Gaspic et al. (2004)	1		1																			
Tranchina et al. (2004; 2005)	2		2	2	2																	
Maserti et al. (2005)	1	1																				
Gosselin et al. (2006)	1		1	1	1	1		1				1				1						
Fourqurean et al. (2007)							1					1										
Conti et al. (2007)	1		1	1	1	1																
Lafabrie et al. (2007b)		1	1	1		1		1						1								
Lafabrie et al. (2008a, 2008b)	2	2	2			2		2						2								
Lopez y Royo et al. (2009)	1	1	1	1				1									1					
Conti et al. (2010)	1		1	1	1	1																
Number of reference	38	31	29	27	25	22	14	13	7	4	4	3	3	2	3	3	2	2	2	2	1	1
	Cd	**Hg**	**Pb**	**Cu**	**Zn**	**Cr**	**Fe**	**Ni**	**Cs**	**K**	**Na**	**As**	**Ca**	**Co**	**Mg**	**Se**	**Ag**	**Br**	**Mn**	**Ti**	**Al**	**Am**

Annex A. Non exhaustive number of references concerning trace elements studied in *Posidonia oceanica* tissues; completed (black references) from Pergent-Martini et al. (2000) summary table (grey references).

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3IL		Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
PACA											
Ensuès (1)	**	0,0083 ± 0,0031	129 ± 24	9,1 ± 4,6	0,38 ± 0,06	63,5 ± 6,0	142 ± 16	2,38 ± 0,19	32,5 ± 2,7	13,3 ± 0,6	108,3 ± 8,2
La Vesse (2)	**	0,0077 ± 0,0034	114 ± 33	8,5 ± 2,7	0,30 ± 0,05	60,9 ± 4,2	121 ± 18	2,70 ± 0,18	31,4 ± 1,2	19,8 ± 2,1	169,7 ± 13,8
Corbière (3)	**	0,0078 ± 0,0027	176 ± 21	12,0 ± 3,5	0,44 ± 0,04	59,9 ± 7,3	157 ± 13	2,30 ± 0,25	30,2 ± 1,8	17,0 ± 2,0	134,8 ± 12,9
P. Chèvres (4)	**	0,0053 ± 0,0023	55 ± 8	10,1 ± 0,7	0,29 ± 0,06	44,9 ± 3,6	74 ± 6	2,13 ± 0,28	31,8 ± 2,7	11,9 ± 1,1	93,0 ± 8,4
Riou (5)		~~0,0013~~ ± 0,0017	31 ± 8	1,3 ± 0,2	0,20 ± 0,02	44,4 ± 3,6	63 ± 5	2,55 ± 0,22	38,1 ± 4,0	11,0 ± 1,0	105,2 ± 9,1
Bénat (6)	**	0,0070 ± 0,0014	74 ± 26	1,2 ± 0,2	0,21 ± 0,01	60,7 ± 4,7	87 ± 17	2,88 ± 0,30	34,9 ± 1,6	13,0 ± 1,9	101,7 ± 8,0
Giens (7)	**	0,0070 ± 0,0015	127 ± 24	1,4 ± 0,4	0,23 ± 0,03	66,7 ± 4,5	126 ± 13	3,43 ± 0,22	33,5 ± 1,7	13,0 ± 1,6	135,3 ± 4,7
Cap Roux (8)	**	0,0082 ± 0,0035	85 ± 23	8,9 ± 2,5	0,26 ± 0,01	73,7 ± 5,3	73 ± 12	3,27 ± 0,19	34,6 ± 1,9	10,0 ± 0,7	93,6 ± 5,3
St Raphaël (9)	**	0,0063 ± 0,0022	36 ± 11	3,6 ± 1,2	0,18 ± 0,03	94,6 ± 5,8	53 ± 5	4,41 ± 0,56	47,6 ± 3,7	13,3 ± 1,9	138,6 ± 11,1
Antibes (10)	**	0,0077 ± 0,0035	90 ± 31	28,0 ± 11,1	0,27 ± 0,05	57,3 ± 10,9	93 ± 15	2,51 ± 0,20	28,6 ± 1,9	13,7 ± 1,5	71,4 ± 4,3
Villefranche (11)	**	0,0071 ± 0,0027	57 ± 11	13,6 ± 3,6	0,29 ± 0,02	72,2 ± 20,0	85 ± 6	2,06 ± 0,29	20,5 ± 1,5	23,6 ± 1,7	90,6 ± 13,7
Corsica											
Calvi (I)		~~0,0015~~ ± 0,0014	20 ± 4	9,1 ± 2,2	0,15 ± 0,01	47,9 ± 5,6	45 ± 6	1,79 ± 0,12	27,8 ± 1,7	7,2 ± 1,2	73,4 ± 7,5
Aregno (II)		~~0,0009~~ ± 0,0028	36 ± 12	0,9 ± 0,1	0,14 ± 0,02	52,0 ± 2,5	55 ± 6	2,79 ± 0,09	28,7 ± 0,8	14,6 ± 1,0	114,5 ± 9,4
St Florent (III)		~~0,0041~~ ± 0,0018	96 ± 23	3,1 ± 0,8	0,93 ± 0,24	45,0 ± 1,9	139 ± 23	2,02 ± 0,14	25,1 ± 1,0	8,7 ± 1,3	79,6 ± 8,2
T. Isolaccio (IV)	**	0,0087 ± 0,0032	122 ± 8	16,8 ± 6,5	0,50 ± 0,07	89,7 ± 13,7	134 ± 14	2,43 ± 0,25	28,6 ± 3,2	11,0 ± 0,8	107,3 ± 15,3
Bravone (V)	**	0,0093 ± 0,0027	165 ± 111	1,7 ± 0,2	0,52 ± 0,08	72,1 ± 9,5	150 ± 34	3,42 ± 0,24	27,9 ± 2,6	21,6 ± 2,3	1114,9 ± 320,1
Ajaccio S. (VI)		~~0,0042~~ ± 0,0038	55 ± 11	3,1 ± 1,4	0,20 ± 0,03	57,6 ± 3,1	66 ± 4	2,85 ± 0,17	39,8 ± 0,8	8,2 ± 0,2	102,2 ± 7,5
Ajaccio N. (VII)	**	0,0099 ± 0,0036	132 ± 44	5,6 ± 2,0	0,26 ± 0,02	58,2 ± 3,1	149 ± 31	1,75 ± 0,09	23,7 ± 1,0	7,8 ± 1,0	84,5 ± 9,5

OIL		Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
PACA											
Ensuès (1)	**	0,0092 ± 0,0035	128 ± 30	9,0 ± 3,6	0,37 ± 0,05	66,7 ± 2,9	145 ± 15	2,51 ± 0,16	25,3 ± 1,6	13,9 ± 0,8	119,5 ± 8,8
La Vesse (2)	**	0,0068 ± 0,0023	99 ± 16	12,5 ± 5,4	0,29 ± 0,03	65,2 ± 3,2	114 ± 7	2,76 ± 0,11	26,2 ± 1,0	20,6 ± 0,8	174,2 ± 11,1
Corbière (3)	*	0,0106 ± 0,0022	140 ± 40	11,5 ± 2,4	0,35 ± 0,06	61,4 ± 6,8	136 ± 24	2,40 ± 0,16	22,9 ± 1,3	18,5 ± 1,2	152,5 ± 5,9
P. Chèvres (4)		~~0,0050~~ ± 0,0035	62 ± 12	8,3 ± 1,3	0,27 ± 0,03	42,2 ± 2,2	80 ± 7	1,91 ± 0,11	23,2 ± 1,1	11,9 ± 0,7	90,8 ± 6,6
Riou (5)		~~0,0026~~ ± 0,0023	25 ± 8	1,2 ± 0,1	0,14 ± 0,03	39,3 ± 2,3	56 ± 4	2,11 ± 0,18	27,8 ± 2,8	10,9 ± 0,9	92,7 ± 7,2
Bénat (6)	**	0,0066 ± 0,0047	81 ± 31	1,4 ± 0,2	0,17 ± 0,02	56,2 ± 3,5	87 ± 16	2,34 ± 0,25	26,8 ± 2,9	12,5 ± 1,7	87,2 ± 4,1
Giens (7)	**	0,0066 ± 0,0024	130 ± 42	1,1 ± 0,4	0,18 ± 0,03	58,2 ± 4,7	122 ± 22	2,79 ± 0,30	27,5 ± 1,8	13,9 ± 1,3	110,7 ± 7,2
Cap Roux (8)		~~0,0049~~ ± 0,0035	88 ± 31	4,5 ± 0,9	0,15 ± 0,01	64,7 ± 5,7	64 ± 8	2,65 ± 0,15	24,4 ± 1,7	11,2 ± 1,3	85,5 ± 4,5
St Raphaël (9)		~~0,0051~~ ± 0,0019	38 ± 10	2,7 ± 0,8	0,16 ± 0,04	84,3 ± 6,2	57 ± 18	3,81 ± 0,26	38,7 ± 2,0	13,6 ± 1,1	120,6 ± 8,6
Antibes (10)		~~0,0033~~ ± 0,0019	74 ± 5	23,7 ± 8,2	0,19 ± 0,01	49,9 ± 4,4	78 ± 3	2,18 ± 0,09	21,9 ± 1,6	13,6 ± 1,0	67,4 ± 2,8
Villefranche (11)		~~0,0051~~ ± 0,0035	57 ± 14	11,2 ± 5,3	0,22 ± 0,02	55,5 ± 14,6	76 ± 7	1,59 ± 0,13	13,9 ± 1,9	22,4 ± 2,6	80,9 ± 1,6
Corsica											
Calvi (I)	**	0,0068 ± 0,0038	17 ± 6	7,2 ± 2,4	0,13 ± 0,03	44,2 ± 2,1	41 ± 2	1,59 ± 0,08	23,9 ± 1,6	7,3 ± 0,6	73,7 ± 8,2
Aregno (II)	*	0,0118 ± 0,0035	98 ± 43	0,8 ± 0,1	0,17 ± 0,06	43,8 ± 3,4	76 ± 18	2,27 ± 0,20	23,8 ± 1,6	17,4 ± 2,8	99,8 ± 7,7
St Florent (III)		~~0,0050~~ ± 0,0019	86 ± 14	2,3 ± 0,6	0,82 ± 0,15	38,0 ± 2,3	119 ± 13	1,57 ± 0,09	20,0 ± 0,8	9,1 ± 0,8	74,2 ± 11,9
T. Isolaccio (IV)	**	0,0074 ± 0,0034	102 ± 35	12,4 ± 3,4	0,37 ± 0,11	62,4 ± 1,4	115 ± 24	1,68 ± 0,07	22,5 ± 0,8	11,1 ± 0,8	82,4 ± 14,0
Bravone (V)	**	0,0065 ± 0,0022	110 ± 46	1,6 ± 0,3	0,54 ± 0,26	61,1 ± 4,7	202 ± 91	3,09 ± 0,21	24,5 ± 2,2	22,7 ± 1,5	1332,0 ± 333,3
Ajaccio S. (VI)	**	0,0073 ± 0,0034	67 ± 21	2,2 ± 0,5	0,14 ± 0,01	51,5 ± 3,4	62 ± 5	2,24 ± 0,14	26,8 ± 0,7	8,2 ± 0,5	92,2 ± 12,0
Ajaccio N. (VII)	**	0,0086 ± 0,0042	121 ± 41	9,0 ± 7,7	0,19 ± 0,04	51,2 ± 8,0	122 ± 10	1,35 ± 0,20	19,4 ± 1,4	7,1 ± 0,5	76,3 ± 9,7

Annex B. Spatial variation of trace element concentrations in *Posidonia oceanica* 3rd intermediate leaves (3IL; n = 3-5), other intermediate leaves (OIL; n = 5), blades of adult leaves (BAL; n = 4-5) and sheaths of adult leaves (SAL; n = 3-5). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.

3IL	As		Se	Mo	Ag	Cd		Sn	Sb	Pb	Bi
PACA											
Ensuès (1)	3,72 ± 1,09		0,301 ± 0,035	1,61 ± 0,40	0,98 ± 0,14	2,29 ± 0,15		~~0,005~~ ± 0,006	0,173 ± 0,012	2,40 ± 0,23	0,0158 ± 0,0019
La Vesse (2)	1,89 ± 0,19	*	0,272 ± 0,024	1,14 ± 0,13	1,66 ± 0,20	2,64 ± 0,15	*	0,024 ± 0,010	0,143 ± 0,022	3,24 ± 0,27	0,0221 ± 0,0022
Corbière (3)	2,50 ± 0,38	*	0,223 ± 0,036	1,54 ± 0,17	0,84 ± 0,05	1,92 ± 0,09	*	0,056 ± 0,022	0,211 ± 0,041	4,05 ± 0,33	0,0230 ± 0,0021
P. Chèvres (4)	7,51 ± 0,64	*	0,233 ± 0,039	1,63 ± 0,13	1,03 ± 0,09	2,44 ± 0,26		~~0,005~~ ± 0,009	0,190 ± 0,021	2,69 ± 1,10	0,0403 ± 0,0111
Riou (5)	1,83 ± 0,16	*	0,224 ± 0,038	1,30 ± 0,14	1,08 ± 0,16	3,28 ± 0,14		nd	0,181 ± 0,015	1,36 ± 0,28	0,0145 ± 0,0035
Bénat (6)	1,25 ± 0,12	*	0,242 ± 0,035	1,62 ± 0,20	1,01 ± 0,12	3,09 ± 0,19		nd	0,196 ± 0,045	1,10 ± 0,04	0,0052 ± 0,0013
Giens (7)	0,94 ± 0,12	*	0,261 ± 0,023	1,32 ± 0,10	0,98 ± 0,09	3,26 ± 0,16		nd	0,194 ± 0,016	1,38 ± 0,16	0,0049 ± 0,0007
Cap Roux (8)	1,73 ± 0,43	*	0,229 ± 0,065	1,81 ± 0,16	0,84 ± 0,10	3,04 ± 0,16		nd	0,239 ± 0,025	1,45 ± 0,65	0,0093 ± 0,0020
St Raphaël (9)	1,10 ± 0,13		0,332 ± 0,041	1,67 ± 0,50	1,16 ± 0,20	4,53 ± 0,43	**	0,015 ± 0,095	0,193 ± 0,051	1,00 ± 0,18	0,0042 ± 0,0008
Antibes (10)	2,58 ± 0,35		0,326 ± 0,044	4,56 ± 2,53	0,81 ± 0,08	2,26 ± 0,08		nd	0,204 ± 0,026	1,08 ± 0,20	0,0079 ± 0,0017
Villefranche (11)	1,71 ± 0,09	*	0,242 ± 0,056	6,22 ± 4,26	0,70 ± 0,09	1,19 ± 0,09	*	0,026 ± 0,013	0,252 ± 0,037	3,85 ± 0,78	0,0205 ± 0,0034
Corsica											
Calvi (I)	2,80 ± 0,31		0,324 ± 0,048	5,80 ± 1,88	0,61 ± 0,03	2,70 ± 0,10		nd	0,147 ± 0,010	1,36 ± 0,28	0,0054 ± 0,0005
Aregno (II)	0,89 ± 0,03		0,296 ± 0,088	5,24 ± 2,94	1,28 ± 0,16	3,94 ± 0,12		nd	0,142 ± 0,020	0,91 ± 0,07	0,0035 ± 0,0004
St Florent (III)	0,93 ± 0,07	*	0,146 ± 0,044	1,69 ± 0,25	0,53 ± 0,04	1,72 ± 0,05		nd	0,180 ± 0,020	1,95 ± 0,34	0,0066 ± 0,0008
T. Isolaccio (IV)	1,15 ± 0,20	*	0,227 ± 0,052	4,23 ± 1,26	0,75 ± 0,03	2,27 ± 0,15		nd	0,200 ± 0,019	1,13 ± 0,18	0,0064 ± 0,0008
Bravone (V)	0,98 ± 0,05	*	0,156 ± 0,032	1,62 ± 0,19	1,10 ± 0,11	2,70 ± 0,23	*	0,028 ± 0,016	0,658 ± 0,158	2,20 ± 0,59	0,0049 ± 0,0006
Ajaccio S. (VI)	2,18 ± 0,15	*	0,272 ± 0,045	2,80 ± 0,46	1,23 ± 0,17	2,66 ± 0,09	**	0,019 ± 0,019	0,200 ± 0,014	2,38 ± 0,85	0,0083 ± 0,0016
Ajaccio N. (VII)	1,65 ± 0,12	*	0,152 ± 0,030	2,49 ± 0,50	0,56 ± 0,04	1,68 ± 0,08	*	0,023 ± 0,017	0,212 ± 0,016	5,77 ± 2,02	0,0142 ± 0,0031

OIL	As		Se	Mo	Ag	Cd		Sn	Sb	Pb	Bi
PACA											
Ensuès (1)	3,28 ± 0,23	**	0,343 ± 0,032	1,86 ± 0,30	1,06 ± 0,06	2,36 ± 0,08	*	0,048 ± 0,008	0,205 ± 0,019	2,49 ± 0,23	0,0158 ± 0,0015
La Vesse (2)	1,93 ± 0,14	**	0,306 ± 0,029	1,14 ± 0,06	1,80 ± 0,21	2,68 ± 0,17	*	0,062 ± 0,006	0,161 ± 0,010	3,25 ± 0,23	0,0212 ± 0,0011
Corbière (3)	2,42 ± 0,19	**	0,272 ± 0,037	1,68 ± 0,30	0,98 ± 0,10	2,05 ± 0,13	*	0,085 ± 0,016	0,240 ± 0,014	3,64 ± 0,22	0,0196 ± 0,0015
P. Chèvres (4)	7,54 ± 0,67	**	0,285 ± 0,036	1,83 ± 0,65	1,12 ± 0,13	2,31 ± 0,14	**	0,024 ± 0,013	0,176 ± 0,011	2,44 ± 0,34	0,0431 ± 0,0040
Riou (5)	1,62 ± 0,11		~~0,222~~ ± 0,027	1,14 ± 0,05	1,11 ± 0,08	2,97 ± 0,13		~~0,009~~ ± 0,005	0,162 ± 0,017	1,08 ± 0,18	0,0103 ± 0,0007
Bénat (6)	1,15 ± 0,12		~~0,213~~ ± 0,039	1,55 ± 0,31	1,09 ± 0,19	2,75 ± 0,18		~~0,005~~ ± 0,003	0,178 ± 0,035	1,03 ± 0,13	0,0046 ± 0,0015
Giens (7)	0,83 ± 0,08	**	0,272 ± 0,027	1,18 ± 0,15	1,14 ± 0,07	2,81 ± 0,17		~~0,004~~ ± 0,003	0,148 ± 0,008	1,17 ± 0,14	0,0045 ± 0,0004
Cap Roux (8)	1,22 ± 0,08		~~0,159~~ ± 0,038	1,63 ± 0,07	1,05 ± 0,13	2,90 ± 0,24		~~0,010~~ ± 0,003	0,184 ± 0,016	0,86 ± 0,04	0,0037 ± 0,0003
St Raphaël (9)	1,07 ± 0,11	**	0,268 ± 0,036	1,63 ± 0,29	1,27 ± 0,08	4,17 ± 0,26		~~0,000~~ ± 0,004	0,170 ± 0,010	0,91 ± 0,08	0,0045 ± 0,0012
Antibes (10)	2,33 ± 0,13	**	0,269 ± 0,035	3,26 ± 0,64	0,82 ± 0,07	2,09 ± 0,08		~~0,006~~ ± 0,011	0,181 ± 0,013	0,89 ± 0,09	0,0077 ± 0,0014
Villefranche (11)	1,61 ± 0,15		~~0,231~~ ± 0,026	3,86 ± 2,76	0,69 ± 0,03	1,07 ± 0,06	*	0,038 ± 0,021	0,219 ± 0,010	3,03 ± 0,60	0,0177 ± 0,0029
Corsica											
Calvi (I)	2,58 ± 0,26	**	0,356 ± 0,019	5,44 ± 1,42	0,67 ± 0,05	2,63 ± 0,08		nd	0,170 ± 0,018	1,44 ± 0,36	0,0044 ± 0,0004
Aregno (II)	0,92 ± 0,05	**	0,282 ± 0,054	5,70 ± 4,75	1,50 ± 0,17	3,50 ± 0,05	**	0,035 ± 0,048	0,126 ± 0,020	0,88 ± 0,20	0,0027 ± 0,0003
St Florent (III)	0,85 ± 0,05		~~0,230~~ ± 0,031	1,56 ± 0,35	0,66 ± 0,05	1,62 ± 0,09		~~0,005~~ ± 0,009	0,143 ± 0,008	1,46 ± 0,25	0,0050 ± 0,0008
T. Isolaccio (IV)	0,99 ± 0,11		~~0,199~~ ± 0,039	2,59 ± 0,69	0,94 ± 0,05	2,05 ± 0,15		~~0,004~~ ± 0,002	0,153 ± 0,009	0,75 ± 0,05	0,0062 ± 0,0017
Bravone (V)	1,21 ± 0,32		~~0,184~~ ± 0,045	1,49 ± 0,13	1,24 ± 0,15	2,72 ± 0,08	*	0,062 ± 0,017	0,705 ± 0,156	2,43 ± 0,61	0,0045 ± 0,0012
Ajaccio S. (VI)	1,95 ± 0,29	**	0,311 ± 0,084	3,22 ± 0,87	1,29 ± 0,16	2,59 ± 0,12	*	0,039 ± 0,013	0,206 ± 0,049	1,78 ± 0,59	0,0075 ± 0,0015
Ajaccio N. (VII)	1,66 ± 0,15	**	0,267 ± 0,022	2,69 ± 0,38	0,58 ± 0,04	1,59 ± 0,13	*	0,044 ± 0,026	0,191 ± 0,020	4,51 ± 1,46	0,0114 ± 0,0031

Annex B (*Continued*). Spatial variation of trace element concentrations in *Posidonia oceanica* 3rd intermediate leaves (3IL; n = 3-5), other intermediate leaves (OIL; n = 5), blades of adult leaves (BAL; n = 4-5) and sheaths of adult leaves (SAL; n = 3-5). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.

BAL		Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
PACA											
Ensuès (1)		0,0102 ± 0,0013	91 ± 21	12,3 ± 2,1	0,36 ± 0,05	72,4 ± 2,3	121 ± 25	2,74 ± 0,15	36,5 ± 1,8	10,1 ± 0,7	100,5 ± 4,3
La Vesse (2)		0,0105 ± 0,0043	132 ± 50	15,0 ± 3,3	0,40 ± 0,10	78,9 ± 8,3	133 ± 27	3,48 ± 0,26	42,7 ± 4,2	17,4 ± 1,1	156,8 ± 7,8
Corbière (3)		0,0103 ± 0,0028	123 ± 25	20,1 ± 8,0	0,40 ± 0,07	67,4 ± 10,6	132 ± 18	2,76 ± 0,39	35,0 ± 3,6	17,1 ± 1,8	135,0 ± 11,6
P. Chèvres (4)	*	0,0088 ± 0,0018	65 ± 7	40,1 ± 7,3	0,47 ± 0,01	58,1 ± 3,6	88 ± 6	2,76 ± 0,12	37,4 ± 1,7	11,7 ± 0,6	103,5 ± 4,2
Riou (5)	*	0,0069 ± 0,0035	21 ± 6	2,6 ± 0,3	0,20 ± 0,02	50,2 ± 6,5	54 ± 7	2,89 ± 0,29	42,5 ± 1,7	9,3 ± 1,1	99,2 ± 9,3
Bénat (6)	*	0,0094 ± 0,0026	88 ± 26	2,6 ± 0,3	0,27 ± 0,05	79,0 ± 7,6	95 ± 8	3,90 ± 0,54	45,1 ± 6,3	11,2 ± 2,2	100,3 ± 12,4
Giens (7)		0,0119 ± 0,0030	154 ± 30	2,7 ± 1,0	0,31 ± 0,04	90,7 ± 5,6	137 ± 21	4,52 ± 0,41	45,9 ± 2,2	10,6 ± 1,1	143,1 ± 6,8
Cap Roux (8)		0,0139 ± 0,0017	156 ± 25	12,8 ± 2,6	0,35 ± 0,02	93,7 ± 10,4	89 ± 6	4,26 ± 0,42	46,7 ± 4,0	8,1 ± 0,7	95,0 ± 6,6
St Raphaël (9)	*	0,0091 ± 0,0022	41 ± 12	4,5 ± 1,3	0,26 ± 0,04	118,4 ± 17,3	53 ± 7	6,17 ± 0,77	66,0 ± 5,6	10,4 ± 1,6	138,2 ± 14,9
Antibes (10)		0,0114 ± 0,0019	114 ± 38	24,3 ± 8,6	0,36 ± 0,06	61,0 ± 6,9	98 ± 17	3,12 ± 0,24	38,8 ± 3,4	12,5 ± 1,3	69,6 ± 7,8
Villefranche (11)		0,0122 ± 0,0032	60 ± 7	16,4 ± 5,9	0,36 ± 0,03	65,4 ± 12,0	96 ± 5	2,86 ± 0,27	30,2 ± 3,8	22,1 ± 1,2	98,4 ± 3,0
Corsica											
Calvi (I)	*	0,0083 ± 0,0012	20 ± 4	12,8 ± 1,9	0,23 ± 0,02	63,3 ± 7,4	43 ± 2	2,71 ± 0,08	36,5 ± 1,5	5,9 ± 0,4	76,7 ± 4,6
Aregno (II)		~~0,0026~~ ± 0,0024	39 ± 12	2,2 ± 0,3	0,19 ± 0,02	73,5 ± 3,5	53 ± 4	4,37 ± 0,16	40,4 ± 1,9	13,6 ± 1,4	133,2 ± 9,1
St Florent (III)	**	0,0033 ± 0,0027	89 ± 36	3,7 ± 0,6	0,90 ± 0,22	63,1 ± 7,4	136 ± 35	2,85 ± 0,05	31,6 ± 2,8	8,1 ± 1,2	79,0 ± 6,1
T. Isolaccio (IV)		0,0111 ± 0,0028	84 ± 18	29,3 ± 9,7	0,49 ± 0,07	129,1 ± 15,8	109 ± 11	3,39 ± 0,21	38,1 ± 1,2	8,8 ± 0,9	100,9 ± 15,1
Bravone (V)		0,0124 ± 0,0015	146 ± 31	3,6 ± 0,5	0,54 ± 0,04	111,9 ± 9,9	148 ± 19	5,22 ± 0,19	40,4 ± 2,1	22,6 ± 2,6	1655,0 ± 337,9
Ajaccio S. (VI)	*	0,0060 ± 0,0020	92 ± 22	5,2 ± 1,5	0,25 ± 0,05	69,8 ± 5,1	83 ± 10	3,52 ± 0,22	42,4 ± 2,1	8,2 ± 0,4	97,3 ± 7,8
Ajaccio N. (VII)		0,0144 ± 0,0035	168 ± 61	7,3 ± 3,1	0,31 ± 0,06	72,6 ± 11,1	157 ± 26	2,11 ± 0,18	26,3 ± 1,9	6,8 ± 0,3	74,4 ± 3,5

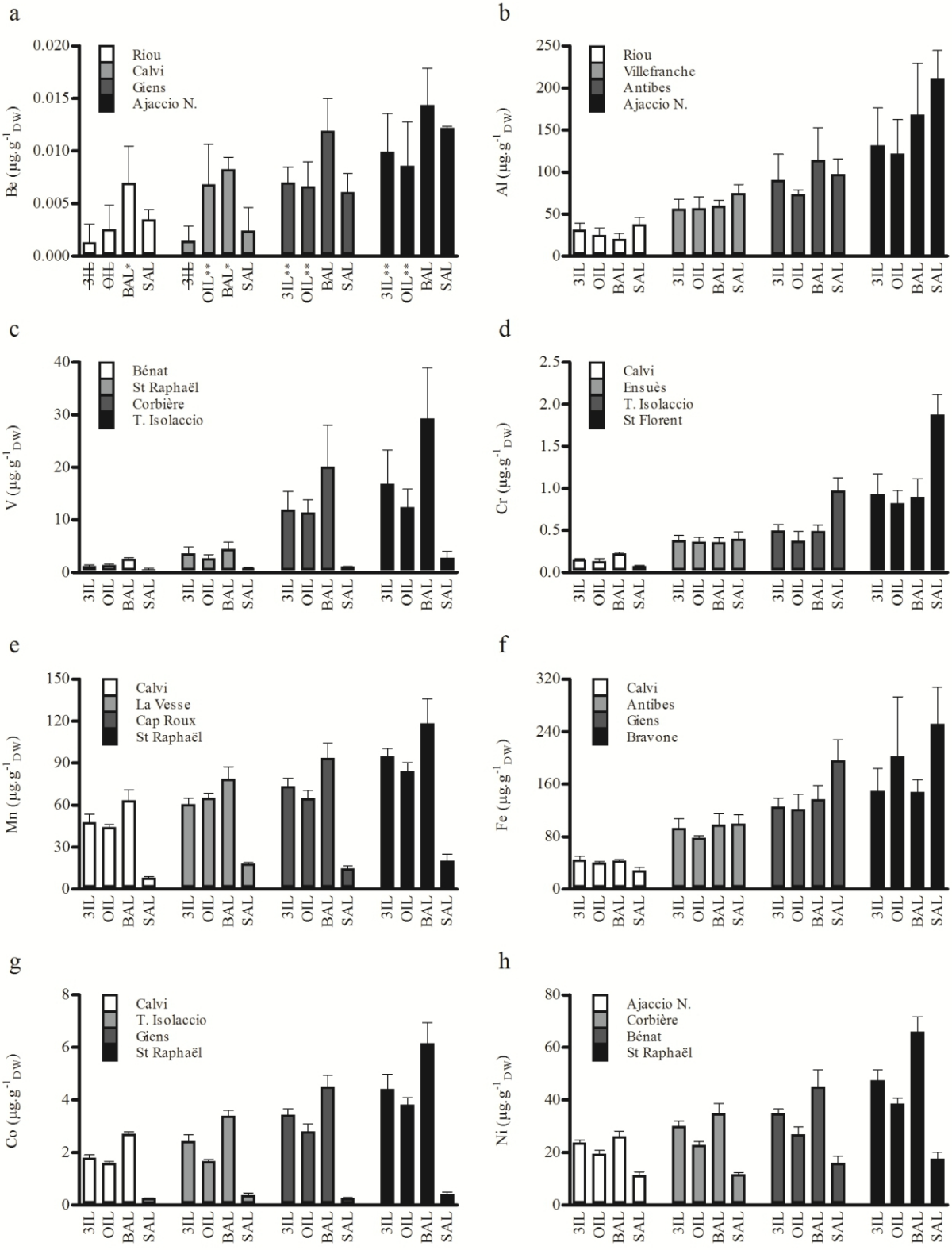
SAL	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
PACA										
Ensuès (1)	0,0080 ± 0,0002	161 ± 20	1,7 ± 0,4	0,40 ± 0,08	18,4 ± 0,6	193 ± 15	0,32 ± 0,03	16,5 ± 0,2	19,9 ± 0,3	48,9 ± 7,1
La Vesse (2)	0,0042 ± 0,0000	95 ± 5	2,2 ± 1,3	0,25 ± 0,04	18,4 ± 0,7	105 ± 5	0,30 ± 0,03	13,3 ± 0,7	27,5 ± 3,1	55,0 ± 4,5
Corbière (3)	0,0043 ± 0,0000	119 ± 33	1,1 ± 0,1	0,23 ± 0,06	14,8 ± 0,3	113 ± 20	0,25 ± 0,03	11,7 ± 0,7	22,8 ± 2,4	47,3 ± 0,6
P. Chèvres (4)	0,0042 ± 0,0000	74 ± 13	2,2 ± 0,1	0,37 ± 0,10	16,8 ± 1,9	89 ± 7	0,30 ± 0,04	14,9 ± 1,0	13,5 ± 0,5	46,0 ± 2,3
Riou (5)	0,0035 ± 0,0009	38 ± 8	0,8 ± 0,2	0,19 ± 0,01	12,5 ± 0,3	56 ± 12	0,26 ± 0,04	18,2 ± 0,4	16,4 ± 0,5	43,3 ± 2,0
Bénat (6)	0,0057 ± 0,0024	154 ± 44	0,6 ± 0,1	0,19 ± 0,07	12,2 ± 0,2	127 ± 45	0,27 ± 0,03	16,0 ± 2,7	17,6 ± 0,1	28,2 ± 0,2
Giens (7)	0,0061 ± 0,0018	211 ± 31	0,9 ± 0,2	0,25 ± 0,03	15,4 ± 1,6	197 ± 31	0,27 ± 0,03	11,2 ± 2,1	19,1 ± 3,2	30,1 ± 1,5
Cap Roux (8)	0,0059 ± 0,0017	153 ± 43	0,7 ± 0,2	0,14 ± 0,03	14,7 ± 2,1	74 ± 16	0,25 ± 0,02	10,2 ± 0,9	14,3 ± 1,4	24,8 ± 1,4
St Raphaël (9)	0,0038 ± 0,0020	70 ± 22	0,8 ± 0,2	0,19 ± 0,06	20,4 ± 4,5	64 ± 15	0,41 ± 0,09	17,5 ± 2,5	16,8 ± 2,3	35,2 ± 3,2
Antibes (10)	0,0035 ± 0,0017	98 ± 18	2,2 ± 1,2	0,20 ± 0,04	15,0 ± 2,5	100 ± 13	0,30 ± 0,04	12,3 ± 0,2	22,5 ± 2,3	33,6 ± 2,8
Villefranche (11)	0,0014 ± 0,0023	75 ± 10	1,0 ± 0,4	0,48 ± 0,50	8,2 ± 1,1	78 ± 4	0,22 ± 0,01	9,3 ± 0,3	25,6 ± 3,2	27,5 ± 0,5
Corsica										
Calvi (I)	0,0024 ± 0,0022	29 ± 7	0,5 ± 0,1	0,07 ± 0,01	8,3 ± 0,8	28 ± 5	0,27 ± 0,01	15,9 ± 3,1	8,2 ± 1,9	28,4 ± 3,6
Aregno (II)	0,0096 ± 0,0062	164 ± 96	1,1 ± 0,5	0,54 ± 0,46	9,2 ± 1,7	138 ± 103	0,24 ± 0,04	12,7 ± 2,1	17,0 ± 1,5	24,3 ± 2,9
St Florent (III)	0,0053 ± 0,0023	223 ± 10	1,1 ± 0,3	1,87 ± 0,24	10,4 ± 0,7	182 ± 19	0,26 ± 0,01	11,6 ± 1,5	14,2 ± 1,1	33,4 ± 3,5
T. Isolaccio (IV)	0,0069 ± 0,0024	277 ± 61	2,7 ± 1,3	0,98 ± 0,15	28,3 ± 4,2	315 ± 56	0,38 ± 0,08	11,3 ± 2,1	15,2 ± 2,0	30,2 ± 4,7
Bravone (V)	0,0050 ± 0,0035	219 ± 49	1,0 ± 0,2	0,95 ± 0,25	17,1 ± 2,5	251 ± 56	0,42 ± 0,05	8,8 ± 1,0	24,3 ± 3,5	356,5 ± 239,2
Ajaccio S. (VI)	0,0194 ± 0,0089	417 ± 178	2,0 ± 0,6	0,24 ± 0,06	16,0 ± 3,0	145 ± 59	0,30 ± 0,05	13,8 ± 1,0	9,7 ± 1,4	30,8 ± 1,3
Ajaccio N. (VII)	0,0122 ± 0,0002	212 ± 33	1,8 ± 1,3	0,15 ± 0,03	13,8 ± 1,3	146 ± 28	0,20 ± 0,03	11,4 ± 1,3	9,7 ± 1,9	29,7 ± 3,7

Annex B (*Continued*). Spatial variation of trace element concentrations in *Posidonia oceanica* 3rd intermediate leaves (3IL; n = 3-5), other intermediate leaves (OIL; n = 5), blades of adult leaves (BAL; n = 4-5) and sheaths of adult leaves (SAL; n = 3-5). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.

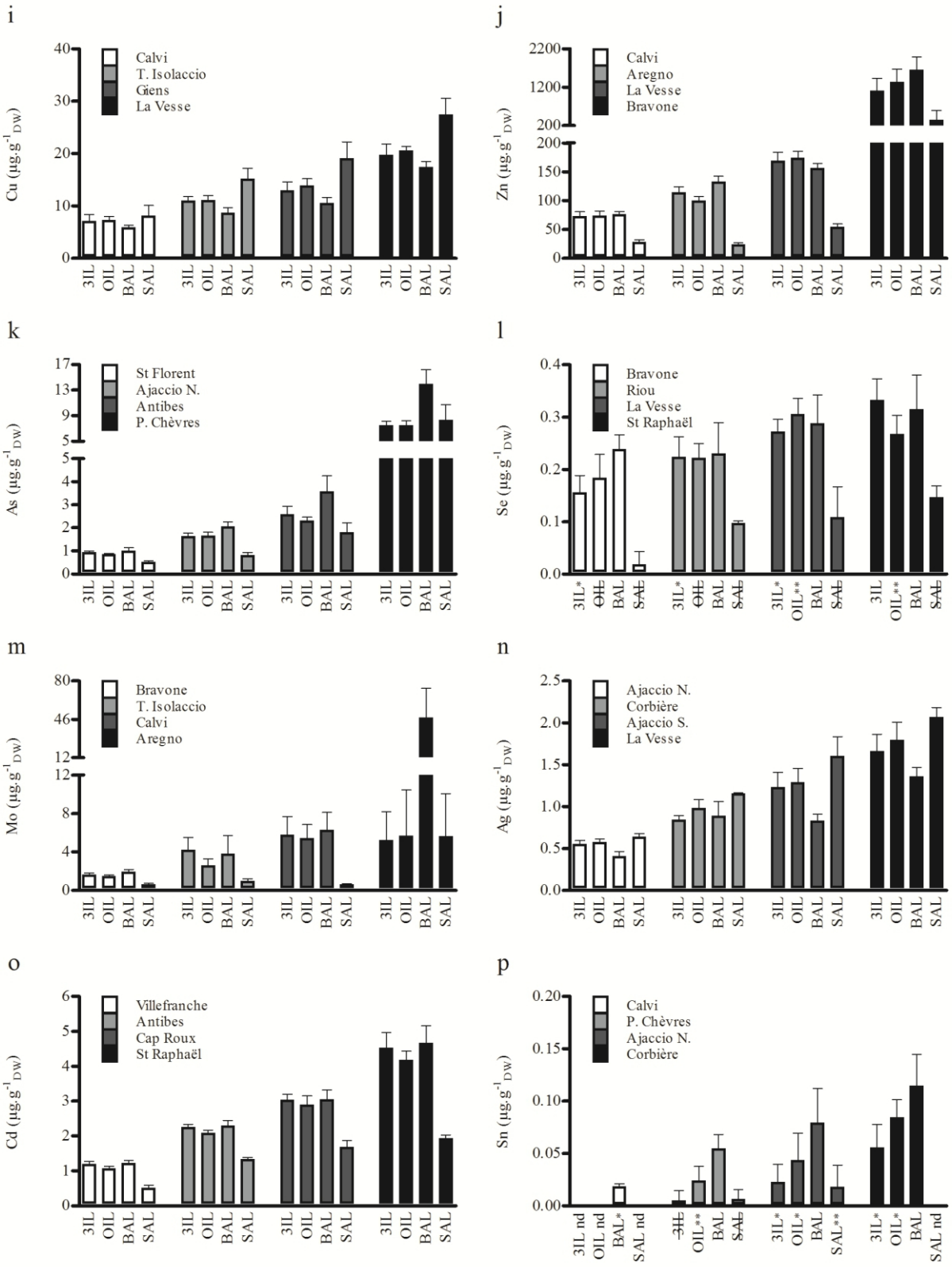
BAL	As		Se	Mo	Ag	Cd		Sn		Sb		Pb		Bi	
PACA															
Ensuès (1)	3,76 ± 0,52		0,285 ± 0,043	1,63 ± 0,28	0,56 ± 0,11	1,99 ± 0,12	*	0,041 ± 0,013		0,227 ± 0,010		3,21 ± 0,23		0,0207 ±	0,0011
La Vesse (2)	2,57 ± 0,37		0,288 ± 0,055	1,33 ± 0,14	1,36 ± 0,10	2,35 ± 0,11		0,105 ± 0,018		0,199 ± 0,007		6,08 ± 0,74		0,0393 ±	0,0052
Corbière (3)	2,81 ± 0,27		0,240 ± 0,047	1,95 ± 0,20	0,89 ± 0,17	1,49 ± 0,11		0,115 ± 0,030		0,257 ± 0,039		6,12 ± 1,12		0,0320 ±	0,0045
P. Chèvres (4)	13,97 ± 2,22		0,386 ± 0,051	4,16 ± 3,16	0,59 ± 0,06	1,97 ± 0,09		0,055 ± 0,014		0,239 ± 0,009		5,81 ± 0,58		0,0700 ±	0,0044
Riou (5)	2,01 ± 0,22		0,231 ± 0,059	1,40 ± 0,28	0,70 ± 0,11	2,95 ± 0,20	*	0,017 ± 0,008		0,216 ± 0,021		1,82 ± 0,28		0,0181 ±	0,0026
Bénat (6)	1,46 ± 0,14		0,220 ± 0,033	2,03 ± 0,45	0,95 ± 0,19	3,21 ± 0,36	*	0,024 ± 0,004		0,232 ± 0,029		1,83 ± 0,20		0,0067 ±	0,0007
Giens (7)	1,15 ± 0,08		0,178 ± 0,018	1,46 ± 0,17	0,75 ± 0,03	3,35 ± 0,19	*	0,026 ± 0,004		0,232 ± 0,016		2,28 ± 0,27		0,0066 ±	0,0010
Cap Roux (8)	2,15 ± 0,17		0,217 ± 0,031	2,04 ± 0,22	0,55 ± 0,04	3,05 ± 0,26	*	0,037 ± 0,012		0,272 ± 0,024		1,77 ± 0,14		0,0081 ±	0,0013
St Raphaël (9)	1,34 ± 0,14		0,315 ± 0,065	1,78 ± 0,29	0,72 ± 0,09	4,67 ± 0,48	*	0,020 ± 0,003		0,233 ± 0,029		1,52 ± 0,26		0,0067 ±	0,0009
Antibes (10)	3,59 ± 0,67		0,297 ± 0,024	3,28 ± 1,32	0,74 ± 0,08	2,29 ± 0,16	*	0,027 ± 0,005		0,238 ± 0,021		1,80 ± 0,24		0,0118 ±	0,0021
Villefranche (11)	2,00 ± 0,21		0,257 ± 0,045	3,81 ± 1,58	0,75 ± 0,11	1,22 ± 0,07		0,098 ± 0,009		0,331 ± 0,024		5,48 ± 1,07		0,0379 ±	0,0041
Corsica															
Calvi (I)	3,57 ± 0,58		0,331 ± 0,036	6,31 ± 1,80	0,40 ± 0,06	2,64 ± 0,13	*	0,019 ± 0,003		0,249 ± 0,015		2,03 ± 0,20		0,0082 ±	0,0007
Aregno (II)	1,05 ± 0,02		0,330 ± 0,038	47,63 ± 25,51	0,64 ± 0,03	4,40 ± 0,10	*	0,023 ± 0,029		0,262 ± 0,008		1,45 ± 0,08	*	0,0047 ±	0,0006
St Florent (III)	1,01 ± 0,12		0,194 ± 0,045	1,52 ± 0,14	0,41 ± 0,03	1,89 ± 0,11	*	0,026 ± 0,011		0,255 ± 0,127		3,00 ± 0,61		0,0089 ±	0,0022
T. Isolaccio (IV)	1,38 ± 0,29		0,244 ± 0,086	3,79 ± 1,90	0,46 ± 0,05	2,54 ± 0,15	*	0,025 ± 0,011		0,225 ± 0,020		1,76 ± 0,13		0,0085 ±	0,0008
Bravone (V)	1,25 ± 0,11		0,239 ± 0,027	1,93 ± 0,26	1,02 ± 0,08	2,63 ± 0,09		0,059 ± 0,040		0,875 ± 0,178		3,47 ± 0,60		0,0073 ±	0,0005
Ajaccio S. (VI)	2,55 ± 0,22		0,274 ± 0,019	4,21 ± 0,84	0,83 ± 0,08	2,63 ± 0,28		0,072 ± 0,060		0,247 ± 0,019		3,13 ± 0,53		0,0153 ±	0,0027
Ajaccio N. (VII)	2,07 ± 0,19		0,184 ± 0,023	2,60 ± 0,22	0,41 ± 0,06	1,75 ± 0,05		0,080 ± 0,032		0,228 ± 0,034		5,91 ± 0,79		0,0185 ±	0,0029

SAL	As		Se	Mo	Ag	Cd		Sn		Sb		Pb		Bi	
PACA															
Ensuès (1)	2,89 ± 0,27		~~0,162~~ ± 0,009	0,77 ± 0,14	1,56 ± 0,14	1,34 ± 0,12		nd	*	0,019 ± 0,007		0,83 ± 0,05	*	0,0072 ±	0,0016
La Vesse (2)	1,74 ± 0,33		~~0,109~~ ± 0,058	0,78 ± 0,27	2,07 ± 0,12	0,84 ± 0,03		nd	*	0,022 ± 0,010		1,09 ± 0,13	*	0,0077 ±	0,0009
Corbière (3)	2,28 ± 0,20		~~0,095~~ ± 0,029	0,57 ± 0,00	1,16 ± 0,00	0,87 ± 0,03		nd	*	0,016 ± 0,008		1,23 ± 0,12	*	0,0070 ±	0,0013
P. Chèvres (4)	8,34 ± 2,36		~~0,156~~ ± 0,009	0,82 ± 0,09	1,32 ± 0,05	1,40 ± 0,07		~~0,007~~ ± 0,009	*	0,023 ± 0,000		1,08 ± 0,13		0,0248 ±	0,0003
Riou (5)	1,09 ± 0,17		~~0,098~~ ± 0,003	0,57 ± 0,07	1,55 ± 0,12	1,86 ± 0,04		nd	**	0,011 ± 0,001		0,60 ± 0,09	*	0,0078 ±	0,0020
Bénat (6)	0,95 ± 0,10		~~0,159~~ ± 0,034	0,78 ± 0,23	1,22 ± 0,02	1,23 ± 0,03		nd	**	0,011 ± 0,005		0,40 ± 0,18		~~0,0002~~ ±	0,0006
Giens (7)	0,74 ± 0,18		~~0,107~~ ± 0,019	0,69 ± 0,07	1,44 ± 0,04	1,21 ± 0,09		nd	*	0,017 ± 0,008		0,64 ± 0,10		~~0,0010~~ ±	0,0006
Cap Roux (8)	0,95 ± 0,19		~~0,092~~ ± 0,022	0,79 ± 0,07	1,12 ± 0,13	1,68 ± 0,19		nd	**	0,009 ± 0,003		0,40 ± 0,05	**	0,0029 ±	0,0015
St Raphaël (9)	0,93 ± 0,16		~~0,147~~ ± 0,021	1,03 ± 0,16	1,44 ± 0,12	1,94 ± 0,09		nd	*	0,022 ± 0,020		0,51 ± 0,15	*	0,0047 ±	0,0012
Antibes (10)	1,82 ± 0,41		~~0,059~~ ± 0,015	0,81 ± 0,24	1,25 ± 0,05	1,33 ± 0,06		nd	*	0,016 ± 0,004		0,43 ± 0,06	**	0,0030 ±	0,0006
Villefranche (11)	1,81 ± 0,18		~~0,071~~ ± 0,051	0,63 ± 0,04	0,65 ± 0,05	0,52 ± 0,08		nd	**	0,011 ± 0,002		0,99 ± 0,06	*	0,0070 ±	0,0009
Corsica															
Calvi (I)	1,42 ± 0,21		~~0,130~~ ± 0,049	0,61 ± 0,10	0,69 ± 0,04	1,19 ± 0,13		nd ±		nd		0,30 ± 0,16		~~0,0011~~ ±	0,0004
Aregno (II)	0,84 ± 0,14		~~0,155~~ ± 0,025	5,63 ± 4,45	1,38 ± 0,17	1,32 ± 0,15		0,120 ± 0,264		~~0,004~~ ± 0,013		0,32 ± 0,17		~~0,0011~~ ±	0,0003
St Florent (III)	0,53 ± 0,05		~~0,188~~ ± 0,008	0,42 ± 0,02	0,83 ± 0,09	0,86 ± 0,12		nd ±		nd		0,48 ± 0,18	**	0,0025 ±	0,0011
T. Isolaccio (IV)	1,27 ± 0,22		nd	1,00 ± 0,22	1,21 ± 0,10	1,10 ± 0,05		nd ±	*	0,014 ± 0,011		0,45 ± 0,06		~~0,0018~~ ±	0,0007
Bravone (V)	1,25 ± 0,31		~~0,018~~ ± 0,025	0,61 ± 0,11	1,30 ± 0,15	1,05 ± 0,07	**	0,012 ± 0,041		0,228 ± 0,127		0,77 ± 0,22	**	0,0035 ±	0,0006
Ajaccio S. (VI)	1,77 ± 0,34		~~0,177~~ ± 0,077	2,17 ± 1,57	1,60 ± 0,23	1,04 ± 0,07	**	0,014 ± 0,020	*	0,014 ± 0,003		0,68 ± 0,17	*	0,0054 ±	0,0023
Ajaccio N. (VII)	0,81 ± 0,12	**	0,220 ± 0,024	0,93 ± 0,16	0,64 ± 0,04	0,75 ± 0,06	**	0,018 ± 0,021	*	0,018 ± 0,009		0,90 ± 0,28	**	0,0031 ±	0,0013

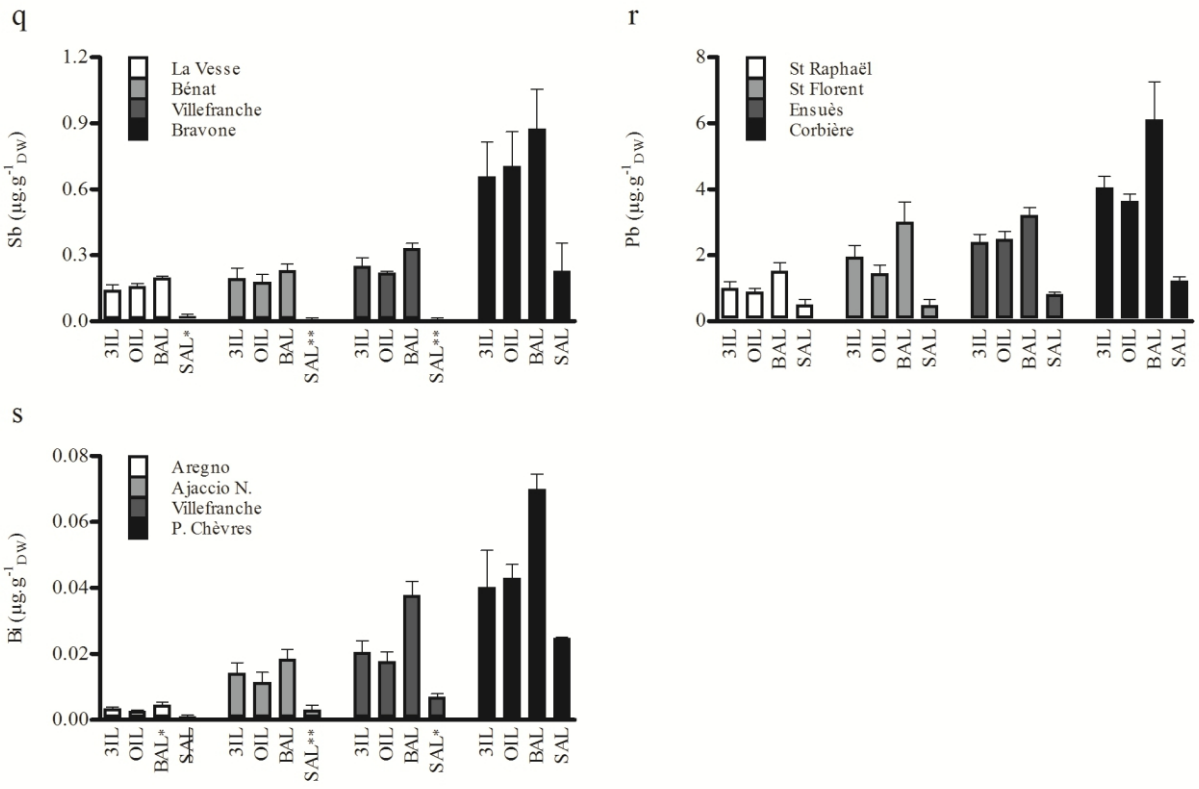
Annex B (*Continued*). Spatial variation of trace element concentrations in *Posidonia oceanica* 3rd intermediate leaves (3IL; n = 3-5), other intermediate leaves (OIL; n = 5), blades of adult leaves (BAL; n = 4-5) and sheaths of adult leaves (SAL; n = 3-5). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. *, **, struck-through values and nd represent concentrations < L_Q, < L_D, < L_C and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.



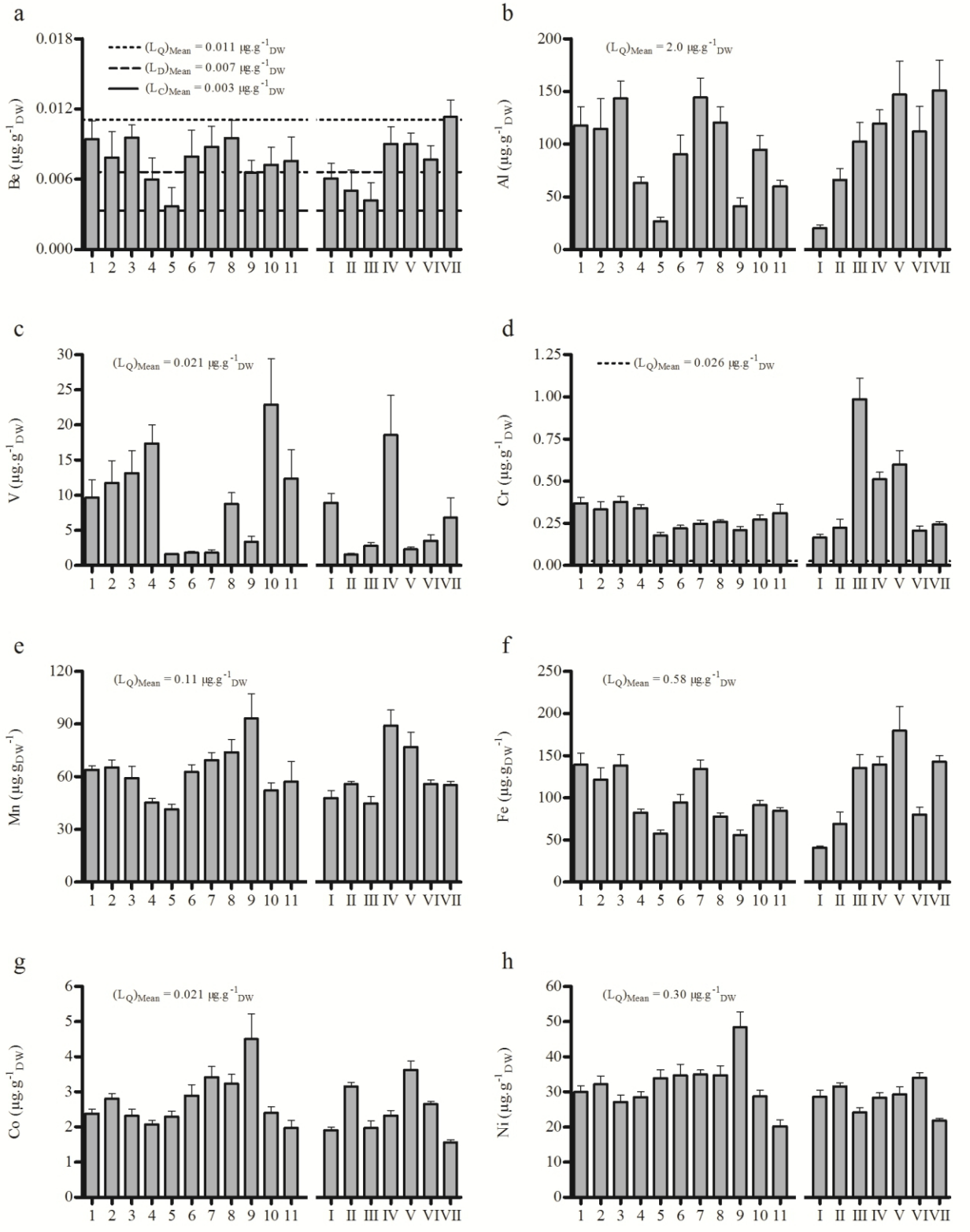
Annex C. *Posidonia oceanica* compartmentalization of Be, Al, V, Cr, Mn, Fe, Co and Ni (a–h) at 4 of the 18 sites. Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. X-labels indicate the different compartments: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL); *, **, struck-through compartments and nd represent concentrations $< L_Q$, $< L_D$, $< L_C$ and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.



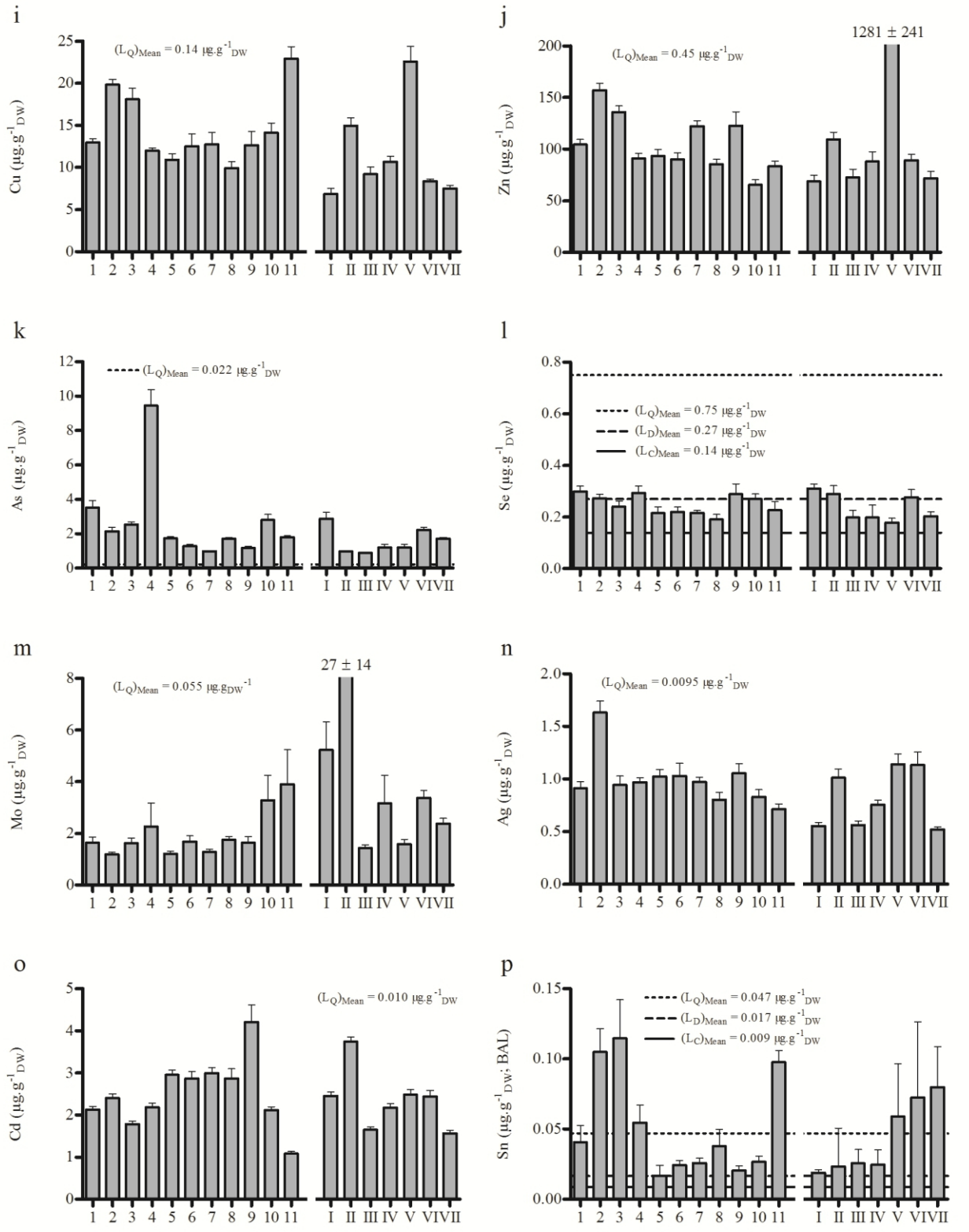
Annex C (*Continued*). *Posidonia oceanica* compartmentalization of Be, Al, V, Cr, Mn, Fe, Co and Ni (i-p) at 4 of the 18 sites. Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. X-labels indicate the different compartments: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL); *, **, struck-through compartments and nd represent concentrations $< L_Q$, $< L_D$, $< L_C$ and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.



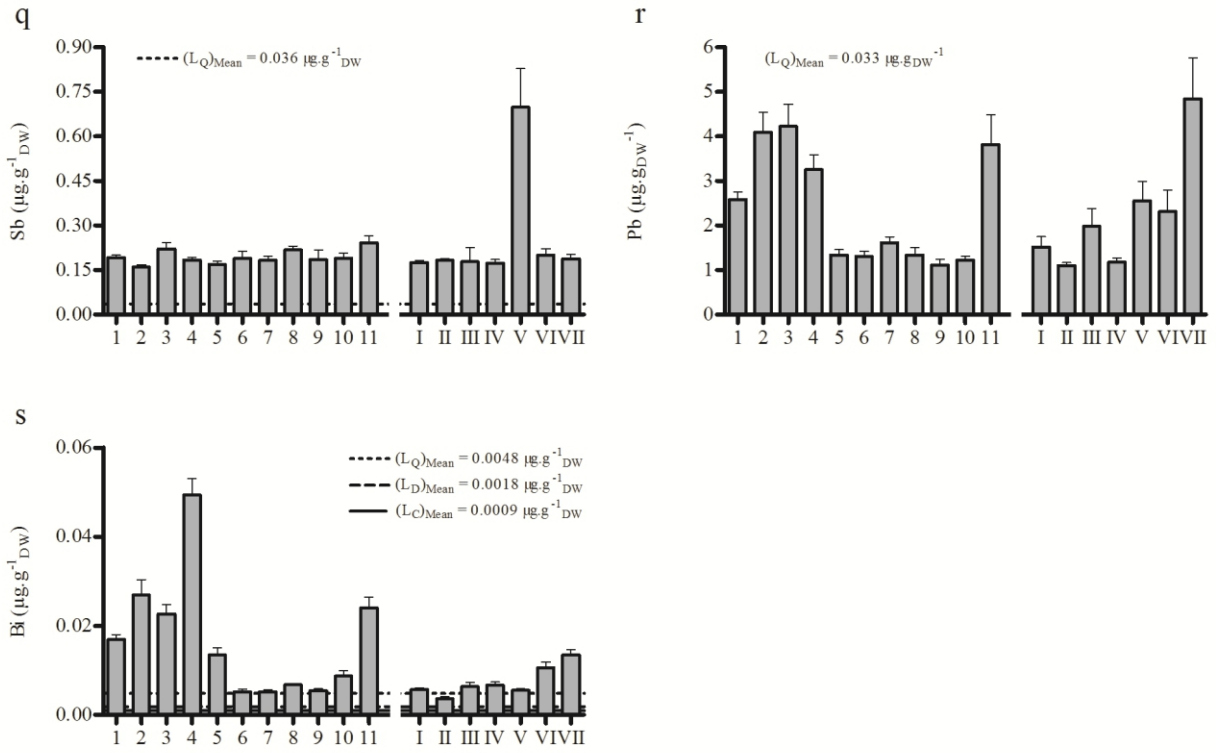
Annex C (*Continued*). *Posidonia oceanica* compartmentalization of Sb, Pb and Bi (q-s) at 4 of the 18 sites. Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$. X-labels indicate the different compartments: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL); *, **, struck-through compartments and nd represent concentrations $< L_Q$, $< L_D$, $< L_C$ and not detected, respectively. Number of replicates varies from 3 to 5, depending on site and compartment.



Annex D. Spatial variation of Be, Al, V, Cr, Mn, Fe, Co and Ni (a–h) concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.



Annex D (*Continued*). Spatial variation of Cu, Zn, As, Se, Mo, Ag, Cd and Sn (BAL) (i-p) concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.



Annex D (*Continued*). Spatial variation of Sb, Pb and Bi (q–s) concentrations in *Posidonia oceanica* shoots at the 18 sites: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica). Concentrations are expressed as mean ± standard deviation in $\mu g.g^{-1}_{DW}$.

Chapter 3

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TE kinetics in *Posidonia oceanica*

Experimental *in situ* exposure of *Posidonia oceanica* to 15 trace elements

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Abstract

Posidonia oceanica (L.) Delile were *in situ* experimentally exposed to a mix of 15 TEs (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Pb and Bi). Mesocosms were deployed in the Calvi Bay (Corsica, France), a reference station for the Northwestern Mediterranean. Enclosed plants were exposed (i) to a moderate level of dissolved chemicals, to measure element fluxes at low environmental contaminant concentrations or (i) to an acute level, similar to highly contaminated seagrass meadows. TEs concentrations were measured by DRC ICP-MS in *P. oceanica* leaves and rhizomes, in epiphytes, in water and in sediments sampled at specific time intervals. In both cases, *P. oceanica* immediately accumulated pollutants from the beginning of experiments; once contaminations ended, TE concentrations came back to their original levels within two weeks, or at least showed a clear decrease. Leaves exhibited different uptake kinetics for many elements (e.g. Cr, Cu, Ag, Bi etc.): the younger growing leaves forming new tissues incorporated chemicals more rapidly than the older senescent leaves. Epiphytes also exhibited a net uptake of most chemicals at the end of both exposures, partly similar to that of *P. oceanica* shoots. The principal route of TE uptake was through the water column, as no contamination of superficial sediments was observed. However, rhizomes indirectly accumulated many chemical during the overall experiments through acropetal translocation processes. TE observed kinetics also highlighted some general aspect of essential micronutrient dynamics, and showed the essential role played by *P. oceanica* in the cycling of TEs in the Mediterranean.

Keywords: Posidonia oceanica, trace elements, *in situ* exposures, uptake and loss kinetics.

1. Introduction

The Mediterranean Sea is a semi-enclosed basin largely submitted to anthropogenic disturbances. Urban coastal development, tourism, industries, shipping, etc., threaten and pollute Mediterranean coastal environments (Laubier 2005). The UNEP (1999, 2002) recently identified 101 geographic sites impacted by industrial and domestic pollutions. The contribution of these hot spots is highly variable; only a limited number of them are responsible for the major part of the pollution. Thus, eight hot spots mainly concentrated in the eastern Mediterranean basin are responsible for major discharges of Hg, Cd, Pb, Cr, Cu, Zn and Ni (Laubier 2005).

Trace element (TE) loadings in coastal areas can be monitor through direct chemical analysis of environmental matrices such as sediment and water, or through the use of bioindicator (Zhou et al. 2008). Fränzle (2006) defined a bioindicator in a general ecological sense as "organelles, organisms or groups of organisms suited to determine qualitatively or quantitatively the state of the environment".

Posidonia oceanica (L) Delile, the endemic marine magnoliophyte of the Mediterranean, forms monospecific meadows along its coasts from the surface to depths of about 40 m (Boudouresque and Meinesz 1982, Gobert et al. 2006). This seagrass presents characters of a good bioindicator: this sessile organism is abundant, widely distributed, easy to sample; it effectively accumulates high levels of pollutants and occupies an important position in the food chain (Wright and Welbourn 2002).

Posidonia oceanica has largely been used for trace metals biomonitoring (Cr, Fe, Ni, Cu, Zn, Cd, Pb and Hg) since decades, as reviewed by Pergent Martini and Pergent (2000) and, more recently, by Luy *et al.* (2012). Moreover, these latest authors have also demonstrated that *P. oceanica* above-ground tissues could effectively be used for the monitoring of a series of little studied and potentially toxic TEs (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi), and that abnormally high levels of chemicals compared to baseline values could precisely be linked to specific human activities. Once an organism is determined as a potential tool for the monitoring of coastal pollution, it is interesting to experimentally investigate how this bioindicator will respond to environmental changes such as pollutant loadings. However, present information for the genus *Posidonia* is limited mainly to plant contaminant levels, while virtually nothing is known about kinetics of TE fluxes through *P.*

oceanica communities. These observations made by Warnau (1996) in the mid-1990s are still topical as, to our knowledge, no other kinetic studies have been driven since then. Furthermore, *P. oceanica* is expected to play a major role in the cycling of TEs in Mediterranean coastal areas due to its wide abundance, high productivity and capacity to accumulate chemicals (Sanz-Lázaro et al. 2012).

The aim of this study was to investigate kinetic parameters of TE uptake and loss by *P. oceanica* in order to further assess its value as a bioindicator organism for quantitatively identifying coastal pollution. TE exposures were experimented in mesocosms deployed *in situ*; extrapolating laboratory findings to the field can be unreliable (Ralph et al. 2006) because naturally occurring factors in water and sediments can either reduce or increase chemical bioavailability, which cannot easily be simulated in the laboratory (Lewis and Devereux 2009). Isolated portions of the seagrass bed were contaminated using multi-element exposures at two experimental levels: (i) a moderate level, in order to measure element fluxes at realistic low environmental contaminant concentrations, and (ii) an acute level, similar to highly contaminated seagrass meadows. Kinetics were distinctly followed in entire shoots, in young and old leaves and in rhizomes. Bioavailable pollutant levels within mesocosms were also followed throughout the experiment, and contamination of superficial sediments and leaf epiphytes were monitored.

2. Materials and methods

2.1. Continuous field survey

To determine the mean natural TE levels of the studied seagrass bed and to validate the election of the Calvi Bay, Northwestern Corsica (France; Fig. 1), as a reference site for the monitoring of trace element pollution in the Northwestern Mediterranean (as suggested in previous studies punctual in time; e.g. Luy et al. 2012), P. oceanica shoots and superficial sediment samples were seasonally collected between years 2008 and 2010 at 10 m depth, in front of the oceanographic station STARESO.

2.2. Experimental design

In June 2009, isolated portions of a *Posidonia oceanica* seagrass bed were experimentally contaminated *in situ* with a cocktail of 15 trace elements (TEs): Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ag, Cd, As, Mo, Pb and Bi. The experiment was driven at 10 m depth in the Calvi Bay, in front of the oceanographic station STARESO (Fig. 1).

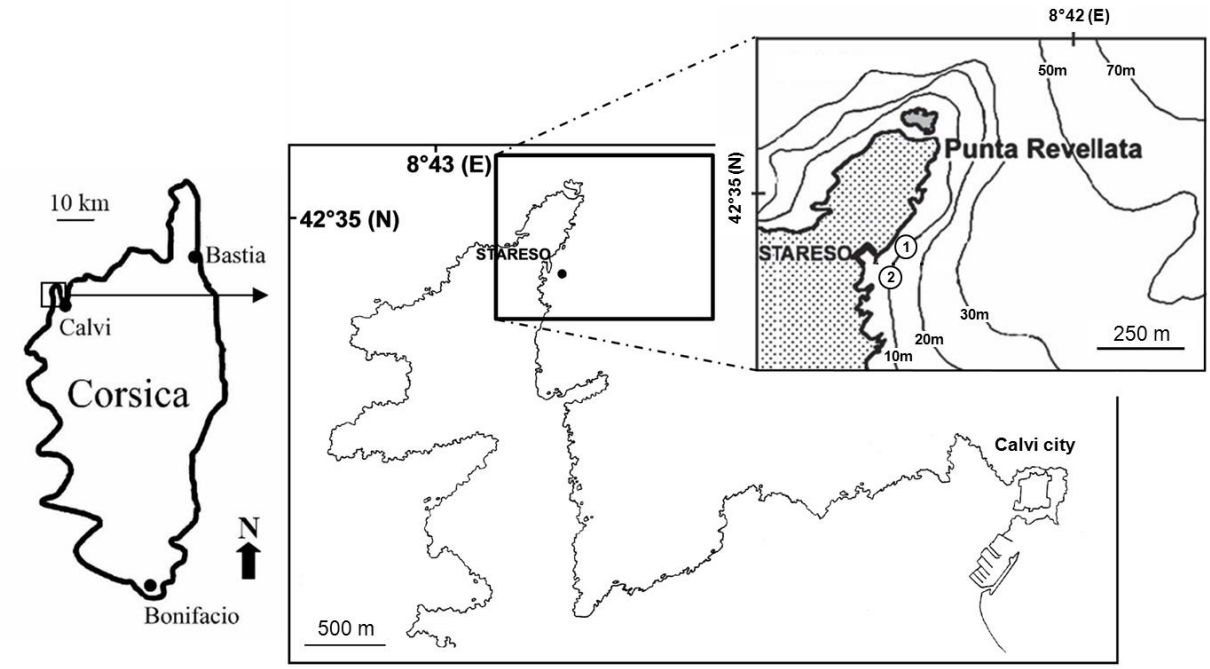

Fig. 1. Location of the *in situ* experimental contaminations near the oceanographic station STARESO, in the Calvi Bay (NW Corsica, France). Numbered circles on the 10 m isobaths in the STARESO area zoom show the emplacement of mesocosms contaminated at moderate (1) or acute (2) levels, respectively.

Two levels of contaminations were intended: (i) a moderate and (ii) an acute level. The moderate level of contaminants corresponded to a final TE concentration in average 3.7 times higher than the world ocean average concentration (and more than 20 times the Calvi Bay concentration); the acute level of contaminants corresponded to a final TE concentration 28.8 times higher (and more than 150 times the Calvi Bay concentration). The world ocean TE average concentration was calculated by compiling data from the literature (Table 1).

Table 1.
Non-exhaustive compilation of surface seawater trace element concentrations (ppb) available in the litterature. A) world mean seawater composition. B) Trace element concentrations in locations sampled outside the Mediterranean. C) Trace element concentrations in locations sampled inside the Mediterranean. Mean concentrations are calculated without values considered as high (grey cells). nd = not determined.

	Geographic site	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi	Ref.
A)	seawater mean composition	2,60	1,00	1,90	0,200	3,400	0,40	0,3900	6,600	0,900	5,000	10,0	0,2800	0,1100	0,030	2,0E-02	[1]
	Pacific Ocean	1,55	0,11		0,277	0,042	0,14	0,0070	0,151	0,035	0,007	10,4	0,0002	0,0003	0,014	3,7E-05	[2]
	Atlantic Ocean		0,81	1,24		0,098	0,14		0,130	0,072	0,003			0,0002	0,551		
	seawater mean composition	1,76	0,31	2,23	0,261	0,259	0,07	0,0012	0,549	0,217	0,331	10,4	0,0026	0,0818	0,003	4,3E-06	[3]
	seawater mean composition	1,50	0,51	2,50	0,302	2,010	0,20	0,0501	0,200	0,102	0,098	9,6	0,0399	0,0100	0,003		[4]
	seawater mean composition	1,78	0,06	1,74	0,215	0,029	0,02	0,0012	0,486	0,197	0,338	10,4	0,0023	0,0698	0,002		[5]
B)	Boston Light-Ship (Massachusetts, USA)															1,6E-02	
	Pacific Deep Ocean															4,1E-02	[6]
	Bahia Honda Key (Florida, USA)															9,1E-02	
	Northeast Atlantic Ocean			1,19								10,7					[7]
	Pacific Ocean															5,3E-05	
	Scripps Pier, La Jolla (California, USA)															6,9E-05	[8]
	San Diego Bay, San Diego (California, USA)															6,3E-04	
	Mission bay, San Diego (California, USA)															4,6E-04	
	North USA Atlantic inshore waters		0,22													5,4E-05	
	Gulf Stream (North Carolina, USA)		0,42														
	Sargasso Sea		1,12													7,4E-05	[9]
	Carribean Sea		1,12													5,4E-05	
	Panama Bassin		2,10														
	Northeast Pacific Ocean											10,3					[10]
	Tokyo Bay (Japan)		6,50		0,070	14,40	36,1	0,1400	1,500	0,310	2,000				1,700		
	Hiroshima Bay (Japan)		9,60		0,090	1,270	1,06	0,1400	0,180	0,190	0,800				nd		[11]
	Sagami Bay (Japan)		0,95		0,070	0,660	1,05	nd	nd	0,067	0,120				nd		
	Atlantic Ocean	1,20		1,66													[12]
	Nagoya port (Japan)	0,71	5,60		0,250	4,000	54,0	0,3300	11,00	1,000	5,700				0,088		[13]
	Marina IV cruise, Seine Bay (France)	1,32															[14]
	Tianjin (China)						0,01	0,0271		2,758			0,0324	0,1275	0,059	8,7E-03	
	Dalian (China)						0,01	0,0777		1,152			0,0189	0,0472	0,057	8,1E-06	[15]
	Qingdao (China)						0,05	0,0810		0,276			0,0543	0,0345	0,081	3,1E-06	
	Qinghuangdao (China)						0,07	0,0522		0,132			0,0203	0,0700	0,077	1,1E-05	
	North Atlantic					0,056			0,170	0,073	0,141			0,0031	0,019		[16]
	Louisiane Shelf (USA)			0,99													[17]
	Northern French coastal waters (France)			2,02													[18]
	Chao Phraya estuary (Gulf of Thailand)											11,9					[19]
	Huelva Estuary (SW Spain): winter min	10,1															[20]
	Huelva Estuary (SW Spain): winter max	17,8															
	Shibukawa Sea (Okayama, Japan)															2,3E-02	[21]
	Shibukawa Sea (Okayama, Japan)											9,4					[22]
	German Bight offshore (North Sea)						0,66					9,9					[23]
	Huelva Estuary (Spain)	13,0					141,0		5,900	76,00	309,0			2,8000			[24]
	North East Atlantic Ocean			2,27					0,478	0,254							[25]
	20 sites in Bohai Bay (China): min										15,20			0,1070			[26]
	20 sites in Bohai Bay (China): max										24,30			0,1820			
C)	Gulf of Elefsis (Greece)										18,30						[27]
	Tyro Basin (Cretan Sea)	1,52		1,78								15,5					[28]
	Bannock Basin (Cretan Sea)	1,50		1,70								14,8					
	Athens Sewage Outfall (Greece)												1,0100				[29]
	Inner Saronikog Gulf (Greece)												0,3000				
	Open Saronikos Gulf (Greece)												0,2700				
	Aegean Sea												0,1800				
	Western Strait 2004 (Aegean Sea, Greece)				0,063		0,42	0,0190	0,327	0,194				0,0220	0,490		[30]
	Cretan Sea 2004 (Aegean Sea, Greece)				0,059		0,31	0,0122	0,289	0,171				0,0182	0,416		
	Eastern Strait 2004 (Aegean Sea, Turkey)				0,045		0,43	0,0162	0,268	0,306				0,0232	0,408		
	Mersin Bay (Turkey)					4,500			4,800	1,940	2,800			0,3700	1,200		[31]
	Northwestern Mediterranean Sea		1,65														[32]
	Gulf of Cadiz					0,098			0,199	0,129	0,666			0,0119	0,028		[16]
	Alboran Sea					0,144			0,178	0,084	0,188			0,0037	0,015		
	Western Mediterranean Bassin					0,077			0,187	0,104	0,171			0,0096	0,026		
	Sicilian Strait					0,088			0,188	0,101	0,159			0,0080	0,025		
	Favigna Island (Sicily, Italy)				0,090					0,630	3,100			0,1200	0,570		[33]
	Canari (Corsica, France)				0,152			0,0170	1,380					0,0160	0,048		[34]
	Livorno (Toscana, Italy)				0,616			0,0080	0,197					0,0060	0,038		
	Porto-Tores (Sardinia, Italy)				0,282			0,0160	0,378					0,0090	0,075		
	4 sites in Sardinia (Italy): min.								0,033	0,005				0,0010	0,004		[35]
	4 sites in Sardinia (Italy): max.								0,120	0,080				0,0350	0,147		
	Ustica Island (Italy)				0,210					1,210	13,350			0,2000	0,970		[36]
	Linosa Island (Italy)				0,160					2,340	13,000			0,3300	0,970		[37]
	Algeciras Bay (Spain)	1,70					2,10		0,600	0,500	9,800			0,0200			[24]
	number of data	14	16	12	18	16	20	18	26	31	24	12	13	31	29	18	
	mean concentration	1,56	0,80	1,77	0,190	1,115	0,42	0,0770	0,372	0,315	1,272	11,1	0,100	0,048	0,131	1,2E-04	
	maximal concentration	17,8	9,60	2,50	0,616	14,40	141,0	0,3900	11,00	76,00	309,0	15,5	1,010	2,800	1,700	9,1E-02	
		As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi	

References: [1] Turekian 1968, [2] Quinby-Hunt and Wilde 1986-87, [3] Li 1991, [4] Fischer and Wartel 1992, [5] Bruland and Lohan 2004, [6] Gilbert and Hume 1973, [7] Morris 1975, [8] Lee 1982, [9] Measures et al. 1984, [10] Collier 1985, [11] Akagi et al. 1985, [12] Middelburg et al. 1988, [13] Sawatari et al. 1995, [14] Michel et al. 1999, [15] Wen et al. 1999, [16] Yoon et al. 1999, [17] Shiller and Mao 1999, [18] Abbasse et al. 2002, [19] Dalai et al. 2005, [20] Sánchez-Rodas et al. 2005, [21] Oshita et al. 2007, [22] Sabarudin et al. 2007, [23] Dellwig et al. 2007, [24] Morillo and Usero 2008, [25] Santos-Echeandia et al. 2008, [26] Zhang et al. 2010, [27] Scoullos 1981, [28] Van der Weijden et al. 1990, [29] Grimanis et al. 1994, [30] Voutsinou-Taliadouri et al. 1997, [31] Elçi et al. 1997, [32] Chou and Wollast 1997, [33] Campanella et al. 2001, [34] Lafabrie et al. 2007, [35] Schintu et al. 2008, [36] Conti et al. 2007, [37] Conti et al. 2010.

Table 2.

A) Theoretical TE concentrations (ppb) within mesocosms after injections (considering that 100% of injectd chemicals are bioavailable). B) Average TE concentrations (ppb) in *P. oceanica* control meadow and mesocosms measured with DGTs; grey values for V, Mo and Bi are total TE contents accumulated in DGT chelex resins. C) Bioavailable fractions (%) of chemicals initially injected within mesocosms. D) Contamination factors between *P. oceanica* control meadow and mesocosms. nd = not determined.

A.	**injected TEs**	**As**	**Al**	**V**	**Cr**	**Fe**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**	**Mo**	**Ag**	**Cd**	**Pb**	**Bi**
	moderate level	9,4	8,9	22,0	2,3	4,7	4,4	0,9	4,4	4,5	56,9	88,5	2,4	3,9	4,6	0,16
	acute level	94,4	90,1	221,7	23,0	47,2	44,2	9,0	44,8	45,5	572,8	891,3	24,3	39,0	46,3	1,63
B.	**measured TEs**															
	control	nd	0,06	0,44	0,13	0,23	0,23	0,01	0,15	0,04	3,5	0,6	0,0003	0,009	0,05	nd
	moderate level	nd	2,00	2,60	0,16	1,46	0,34	0,20	1,14	0,94	16,4	7,7	0,0184	0,306	1,00	0,15
	acute level	nd	21,73	51,06	1,10	11,39	2,72	1,64	8,35	8,56	129,9	137,6	0,0586	2,548	5,34	0,59
C.	**bioavailability**															
	moderate level	-	22%	-	7,1%	31%	7,8%	22%	26%	21%	29%	-	0,76%	7,9%	22%	-
	acute level	-	24%	-	4,8%	24%	6,2%	18%	19%	19%	23%	-	0,24%	6,5%	12%	-
D.	**conta. factors**															
	moderate level	nd	35,1	5,9	1,3	6,2	1,5	38,3	7,8	24,0	4,7	13,5	55,1	34,7	21,2	nd
	acute level	nd	381,2	115,2	8,7	48,8	12,0	319,0	57,3	218,2	37,4	241,5	175,4	289,4	112,8	nd

Moderate levels of pollutants corresponded to what can be measured in contaminated sites of the Mediterranean such as Canari (Corsica, France; Ni contamination; Lafabrie et al. 2007), Gulf of Elefsis (Greece; Zn contamination; Scoullos 1981) or Favigna Island (Siciliy, Italy; Cd contamination; Campanella et al. 2001). Bulk multielement solutions of pollutants were prepared by diluting 1000 ppm TE standard solutions (Certipur grade, Merck). The theoretical concentrations within mesocosms after injections are given in Table 2A.

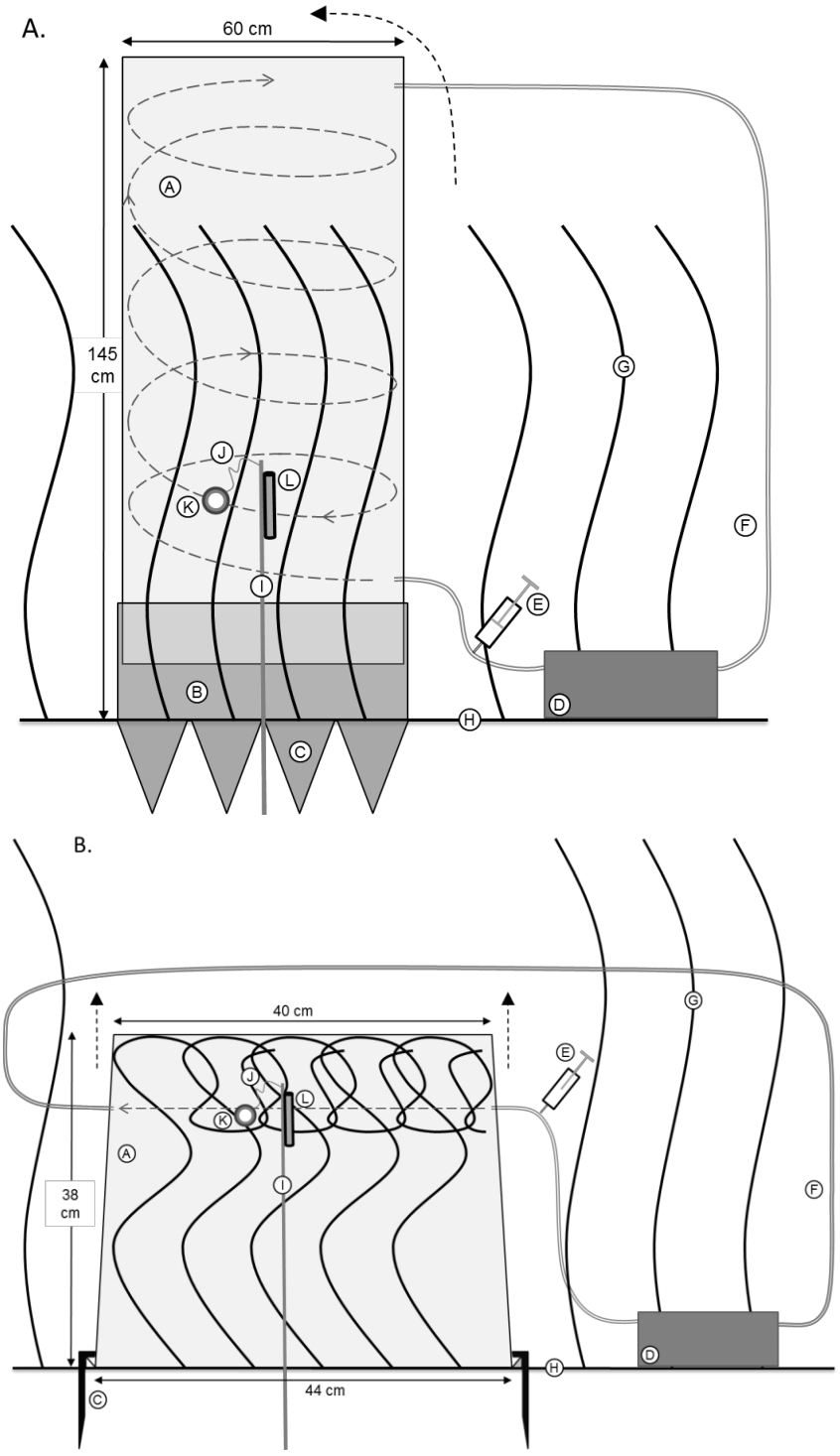

Fig. 2. Schematic designs of mesocosms used for the moderate (A – PVC cylinder) and acute (B – PP trapezoidal box) contaminations. Circled letters correspond to: a) movable PVC cylinder or PP box; b) fixed circular basis of PVC cylinder; c) buried teeth of PVC cylinder circular basis or stainless steel moorings of PP box; d) submersible pump; e) syringe for pollutant injection; f) recirculating pipe; g) *P. oceanica* shoot; h) sedimentary interface; i) plastic stage holding l) the Minilog (temperature logger) and the k) DGT, fixed to the stage with j) a nylon fishing line. Mesocosm dimensions are annotated on figures. Black dotted arrows symbolise mesocosms opening for DGT replacement and shoot sampling during the contamination period. Grey dotted arrows symbolize the water circulation within mesocosms. The 2 taut nylon boots of mesocosm A fixed to 4 cross-shaped stainless steel moorings are not shown.

Ninety-height *P. oceanica* shoots were contained in the moderately contaminated cylindrical mesocosm (Fig. 2A). It was made of a plastic jagged basis buried in the sediment up to the base of its teeth. This basis was surmounted by a 120 cm high (total height = 145 cm) and 60 cm width removable polyvinyl chloride (PVC) cylinder, closed to its top. The all structure was maintained with 2 taut nylon boots intersecting on the cylinder top and tightly fixed to 4 cross-shaped stainless steel moorings (not shown on Fig. 2A). The water volume inside the mesocosm was equal to 410 litres. A continuous circular ascending water courant was created with a submerged pump recirculating all the water volume in 1h25. During 5 days, the pollutant solution was injected every 12 hours (at 9.00 am and 9.00 pm) through the recirculating water pipe with the aid of a syringe. Three *P. oceanica* shoots were sampled every morning before pollutant injection, to study their contamination kinetics, by toppling over the mesocosm what renewed water inside. The experimental cylindrical-shaped mesocosm was removed after 6 days; *P. oceanica* shoots were sampled during 15 more days, to study their decontamination kinetics, and in November 2009 and March 2010 as post-controls.

In the second experimental mesocosm, 91 *P. oceanica* shoots were contaminated at acute levels. The mesocosm consisted of a 54 litres polypropylene (PP) trapezoidal box (surface on sediment: 44 cm x 35 cm; height: 38 cm) set down with its opening side facing the sediment (Fig. 2B). The mesocosm was maintained with four cross-shaped stainless steel moorings. The water current was horizontal through the *P. oceanica* shoots enclosed in the mesocosm. The complete water volume cycle trough the recirculating closed system took 11 minutes. The contamination period lasted 24 hours. The 12 first hours, chemicals were injected every 3 hours (from 9 am to 9 pm). The last contamination went on all night (from 9 pm until 9 am). Three *P. oceanica* shoots were sampled before every pollutant injection, to study the contamination kinetics of *P. oceanica*, by toppling over the mesocosm what renewed water inside. After the mesocosm removal, *P. oceanica* shoots were sampled during 15 more days, to study their decontamination kinetics of, and in November 2009 and March 2010 as post-controls.

2.3. *Posidonia oceanica* sample processing

Posidonia oceanica shoots were treated according to the biometric method proposed by Giraud (1979). Epiphytes were scraped from leaves with the aid of a ceramic scalpel blade.

Furthermore, each shoot was sorted and noted as follows: intermediate leaves (IL), adult leaves (AL), rhizome 2 first cm (Rz; scales removed) and epiphytes (Ep). Sorted tissues were lyophilized (BenchTop 3L, VirTis Company Inc.), weighed and ground in an agate mortar. Contrary to rhizomes and epiphytes, *P. oceanica* leaves were cryogenic ground and then re-lyophilized to eliminate condensed ambient water vapour. Dried powders were mineralized in Teflon bombs in a closed microwave digestion labstation (Ethos D, Milestone Inc.). The digestion procedure performed was a nitric acid mineralization (HNO_3/H_2O_2; suprapure grade, Merck). Finally, mineralisats were diluted to an appropriate volume of 50 cm^3 prior to be analysed. TE levels measured in *P. oceanica* intermediate and adult leaves were balanced by their respective dry weight to calculate shoot TE levels.

2.4. Bioavailable trace element concentrations

Bioavailable TEs present in mesocosms were measured throughout the used of diffusive gradients in thin films (DGTs) devices (DGT Research Ltd. UK). DGT technique is based on diffusion of metals through a diffusive layer until a cation-exchange resin selective for trace metals (Davison and Zhang 1994, Zhang and Davison 1995). DGT units, fixed with a nylon fishing line to a plastic stage buried in the sediment (Figs. 2A, B), could float freely in the *P. oceanica* canopy. As trace metal diffusion coefficients through the diffusive layer depend on water temperature, average temperatures were recorded with Minilog temperature data loggers (VEMCO Division, AMIRIX Systems Inc., Canada). DGT units were replaced every day (at 9.00 am) for the moderate contamination (5 DGTs deployed), every two days in the reference site (4 DGTs deployed), and after 12 h for the acute contamination (2 DGTs deployed). Collected DGTs were carefully rinced with milliQ water and stored at 4°C. Elution of metals from the Chelex 100 binding phase was carried out by immersing the resin in 1.0 M HNO_3 (Suprapure grade, Merck) for minimum 24 hours prior to be analysed.

2.5. Sediment samples collect and processing

Superficial sediments (1^{st} oxic centimetre) were collected at the end of the contamination period, in both experimental mesocosms, and in the reference surrounding seagrass bed. Frozen sediments were lyophilized (BenchTop 3L, VirTis Company Inc.) and sifted through PP sieves with nylon mesh. The different fraction of the sediment corresponding to medium to

very coarse sand (0.5-2 mm), very fine to medium sand (0,625-0,5 mm) and mud (<0,625 mm) (Wentworth 1922) were eluted for 4 hours at room temperature with 30 ml of HCl 1N (Suprapure grade, Merck), according to Townsend et al. (2007). Eluats were then diluted to an appropriate volume of 50 cm^3, centrifuged 10 minutes at 2000 rpm and separated from their remaining culot prior to be analysed. A 4 h extraction time in HCl 1N ensures the removal of the available anthropogenic pollutants (i.e. TEs injected in mesocosms) while not favouring the extraction of natural geogenic metals (Snape et al. 2004), and is preferred to a total HF sediment digestion. TE levels measured in the 3 size fractions of dry sifted sediments were balanced by their respective percentage to calculate total sediment (< 2 mm) TE levels.

2.6. Trace element analysis

TE analyses were carried out by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). This instrument uses ion-molecule reactions to overcome spectral overlaps and requires selection of the appropriate reaction gas (Olesik and Jones 2006): no reaction gas in standard mode (for ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{208}Pb and ^{209}Bi), NH_3 (for ^{27}Al, ^{51}V, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu and ^{66}Zn) or H_2 (for ^{75}As) in DRC modes.

Analytical accuracy was checked by analysing Certified Reference Materials (Table 3) from the Institute for Reference Materials and Measurements (IRMM; Commission of the European Communities): BCR 60 (*Lagarosiphon major*; aquatic plant) and BCR 62 (*Olea europaea*; olives leaves); Institute of Geophysical and Geochemical Exploration (IGGE; Langfang, China): GBW 07603 (brush branch and leaves, aquatic and terrestrial biological products); National Institute of Agronomic Research (INRA; France): V463 (maize); and National Research Council Canada (NRCC; Ottawa, Ontario, Canada): PACS-2 (harbour sediments). PACS-2 TE concentrations were compared to Townsend et al. (2007) mean values obtained from as many as 25 individual HCl 1M elutions over a 5 year period.

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. Concentrations below mean limits are highlighted in the text or in Tables and Annex A.

Table 3.

Evaluation of analytical accuracy through certified reference materials (CRMs). Italic values represent indicative values of CRMs; n represents number of replicates. PACS-2 CRM values are from Towsend et al. (2007).

	As	Al	V	Cr	Fe	Mn	Co	Ni
CRM values								
BCR 60	*8*	4180 ± 120	*6*	*26*	*2380*	1760 ± 60	*4*	*40*
BCR 62	*0,2*	448 ± 18	*1*	*2*	*280*	57,0 ± 2,4	*0,2*	*8*
GBW 07603	1,25 ± 0,15	2000 ± 300	2,40 ± 0,40	2,6 ± 0,2	1070 ± 57	61 ± 5	0,41 ± 0,05	1,7 ± 0,3
V463		*172 ± 13*		*3,37 ± 0,61*	*366 ± 25*	*24,87 ± 0,78*	*0,18 ± 0,06*	*3,37 ± 0,18*
PACS-2	*14 ± 2*	*3400 ± 330*	*38 ± 5*	*12 ± 1*	*8260 ± 570*	*53 ± 4*	*2,8 ± 0,2*	*9 ± 0,9*
Our values								
BCR 60 (n=11)	6,4 ± 0,2	1823 ± 443	4,4 ± 0,3	17,5 ± 2,5	1913 ± 88	1571 ± 126	4,2 ± 0,1	45,2 ± 2,7
BCR 62 (n=11)	0,20 ± 0,02	370 ± 29	0,82 ± 0,05	1,8 ± 0,5	302 ± 21	55 ± 3	0,34 ± 0,07	7,5 ± 1,9
GBW 07603 (n=18	1,33 ± 0,10	1189 ± 224	2,0 ± 0,2	1,8 ± 0,1	963 ± 34	65 ± 2	0,59 ± 0,09	5,7 ± 2,1
V463 (n=13)	0,11 ± 0,02	129 ± 21	0,27 ± 0,03	1,63 ± 0,34	348 ± 23	24 ± 1	0,14 ± 0,02	3,1 ± 1,0
PACS-2 (n=12)	*8,6 ± 3,0*	*3710 ± 230*	*35 ± 1*	*12,7 ± 0,5*	*9685 ± 464*	*64 ± 4*	*2,6 ± 0,1*	*8,4 ± 0,4*

	Cu	Zn	Mo	Ag	Cd	Pb	Bi
CRM values							
BCR 60	51,2 ± 1,9	313 ± 8	*2*	*0,2*	2,20 ± 0,10	64 ± 4	
BCR 62	46,6 ± 1,8	16,0 ± 0,7	*0,2*	*0,2*	0,10 ± 0,02	25,0 ± 1,5	
GBW 07603	6,6 ± 0,8	55 ± 4	0,28 ± 0,05	0,049 ± 0,007	*0,38*	47 ± 3	0,023 ± 0,005
V463	*4,72 ± 0,54*	*61,19 ± 1,39*	*0,83 ± 0,08*		*1,66 ± 0,32*		
PACS-2	*215 ± 20*	*293 ± 18*	*1,9 ± 0,2*		*1,9 ± 0,2*	*141 ± 13*	
Our values							
BCR 60 (n=11)	52,1 ± 5,5	299 ± 13	0,89 ± 0,04	0,279 ± 0,025	2,10 ± 0,06	62 ± 1	0,440 ± 0,046
BCR 62 (n=11)	44,6 ± 3,6	15,5 ± 0,9	0,20 ± 0,04	0,026 ± 0,011	0,09 ± 0,01	25 ± 1	0,046 ± 0,013
GBW 07603 (n=18	9,3 ± 3,9	56 ± 2	0,35 ± 0,04	0,057 ± 0,004	0,80 ± 0,08	50 ± 1	0,031 ± 0,007
V463 (n=13)	4,9 ± 0,9	55 ± 3	0,48 ± 0,06	0,029 ± 0,003	1,50 ± 0,14	13 ± 9	0,123 ± 0,034
PACS-2 (n=12)	*169 ± 13*	*273 ± 7*	*1,67 ± 0,38*	*0,23 ± 0,11*	*1,84 ± 0,11*	*155 ± 5*	0,219 ± 0,025

2.7. Statistical and mathematical analysis

Significant differences between initial (before contaminations) and final (at the end of contamination periods) TE concentrations in epiphytes or sediments were highlighted through parametric one-way analysis of variance (ANOVA) followed by Tukey HSD pairwise comparison test of means ($p < 0.05$), after testing for 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 encountered, followed by Dunn pairwise comparison test of means ($p < 0.05$) (Zar 1984).

Different models were investigated to describe the uptake and loss kinetics of the 15 studied TEs by *P. oceanica* shoots, intermediate and adult leaves. Uptake kinetics were described using either a linear regression, an exponentially increasing function, a logarithmically increasing function or a one-phase association function. Loss kinetics were described using either a linear regression, an exponentially decreasing function, or a logarithmically decreasing function. Equations of the different models are: linear regressions: $y = c_1 * x + c_2$ and logarithmic functions: $y = c_1 * \log(x) + c_2$, where c_1 = slope and c_2 = y0; exponential functions: $y = c_1 + c_2 * e^{c3*x}$ and one-phase association functions: $y = c_2 + (c_1 - c_2) * (1 - e^{-c3*x})$, where c_1 = plateau, c_2 = y0 and c_3 = rate constant.

To compare the relative goodness of fit of the different models and to elect the one that best describes the uptake or loss kinetics of each TE by *P. oceanica* shoots and leaves, an Akaike information criterion (AIC) analysis was performed. The AIC expresses the probability that each of the intercompared models is correct. A graphical examination of residuals, evenly distributed above and below zero, further confirmed that each elected function was relevant. In addition, F-statistics tested the overall significance of the regression model (p-values) (Zar 1984).

For the moderate contamination, control shoots (T0) taken a few meters beside the mesocosm were not incorporated in the uptake modelling, as some TEs (Fe, V, As, Al, Mo and Mn) displayed a heterogeneity of their concentrations at small-spatial scale of the same order of magnitude than their uptake kinetic. Contamination factors (ratio between final and initial TE concentrations in *P. oceanica* shoots and leaves) were calculated at the end of both contamination periods, and the total amount of each TE trapped per m^2 of seagrass meadow at

both contamination levels was estimated. In the particular case of rhizomes, showing little significant evolutions of their TE concentrations on the whole, only the linear regression model was applied distinctly for both the contamination and decontamination phases, and for the entire period of the experiment as well.

Mathematical and statistical data treatment was done with Excel (Microsoft, Inc.), STATISTICA (Statsoft, Inc.), GraphPad Prism (GraphPad Software, Inc.) and MATLAB (The MathWorks, Inc.) softwares.

3. Results

3.1. Continuous field survey

3.1.1. Posidonia oceanica compartmentalization

In natural environmental conditions, V, Cr, Mn, Co, As, Mo, Cd and Pb are preferentially accumulated in *P. oceanica* leaves, while rhizomes concentrate more Al, Fe, Ni, Cu, Zn, Ag and Bi. Furthermore, adult leaves concentrate more Al, V, Cr, Mn, Co, Pb and Bi, while intermediate leaves concentrate more Cu, Ag and Cd (differences > 10 %). Both leaf types show similar levels of Fe, Ni, Zn, As and Mo (differences < 10 %). These general annual trends synthetize observations obtained from the present study and from 9 supplementary sampling campaigns realized in the Calvi Bay between years 2008 and 1010, and give standard reference levels in *P. oceanica* leaves and rhizomes for the Northwestern Mediterranean (Table 4A).

Table 4.

A) Mean annual TE concentrations (mean ± SD; n=166) for the Calvi Bay in *P. oceanica* intermediate (IL) and adult (AL) leaves, in integral shoots and in rhizomes (Rz). B) TE concentrations (mean ± SD) in epiphytes scraped from uncontaminated *P. oceanica* shoots (T0; n = 22) and from shoots sampled at the end of the moderate (Tf mod.; n = 4) or acute (Tf acute; n = 4) contamination periods. C) TE concentrations (mean ± SD; n = 6) in mud (<0.625 mm), in very fine to medium (0.625-0.5 mm) or medium to very coarse sands (0.5-2mm), and calculated for total sediment (< 2 mm) of the control *P. oceanica* bed. Concentrations are expressed as mean ± SD in $\mu g.g_{DW}^{-1}$. Letters represent significant differences between sediment fractions, epiphytes or *P. oceanica* compartments. *, ** and struck-through values represent concentrations < L_Q, < L_D and < L_C, respectively.

TE	**A.** *P. oceanica*				**B.** Epiphytes			**C.** Sediments			
	IL	AL	Shoots	Rz	T0	Tf mod.	Tf acute	<0.625	0.625-0.5	0.5-2	<2
As	a 1,53 ± 0,65	a 1,47 ± 0,55	a 1,50 ± 0,56	b 0,84 ± 0,29	a 6,59 ± 1,27	a 4,46 ± 1,15	b 11,19 ± 5,38	a 9,97 ± 1,40	**ab 2,10 ± 0,34	*b 1,76 ± 0,14	*ab 1,95 ± 0,17
Al	a 29 ± 10	a 40 ± 11	a 39 ± 12	b 121 ± 78	a 778 ± 359	a 668 ± 181	b 1616 ± 365	a 527 ± 51	b 141 ± 10	b 122 ± 15	b 134 ± 12
V	a 3,37 ± 2,99	a 3,93 ± 2,24	a 3,78 ± 2,38	b 0,63 ± 0,29	a 10,41 ± 3,59	a 8,03 ± 1,84	a 18,84 ± 15,62	a 9,37 ± 0,78	b 1,52 ± 0,15	b 1,33 ± 0,16	b 1,47 ± 0,15
Cr	a 0,14 ± 0,05	b 0,22 ± 0,07	ab 0,19 ± 0,06	ab 0,18 ± 0,07	a 1,25 ± 0,21	b 2,84 ± 0,53	b 11,00 ± 6,16	a 4,27 ± 0,75	ab 2,09 ± 0,27	c 0,86 ± 0,13	bc 1,38 ± 0,13
Fe	a 43 ± 6	a 47 ± 6	a 45 ± 4	b 81 ± 39	a 420 ± 74	b 264 ± 48	c 630 ± 141	a 1055 ± 98	b 261 ± 19	c 215 ± 16	bc 240 ± 17
Mn	a 36,9 ± 9,4	a 42,4 ± 7,5	a 41,0 ± 6,8	b 4,4 ± 1,1	a 29,4 ± 6,0	a 34,2 ± 4,1	a 34,6 ± 1,5	a 32,5 ± 2,6	b 15,4 ± 2,8	c 8,6 ± 1,9	c 11,5 ± 2,0
Co	a 1,31 ± 0,61	a 1,86 ± 0,62	a 1,67 ± 0,52	b 0,17 ± 0,03	a 0,67 ± 0,11	b 1,01 ± 0,12	a 0,78 ± 0,05	a 0,352 ± 0,029	*b 0,079 ± 0,012	**c 0,031 ± 0,021	*bc 0,053 ± 0,014
Ni	a 23,1 ± 5,8	a 23,2 ± 6,6	a 23,1 ± 6,0	b 37,4 ± 9,1	a 10,1 ± 1,5	b 14,0 ± 2,8	a 10,7 ± 0,6	a 4,25 ± 0,51	**b 0,35 ± 0,10	b ~~0,22~~ ± 0,14	**b 0,30 ± 0,11
Cu	a 9,32 ± 3,81	a 7,43 ± 2,22	a 7,92 ± 2,42	a 9,23 ± 2,11	a 3,5 ± 1,4	b 12,6 ± 6,2	b 18,1 ± 6,7	a 7,36 ± 0,85	b 0,80 ± 0,15	c 0,54 ± 0,12	bc 0,70 ± 0,13
Zn	a 67 ± 15	a 72 ± 19	a 70 ± 16	a 78 ± 20	a 110 ± 15	b 219 ± 12	c 167 ± 20	a 20,75 ± 1,02	b 4,57 ± 0,68	c 3,09 ± 0,50	bc 3,81 ± 0,45
Mo	a 1,94 ± 1,02	a 1,93 ± 0,48	a 1,96 ± 0,62	a 1,32 ± 0,61	a 1,23 ± 0,36	a 1,24 ± 0,27	b 79,07 ± 120,55	a 0,387 ± 0,161	*ab 0,052 ± 0,008	*b 0,028 ± 0,006	*b 0,041 ± 0,008
Ag	a 0,87 ± 0,33	a 0,60 ± 0,31	a 0,69 ± 0,29	b 4,91 ± 1,24	a 0,07 ± 0,02	b 7,34 ± 1,67	b 9,30 ± 2,38	a 0,192 ± 0,046	*b 0,012 ± 0,003	*b 0,014 ± 0,007	*b 0,014 ± 0,005
Cd	a 2,32 ± 0,44	a 2,08 ± 0,38	a 2,18 ± 0,41	b 1,34 ± 0,15	a 2,16 ± 0,41	a 2,80 ± 0,92	a 3,03 ± 0,49	a 0,108 ± 0,016	ab 0,025 ± 0,007	b 0,017 ± 0,005	b 0,021 ± 0,004
Pb	a 0,63 ± 0,17	b 0,98 ± 0,20	b 0,87 ± 0,18	c 0,28 ± 0,13	a 3,82 ± 0,80	b 37,61 ± 21,47	b 46,14 ± 31,39	a 18,49 ± 2,14	b 6,34 ± 0,94	c 4,52 ± 0,49	bc 5,34 ± 0,40
Bi	**a 0,0047 ± 0,0015	*b 0,0078 ± 0,0017	*b 0,0070 ± 0,0018	b 0,0105 ± 0,0100	a 0,045 ± 0,021	b 0,349 ± 0,070	b 1,854 ± 2,044	a 1,163 ± 0,244	b 0,082 ± 0,008	ab 0,124 ± 0,033	ab 0,111 ± 0,017

3.1.2. Trace elements in sediments

TE levels measured in the 3 sediment size fractions and calculated in unsieved sediments of the control *P. oceanica* bed (Table 4C) decrease in the following order: Fe > Al > Mn > Pb, Zn > As, Cr, V, Cu > Ni > Bi > Co, Mo > Cd, Ag. Concentrations of most TEs are significantly higher ($p < 0.05$) in the < 0.0625mm mud fraction than in the 2 other ones. As this fraction only represent 0.8 % in average of the superficial sediment < 2 mm at 10 m depth in the studied *P. oceanica* bed in front of STARESO, its participation to the total TE content is insignificant, and TE profiles are similar to the fine to medium or medium to coarse sand fractions (41.3% and 57.9 % of sediment < 2 mm, respectively).

3.2. Trace element in seawater and sediments of mesocosms

In seawater, mean concentrations of 11 dissolved bioavailable TEs measurable with DGTs decrease in the following order (Table 2B): Zn > Fe, Mn > Ni > Cr > Al > Pb > Cu > Cd > Co > Ag in the canopy water of the *P. oceanica* control bed, Zn > Al > Fe > Ni > Pb > Cu > Mn > Cd > Co > Cr > Ag at moderate contamination levels, and Zn > Al > Fe > Cu > Ni > Pb > Mn > Cd > Co > Cr > Ag at acute contamination levels. For V, As, Mo and Bi, concentrations in *P. oceanica* meadow and mesocosm water cannot be calculated, as we do not know their diffusion coefficients through the diffusive gel (Zhang and Davison 1995). Nevertheless, resins effectively accumulate V, Mo and Bi, and concentrations measured in resin eluats can then be compared to calculate concentration ratios (and not absolute seawater concentrations). For As, strong interferences with ArCl recombinants during ICP-MS analysis do not permit to estimate contamination factors. DGT deployment time was too short in the control *P. oceanica* bed to detect low Bi background concentrations. Apart from these 2 cases, all TEs were above LQ.

Most pollutants injected as acid multielemental solutions are not accessible under dissolved or colloidal forms once diluted in the basic seawater environment of mesocosms: bioavailable TEs are 0.8 (Ag) to 31.1 (Fe) % and 0.2 (Ag) to 24.1 (Fe, Al) % the initial injected amount for the moderate and acute contamination levels, respectively (Table 2C). Contamination factors in the mesocosm water, when compared to the control water, range from 1.3 (Cr) to 55.1 (Ag) for the moderate level and from 8.7 (Cr) to 381.2 (Al) for the acute level (Table 2D).

Contrary to seawater, no contamination of superficial sediments was observed in experimental mesocosms. The small quantities of dissolved TEs added in mesocosms (ppb range) are indeed very low compared to the amounts present in sediments ($\mu g.g^{-1}$ range).

3.3. *Posidonia oceanica* trace element uptake kinetics

TE concentrations in *P. oceanica* shoots and tissues at the successive sampling times are synthetized in Annex A. Graphs of the uptake and loss kinetics of the 15 studied TEs by *P. oceanica* shoots, leaves and rhizomes contaminated at both moderate and acute levels are available in Annex B. For clarity purpose, only kinetics parameters are presented under table format (Tables 5, 6) in the following result section, and are illustrated by some selected typical examples.

3.3.1. Moderate contamination

Shoots of *P. oceanica* moderately contaminated accumulate As, Cu (Fig. 3A), Pb and Bi following an exponential model over the contamination time considered; Mo is late exponentially accumulated after 4 days. Al, V, Cr (Fig. 3B), Ag and Cd are accumulated linearly. Only Fe (Fig. 3C), Co (Fig. 3D), and Zn tend to reach a steady state concentration at the end of the 5 contamination days, following a logarithmic model (Table 5). Finally, Ni and Mn concentrations do not significantly evolve ($p > 0,05$). Mean contamination factor between days C1 and C5 (Ni excluded) is 2.6 ± 1.5, with a minimum for Fe, Mn and Co (1.2) and a maximum for Bi (6.1) (Table 5).

Many TEs are differently accumulated by younger intermediate leaves or senescent adult ones. As, Cu (Fig 3A), Cd, Pb, and Bi accumulations follow an exponential model for intermediate leaves, but a slower continuous linear model for adult leaves (except for Cd). c_1 slope of Al, V and Ag linear accumulations are higher for intermediate leaves than for adult leaves. Fe (Fig. 3C), linearly accumulated by intermediate leaves, tends to reach a plateau in adult leaves. V is late exponentially accumulated by adult leaves, and linearly by intermediate leaves. Mo is exponentially accumulated after 4 days by both leaf types. Finally, Mn, Co (Fig. 3D) and Zn accumulated by intermediate leaves are not significantly uptaken ($p > 0,05$) by adult leaves, and both leaf types do not accumulate Ni. Mean contamination factor between days C1 and C5 (Ni excluded) is 3.0 ± 1.7 for intermediate leaves (min. = 1.2 for Fe; max. =

7.4 for Bi) and 2.0 ± 0.9 for adult leaves (min. = 0.95 for Cd; max. = 3.7 for Bi), as a result of the different uptake kinetics displayed by both leaf types (Table 5).

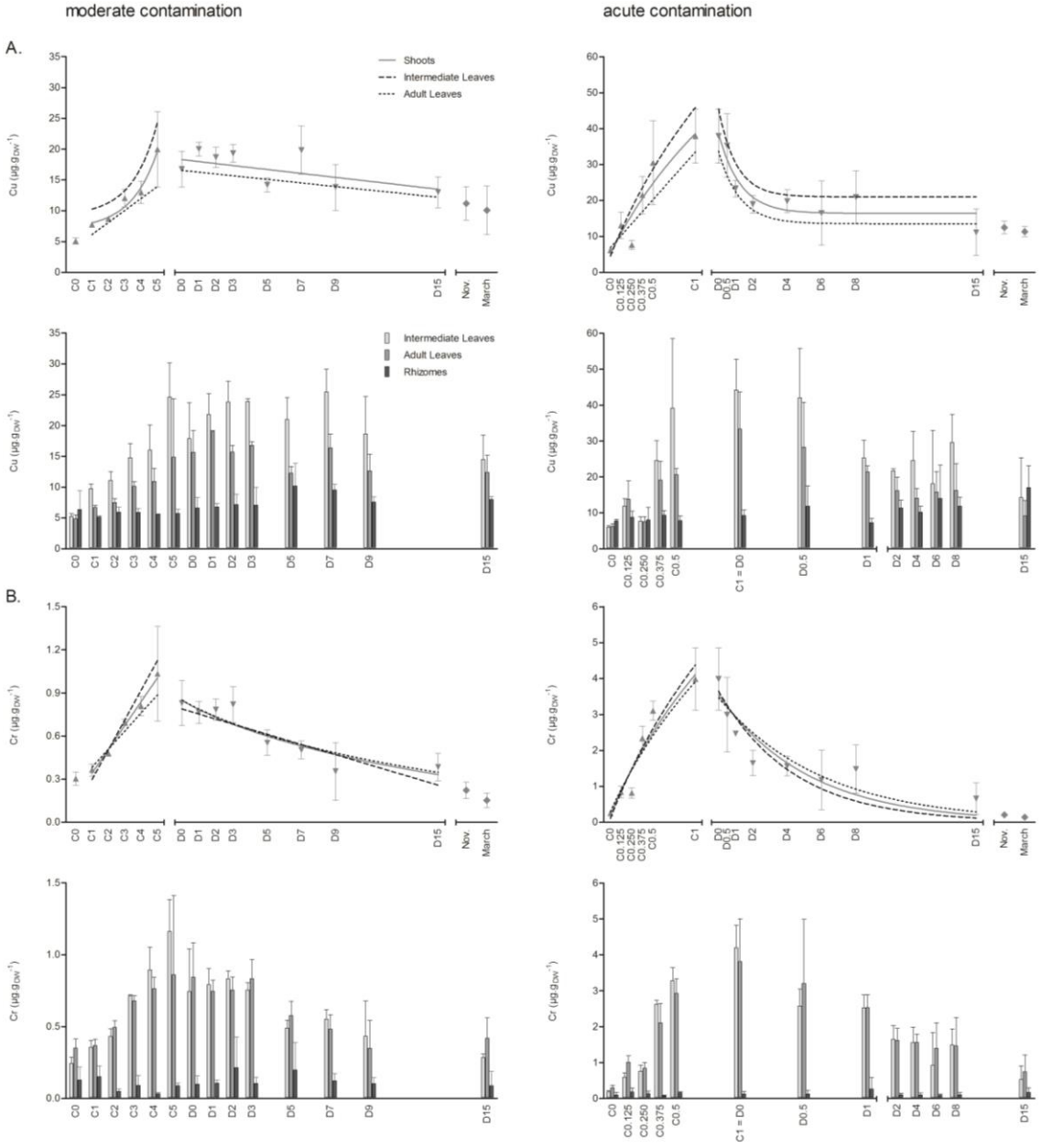

Fig 3. Uptake and loss kinetic models of A) Cu, B) Cr, C) Fe and D) Co in *P. oceanica* shoots (——), intermediate leaves (IL, – – –) and adult leaves (AL, ...) contaminated at moderate (left) or acute (right) levels. For clarity purpose, only concentrations calculated for shoots were indicated on kinetic graphs (▲ uptake, ▼ loss, ◆ November and March post-controls). Histograms of A) Cu and B) Cr tissue compartmentalization between intermediate leaves (light grey ▌), adult leaves (medium grey ▌) and rhizomes (dark grey ▌) during the contamination and decontamination periods at moderate (left) or acute (right) levels are shown beneath their corresponding kinetic graphs. Legends of kinetics and tissue compartmentalizations are given on Cu left graphs as well. On the temporal X-axis, contamination periods C0 to C5 (moderate level) or C0 to C1 (acute level) and decontamination periods D0 to D15 (both levels) are given in days. TE concentrations are expressed in µg.g^{-1} of dry weight. Double bars symbolize standard deviations.

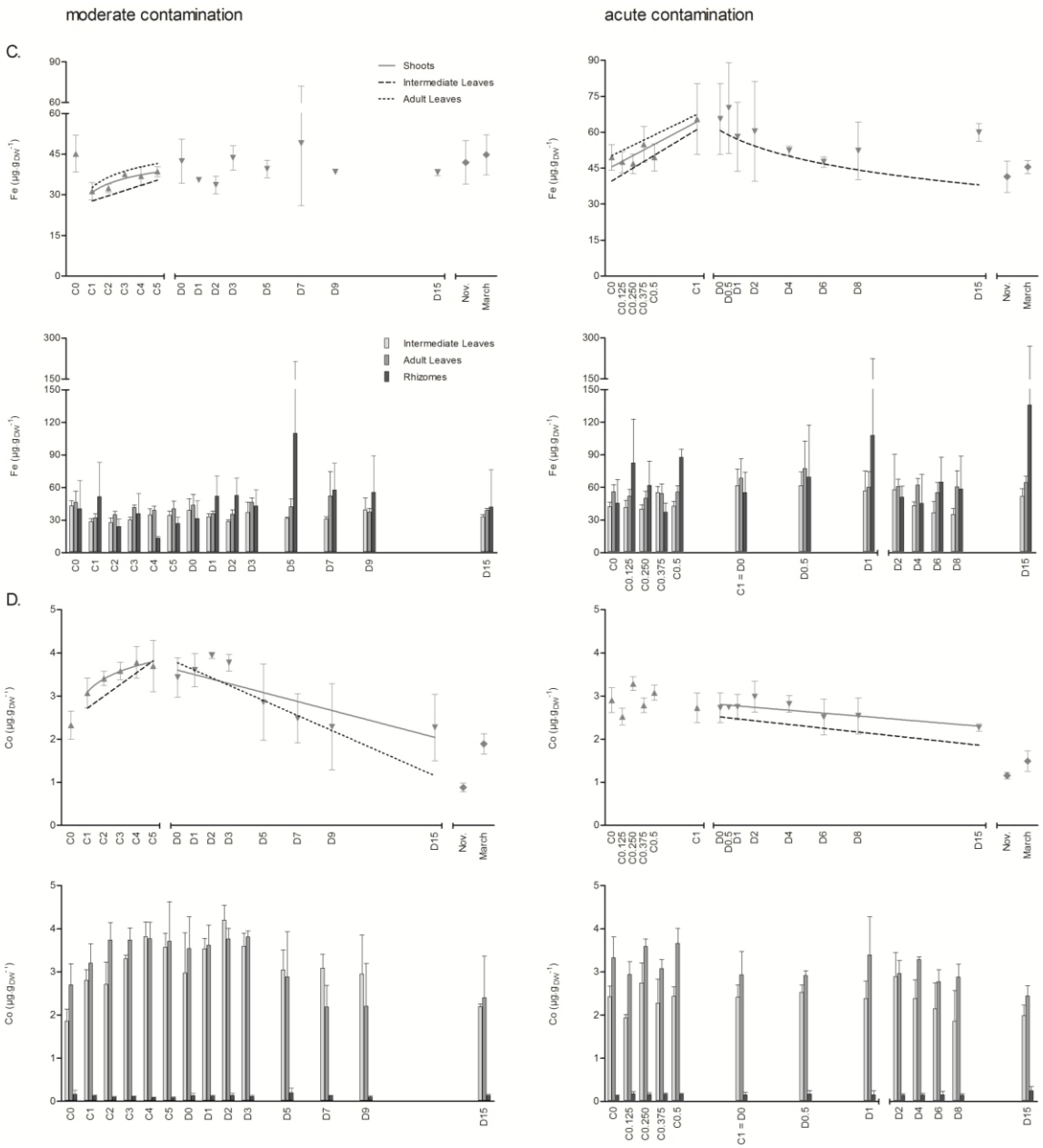

Fig 3 (*Continued*). Uptake and loss kinetic models of A) Cu, B) Cr, C) Fe and D) Co in *P. oceanica* shoots (____), intermediate leaves (IL, _ _ _) and adult leaves (AL, ...) contaminated at moderate (left) or acute (right) levels. For clarity purpose, only concentrations calculated for shoots were indicated on kinetic graphs (▲ uptake, ▼ loss, ◆ November and March post-controls). Histograms of C) Fe and D) Co tissue compartmentalization between intermediate leaves (light grey ▌), adult leaves (medium grey ▌) and rhizomes (dark grey ▌) during the contamination and decontamination periods at moderate (left) or acute (right) levels are shown beneath their corresponding kinetic graphs. Legends of kinetics and tissue compartmentalizations are given on Cu left graphs as well. On the temporal X-axis, contamination periods C0 to C5 (moderate level) or C0 to C1 (acute level) and decontamination periods D0 to D15 (both levels) are given in days. TE concentrations are expressed in µg.g^{-1} of dry weight. Double bars symbolize standard deviations.

Table 5.

Elected models and corresponding equation parameters for the A) uptake and B) loss kinetics of the 15 investigated TEs by *P. oceanica* shoots (Sh), intermediate leaves (IL) and adult leaves (AL). c_1 = slope and c_2 = y0 of linear (lin) regressions and logarithmic (log) functions. c_1 = plateau, c_2 = y0 and c_3 = rate constant of exponential (exp) and one-phase association (1-ph asso) functions. Fitting parameters are indicated (r^2 and p-levels). Contamination factors (c.f.) at the end of both contamination periods and total amounts of TEs trapped per m^2 of seagrass bed are given. Constrains applied to some TE loss kinetic models are detailed.

A. Moderate contamination: uptake kinetics (n=16)

Shoots	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	expo	0,9003	0,03395	0,7588	0,8901	<0,001	2,4	1,3
Al	lin	2,833	9,249	-	0,5385	<0,01	1,9	26,5
V	lin	1,175	0,8549	-	0,4471	<0,01	2,9	3,9
Cr	lin	0,1657	0,1765	-	0,7742	<0,001	2,8	0,20
Fe	log	10,86	30,8	-	0,5635	<0,001	1,2	20,0
Mn	lin	1,631	44,08	-	0,1876	n.s.	1,2	17,5
Co	log	1,006	3,094	-	0,382	<0,05	1,2	0,72
Ni	lin	-0,6308	38,88	-	0,08725	n.s.	0,88	7,2
Cu	expo	7,015	0,5512	0,6271	0,7316	<0,001	2,6	7,3
Zn	log	77,47	140,4	-	0,6178	<0,001	1,4	34,7
Mo	expo	1,363	2,923E-06	2,638	0,7848	<0,001	2,2	1,5
Ag	lin	1,841	-0,2676	-	0,704	<0,001	4,6	1,1
Cd	lin	0,3004	3,094	-	0,8089	<0,001	1,4	1,1
Pb	expo	1,325	0,9725	0,4607	0,82	<0,001	3,9	1,2
Bi	expo	0,00907	0,04328	0,4622	0,8139	<0,001	6,1	0,015
Inter. leaves	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	expo	0,7721	0,01134	1,009	0,9128	<0,001	3,2	1,7
Al	lin	2,994	2,321	-	0,5401	<0,01	2,5	26,2
V	lin	1,405	0,4978	-	0,4505	<0,01	3,4	4,1
Cr	lin	0,207	0,09085	-	0,8632	<0,001	3,3	0,16
Fe	lin	1,921	25,8	-	0,3629	<0,05	1,2	18,5
Mn	expo	41,32	0,06347	1,076	0,7241	<0,001	1,3	16,9
Co	lin	0,2735	2,448	-	0,5441	<0,01	1,3	0,59
Ni	lin	-0,9112	39,72	-	0,1067	n.s.	0,79	6,5
Cu	expo	9,163	0,57	0,6567	0,7462	<0,001	2,5	8,4
Zn	lin	28,01	96,68	-	0,8689	<0,001	1,8	43,1
Mo	expo	1,245	1,779E-05	2,285	0,9105	<0,001	2,2	1,5
Ag	lin	2,416	-0,4468	-	0,7202	<0,001	4,8	1,5
Cd	expo	3,008	0,3065	0,4712	0,8424	<0,001	1,8	1,5
Pb	expo	1,595	1,056	0,5147	0,8496	<0,001	4,7	1,0
Bi	expo	0,04298	0,03361	0,5853	0,8232	<0,001	7,4	0,012
Adult leaves	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	lin	0,275	0,6725	-	0,4157	<0,01	1,9	1,0
Al	lin	2,955	13,15	-	0,2855	<0,05	1,7	25,3
V	expo	3,18	1,502E-05	2,522	0,5235	<0,01	2,4	3,4
Cr	lin	0,1259	0,2572	-	0,4348	<0,01	2,3	0,18
Fe	log	12,67	32,63	-	0,4004	<0,01	1,3	20,9
Mn	log	3,748	49,22	-	0,01337	n.s.	1,1	16,7
Co	log	0,7281	3,336	-	0,137	n.s.	1,2	0,77
Ni	lin	-0,4605	38,41	-	0,04137	n.s.	0,92	7,6
Cu	lin	1,946	4,182	-	0,3686	<0,05	2,2	5,9
Zn	log	18,13	156,1	-	0,03795	n.s.	1,1	26,9
Mo	expo	1,41	1,279E-06	2,776	0,4956	<0,05	2,1	1,4
Ag	lin	1,209	0,4605	-	0,3831	<0,05	3,6	0,76
Cd	lin	-0,02659	3,655	-	0,01616	n.s.	0,95	0,71
Pb	lin	1,082	1,399	-	0,4323	<0,01	2,6	0,91
Bi	lin	0,04636	0,01502	-	0,4455	<0,01	3,7	0,010

Acute contamination: uptake kinetics (n=22, except for Pb in Sh and AL: n=21)

Shoots	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	log	467,1	-7,114	-	0,281	<0,05	88,9	47,7
Al	log	98,78	27,04	-	0,4016	<0,01	2,0	27,9
V	log	92,6	8,307	-	0,2145	<0,05	9,1	12,3
Cr	log	12,94	0,2186	-	0,8504	<0,001	15,5	1,1
Fe	lin	18,83	26,74	-	0,3821	<0,01	1,3	21,4
Mn	log	-21,43	50,01	-	0,1411	n.s.	0,86	12,5
Co	lin	-0,1399	3,063	-	0,02693	n.s.	0,94	0,56
Ni	lin	-4,541	37,54	-	0,2205	<0,05	0,85	7,0
Cu	log	110,7	5,246	-	0,7611	<0,001	6,1	17,3
Zn	1-ph asso	140,1	-1,7E+04	5,842	0,7159	<0,001	1,5	37,0
Mo	log	402,8	104,4	-	0,03269	n.s.	89,8	62,9
Ag	1-ph asso	10,06	-231,5	3,217	0,5589	<0,001	18,3	4,5
Cd	1-ph asso	4,016	-458,5	5,8	0,3446	<0,05	1,4	1,1
Pb	log	123	8,942	-	0,2047	<0,05	39,1	12,1
Bi	log	8,454	-0,07898	-	0,3463	<0,01	419,9	1,0
Inter. leaves	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	log	649	3,439	-	0,1649	n.s.	126,5	69,0
Al	lin	27,55	-10,05	-	0,5125	<0,001	2,4	24,3
V	log	130,8	6,601	-	0,2273	<0,05	10,6	12,8
Cr	log	14,19	0,1083	-	0,8862	<0,001	21,4	1,1
Fe	lin	21,48	18,18	-	0,4187	<0,01	1,5	22,6
Mn	log	-8,485	43,5	-	0,01605	n.s.	0,92	12,1
Co	log	0,363	2,329	-	0,01277	n.s.	1,0	0,46
Ni	lin	-0,2676	27,92	-	0,000624	n.s.	1,0	8,0
Cu	log	137,1	4,547	-	0,7221	<0,001	7,3	24,3
Zn	1-ph asso	145,8	-6802	4,559	0,8058	<0,001	1,8	43,4
Mo	log	567,7	128,3	-	0,02056	n.s.	116,4	80,5
Ag	1-ph asso	10,6	-596,2	4,02	0,5745	<0,001	14,2	4,4
Cd	1-ph asso	4,97	-1003	6,02	0,3413	<0,05	1,6	1,3
Pb	log	210,7	3,039	-	0,3275	<0.01	73,0	16,3
Bi	log	12,09	-0,06081	-	0,2834	<0,05	1685,5	2,8
Adult leaves	model	c_1	c_2	c_3	r^2	p	c. f.	TE ($g.m^{-2}$)
As	lin	98,19	-104,1	-	0,2318	<0.05	62,4	32,8
Al	log	106,3	35,56	-	0,2538	<0,05	1,8	26,2
V	log	67,15	10,29	-	0,1071	n.s.	8,3	11,6
Cr	log	12	0,2949	-	0,7667	<0,001	12,3	1,0
Fe	lin	17,2	33,06	-	0,2719	<0,05	1,2	20,4
Mn	log	-32,34	55,12	-	0,2545	<0,05	0,80	12,1
Co	lin	-0,3384	3,708	-	0,07856	n.s.	0,88	0,58
Ni	lin	-7,978	45,14	-	0,3973	<0,01	0,76	6,3
Cu	lin	26,57	-19,67	-	0,709	<0,001	5,3	14,0
Zn	1-ph asso	135,8	-3,3E+05	9,29	0,3782	<0,05	1,3	32,2
Mo	log	288,8	105,6	-	0,01649	n.s.	72,2	49,8
Ag	log	25,7	1,902	-	0,3941	<0,01	25,4	5,4
Cd	log	1,616	2,885	-	0,1016	n.s.	1,2	0,92
Pb	log	59,57	13,26	-	0,05149	n.s.	22,5	7,9
Bi	log	6,137	-0,04128	-	0,2094	<0,05	189,3	0,52

B. Moderate contamination: loss kinetics (IL: n=26, Sh and AL: n=30, except for V and Mo: n=22-26)

Shoots	model	c_1	c_2	c_3	r^2	p	constrains
As	expo	0,8277	5,208	-0,3522	0,4009	<0,001	
Al	log	-4,964	39,04	-	0,003641	n.s.	
V	expo	2,28	2,3E+04	-1,648	0,21	n.s.	x = D15 excluded
Cr	log	-0,9486	1,587	-	0,6583	<0,001	
Fe	lin	-0,05203	40,92	-	0,0009441	n.s.	
Mn	log	-29,73	68,29	-	0,3371	<0,001	
Co	lin	-0,104	4,225	-	0,4237	<0,001	
Ni	lin	-0,6886	37,73	-	0,3463	<0,001	
Cu	lin	-0,3206	20,21	-	0,2215	<0,01	
Zn	lin	-4,74	234,4	-	0,296	<0,01	
Mo	expo	1,364	52,68	-0,6645	0,5447	<0,001	x = D7, D15 excluded
Ag	lin	-0,1874	9,979	-	0,1553	<0,05	
Cd	lin	-0,1332	5,528	-	0,4273	<0,001	
Pb	expo	1,642	54,21	-0,2574	0,7017	<0,001	
Bi	lin	-0,01746	0,5018	-	0,4388	<0,001	
Inter. leaves	model	c_1	c_2	c_3	r^2	p	constrains
As	log	-1,042	2,099	-	0,1914	<0,5	
Al	log	-13,46	37,26	-	0,03065	n.s.	
V	log	-0,4176	2,635	-	0,006472	n.s.	x = D15 excluded
Cr	lin	-0,03537	1,001	-	0,4193	<0,001	
Fe	log	-7,89	42,92	-	0,03186	n.s.	
Mn	lin	-0,5043	43,14	-	0,05219	n.s.	
Co	lin	-0,06011	3,794	-	0,1209	n.s.	
Ni	lin	-0,2873	35,6	-	0,05	n.s.	
Cu	lin	-0,1627	22,05	-	0,02035	n.s.	
Zn	lin	-0,9044	245,9	-	0,006871	n.s.	
Mo	log	-2,403	3,889	-	0,2258	<0,05	x = D7, D15 excluded
Ag	log	1,125	8,805	-	0,003324	n.s.	
Cd	lin	-0,08353	5,945	-	0,0754	n.s.	
Pb	log	-25,95	35,64	-	0,3914	<0,001	
Bi	lin	-0,01183	0,5775	-	0,08012	n.s.	
Adult leaves	model	c_1	c_2	c_3	r^2	p	constrains
As	expo	0,8213	6,367	-0,3777	0,3502	<0,01	
Al	log	-13,72	52,2	-	0,01963	n.s.	
V	expo	2,334	9,2E+04	-1,829	0,1901	n.s.	x = D15 excluded
Cr	log	-0,9233	1,568	-	0,531	<0,001	
Fe	lin	-0,1084	43,34	-	0,003386	n.s.	
Mn	log	-33,93	73,33	-	0,3303	<0,001	
Co	lin	-0,1747	4,82	-	0,413	<0,001	
Ni	lin	-0,715	37,56	-	0,3237	<0,01	
Cu	lin	-0,2909	18,28	-	0,2366	<0,01	
Zn	log	-107,6	267,6	-	0,3456	<0,001	
Mo	expo	1,422	120,1	-0,7927	0,559	<0,001	x = D7, D15 excluded
Ag	lin	-0,1451	8,832	-	0,05668	n.s.	
Cd	lin	-0,1143	4,847	-	0,5128	<0,001	
Pb	expo	1,929	92,44	-0,3871	0,7968	<0,001	
Bi	lin	-0,01461	0,4044	-	0,5555	<0,001	

Acute contamination: loss kinetics (n=29, except for Mn: n=26 and Pb in Sh and AL: n=25)

Shoots	model	c_1	c_2	c_3	r^2	p	constrains
As	expo	1,925	1,4E+04	-1,553	0,3106	<0,01	c1 = 1.925
Al	log	-23,15	66,61	-	0,09668	n.s.	
V	expo	2,939	8,4E+04	-2,612	0,4807	<0.001	
Cr	expo	-	6,293	-0,191	0,6563	<0,001	without plateau
Fe	log	-17,94	73,15	-	0,1149	n.s.	
Mn	log	-16,66	50,44	-	0,2611	<0,01	x = D15 excluded
Co	lin	-0,03356	2,906	-	0,237	<0.01	
Ni	lin	-0,3394	31,83	-	0,1301	n.s.	
Cu	expo	16,37	242,8	-0,7952	0,6568	<0,001	
Zn	expo	118,1	4,5E+05	-3,385	0,3104	<0,01	
Mo	expo	2,207	1,4E+05	-2,213	0,379	<0,001	c1 = 2,207
Ag	lin	-0,4096	11,65	-	0,2993	<0,01	
Cd	expo	2,995	34,33	-1,268	0,337	<0,01	
Pb	expo	2,222	1545	-1,204	0,4767	<0,001	c1 = 2,222
Bi	expo	0,0778	50,87	-1,011	0,3716	<0,001	c1 = 0.0778
Inter. leaves	model	c_1	c_2	c_3	r^2	p	constrains
As	expo	1,638	5,2E+05	-2,636	0,2106	<0,05	c1 = 1,638
Al	log	-42,06	66,87	-	0,2706	<0,01	
V	expo	2,784	5,1E+06	-3,898	0,5273	<0,001	
Cr	expo	-	7,216	-0,2273	0,7128	<0,001	without plateau
Fe	log	-29,1	74,59	-	0,2075	<0,05	
Mn	lin	-1,508	45,21	-	0,2738	<0,01	x = D15 excluded
Co	lin	-0,04366	2,646	-	0,1985	<0,05	
Ni	lin	-0,37	31,47	-	0,09331	n.s.	
Cu	expo	20,99	430,4	-0,956	0,5201	<0,001	
Zn	expo	110,5	1,6E+05	-2,874	0,4005	<0,01	
Mo	expo	2,068	5,1E+05	-2,576	0,412	<0,001	c1 = 2,068
Ag	lin	-0,3496	12,39	-	0,1478	<0,05	
Cd	expo	3,318	36,23	-1,15	0,294	<0,05	
Pb	expo	2,063	2,5E+04	-2,013	0,5072	<0,001	c1 = 2,063
Bi	expo	0,0954	548,2	-1,689	0,4109	<0,001	c1 = 0,0954
Adult leaves	model	c_1	c_2	c_3	r^2	p	constrains
As	expo	2,099	1914	-0,9877	0,236	<0,01	c1 = 2.099
Al	log	-13,16	69,99	-	0,02039	n.s.	
V	expo	3,132	7699	-1,882	0,3325	<0,01	
Cr	expo	-	5,697	-0,1654	0,5203	<0,001	without plateau
Fe	log	-13,17	74,98	-	0,05515	n.s.	
Mn	log	-15,28	51,06	-	0,1992	<0,05	x = D15 excluded
Co	lin	-0,03504	3,191	-	0,1339	n.s.	
Ni	lin	-0,3574	32,49	-	0,1226	n.s.	
Cu	expo	13,54	243	-0,8291	0,5692	<0,001	
Zn	lin	-1,065	134,2	-	0,1105	n.s.	
Mo	expo	0,7837	3,8E+04	-1,855	0,3145	<0,01	
Ag	log	-7,301	13,3	-	0,2577	<0,01	
Cd	expo	2,716	1351	-2,591	0,3927	<0,01	
Pb	expo	2,989	312,8	-0,7923	0,369	<0,01	
Bi	expo	0,069	14,33	-0,6802	0,2279	<0,01	c1 = 0.069

Rhizome response to moderate contamination is unexpected (Fig. 4). This below-ground tissue do not accumulate pollutants; *a contrario*, their TE contents tend to decrease (slopes significantly negative for As, Co, Ni; $p < 0.05$) during the contamination period, except for Cu, Ag and Bi (Table 6).

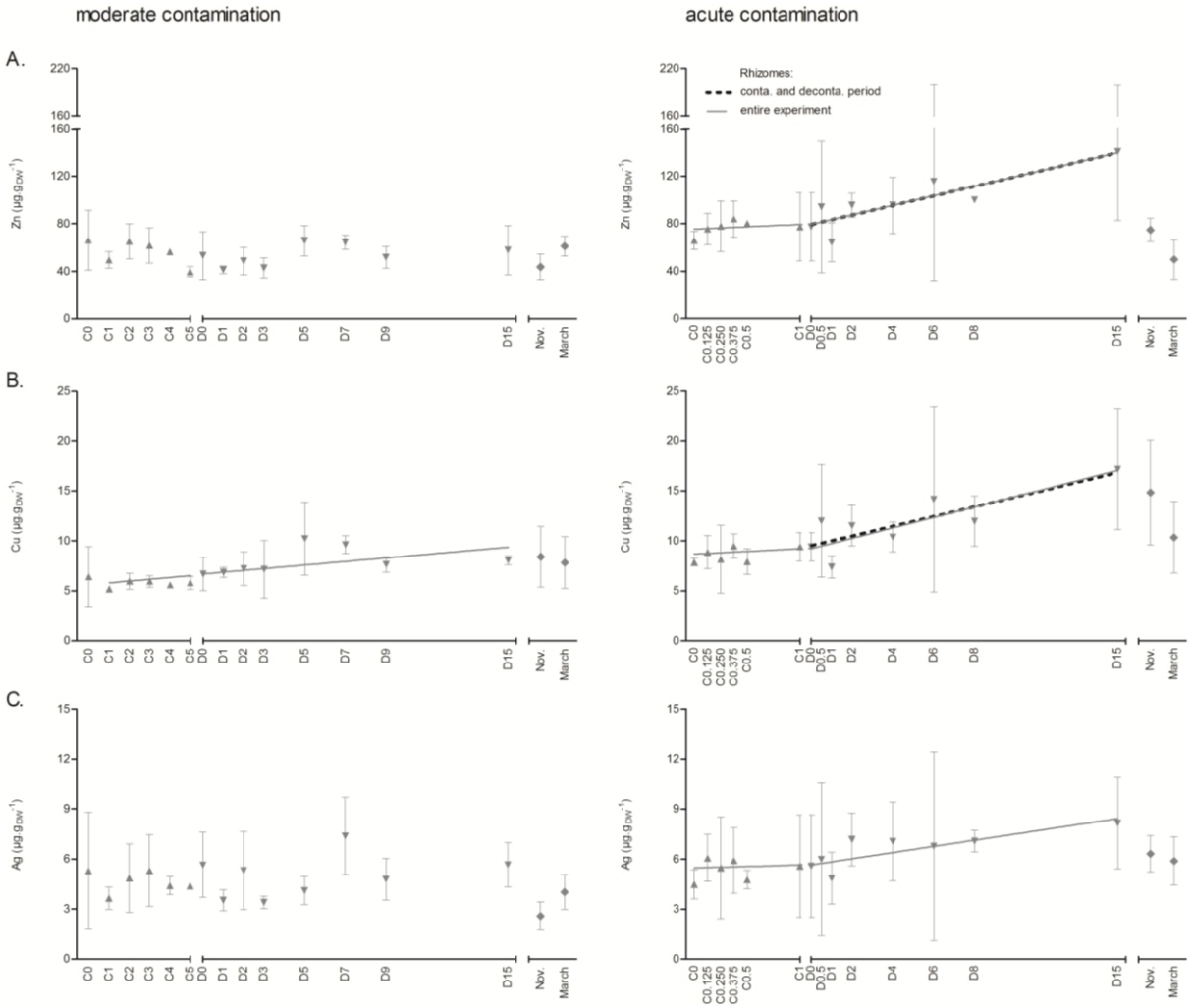

Fig. 4. Kinetics of A) Zn, B) Cu and C) Ag in rhizomes of *P. oceanica* shoots contaminated at moderate (left) or acute (right) levels. Dotted black thick lines (▪▪▪▪) represent linear kinetics observed during the decontamination period; continuous grey thin lines (——) models linear kinetics of the rhizome TE concentration evolution during the entire experiments (i.e. contamination and decontamination periods together). Other symbols and axes are the same than in Figure 3.

Table 6.

Parameters of linear regressions modelling the evolution of the 15 TE concentrations in rhizomes of *P. oceanica* shoots contaminated at A) moderate and B) acute levels. Linear models are applied distinctly to both the contamination "C" and decontamination "D" phases, and to the entire period of the experiments "CD". c_1 = slope and c_2 = y0 of linear regressions. Fitting parameters are indicated (r^2 and p-levels). D15 was excluded from the modelling of Co evolution in the acute experiment, as well as C1=D0 for Mo and C0 and D1 for Bi.

	C (n=14, except for Pb: n=13 and Bi: n=13)				D (n=27, except for Fe: n=26 and Bi: n=25)				CD (n=41, except for Fe: n=40, Pb: n=40 and Bi: n=38)			
A. Moderate	c_1	c_2	r^2	p	c_1	c_2	r^2	p	c_1	c_2	r^2	p
As	-0,0871	0,8666	0,3602	0,0233	-0,0028	0,7145	0,0015	0,8469	0,0007	0,6535	0,0001	0,9430
Al	-6,0380	61,310	0,0757	0,3412	4,3000	45,350	0,0850	0,1401	4,8740	35,710	0,1676	0,0079
V	-0,0589	0,4895	0,0727	0,3512	0,0319	0,3283	0,0571	0,2300	0,0357	0,2607	0,1129	0,0317
Cr	-0,0127	0,1256	0,1057	0,2567	0,0009	0,1205	0,0028	0,7922	0,0026	0,0951	0,0323	0,2607
Fe	-5,5760	48,190	0,1800	0,1306	1,5860	37,450	0,0340	0,3676	2,0210	30,410	0,0883	0,0626
Mn	-0,1154	2,9550	0,0690	0,3642	0,0746	2,7810	0,1367	0,0577	0,0919	2,5080	0,2608	0,0006
Co	-0,0093	0,1382	0,0026	0,0053	0,0000	0,1437	0,0000	0,9789	0,0016	0,1193	0,0468	0,1745
Ni	-2,7490	28,110	0,4502	0,0086	-0,0973	24,370	0,0027	0,7967	0,0690	21,620	0,0021	0,7766
Cu	0,0950	5,4340	0,0638	0,3837	0,1130	6,5610	0,0915	0,1252	0,1782	5,6190	0,2974	0,0002
Zn	-3,0310	63,110	0,1173	0,2307	1,0880	43,020	0,1270	0,0680	0,5530	50,180	0,0463	0,1767
Mo	-0,1346	1,0130	0,2241	0,0873	-0,0090	1,1090	0,0025	0,8051	0,0140	0,7600	0,0097	0,5401
Ag	0,1101	4,2020	0,0149	0,6773	0,0513	4,5680	0,0219	0,4613	0,0634	4,4020	0,0482	0,1681
Cd	-0,0403	1,2890	0,2263	0,0856	0,0173	1,0840	0,1839	0,0256	0,0140	1,1240	0,1805	0,0056
Pb	-0,0320	0,2443	0,2943	0,0554	-0,0006	0,2353	0,0004	0,9214	0,0036	0,1718	0,0229	0,3513
Bi	0,0000	0,0024	0,0005	0,9403	0,0002	0,0024	0,1017	0,1203	0,0003	0,0019	0,2074	0,0041

	C (n=20, except for Mo: n=14 and Bi: n=17)				D (n=27, except for Co: n=24, Mo: n=21 and Bi: n=24)				CD (n=41, except for Co: n=38, Mo: n=35 and Bi: n=35)			
B. Acute	c_1	c_2	r^2	p	c_1	c_2	r^2	p	c_1	c_2	r^2	p
As	-0,0532	0,9268	0,0040	0,7916	0,0106	0,6687	0,0441	0,2931	-0,0003	0,7866	0,0000	0,9756
Al	-17,580	127,100	0,0145	0,6133	7,2130	89,350	0,0771	0,1607	6,7760	93,980	0,0833	0,0672
V	-0,0279	0,5299	0,0012	0,8837	0,0071	0,4125	0,0128	0,5742	0,0031	0,4557	0,0020	0,7805
Cr	-0,0084	0,1534	0,0025	0,8340	-0,0002	0,1489	0,0001	0,9719	0,0001	0,1458	0,0000	0,9732
Fe	-3,7840	65,250	0,0033	0,8090	3,5830	50,090	0,0804	0,1517	3,2090	54,120	0,0783	0,0764
Mn	-0,8232	5,0090	0,0877	0,2048	0,1270	3,0810	0,1419	0,0528	0,0842	3,5340	0,0702	0,0941
Co	0,0009	0,1638	0,0001	0,9640	-0,0014	0,1671	0,0068	0,7008	-0,0015	0,1677	0,0095	0,5610
Ni	-1,5350	31,990	0,0032	0,8126	0,8368	25,390	0,1254	0,0699	0,6491	27,340	0,0709	0,0925
Cu	1,1700	7,0590	0,0760	0,2393	0,4860	8,5320	0,2593	0,0067	0,5212	8,1600	0,3322	< 0,0001
Zn	5,4590	68,650	0,0130	0,6317	4,0260	71,110	0,2136	0,0152	4,0230	71,230	0,2573	0,0007
Mo	0,7508	0,4070	0,0435	0,4741	0,0234	0,9698	0,0275	0,4722	0,0020	1,2060	0,0002	0,9349
Ag	0,3264	4,9620	0,0036	0,8011	0,1700	5,4330	0,0797	0,1536	0,1847	5,2790	0,1047	0,0391
Cd	-0,0752	1,5950	0,0171	0,5822	0,0086	1,4870	0,0267	0,4158	0,0076	1,4990	0,0195	0,3834
Pb	0,0509	0,1782	0,0198	0,5538	-0,0043	0,3083	0,0061	0,6990	-0,0008	0,2721	0,0003	0,9168
Bi	-0,0005	0,0073	0,0047	0,7937	0,0000	0,0060	0,0003	0,9318	0,0000	0,0063	0,0006	0,8899

3.3.2. Acute contamination

Shoots of *P. oceanica* contaminated at acute level accumulate As, Al, V, Cr (Fig. 3B), Cu (Fig. 3A), Pb and Bi following a logarithmic model, but the contamination period was generally not long enough to reach a steady state at the chemical pollutant levels experimentally used, except for Zn, Cd and Ag. The highly variable Mo uptake is poorly modeled with the logarithmic function. Only Fe follows a linear kinetic uptake (Fig. 3C). Finally, Ni concentrations significantly decrease during the 24 hours of acute contamination, contrary to Mn and Co (Fig. 3D). Contamination factors vary highly, with a mean (Ni, Mn and Co excluded) of 57.8 ± 118.5, a minimum for Fe (1.3) and a maximum for Bi (419.9) (Table 5).

Both intermediate and adult leaves accumulate Cr (Fig. 3B) and Bi following a logarithmic model; Zn accumulation reaches a plateau following a one-phase association kinetic and Fe is linearly accumulated (Fig. 3C). The highly variable As, V and Pb uptakes can only be modeled for intermediate or adult leaves; the logarithmic modeling of Mo uptake by leaves is not significant ($p > 0,05$). Al, Cu (Fig. 3A) and Ag uptake kinetic models differ between both leaf types. Cd uptake follows a one-phase association model in intermediate leaves while it is not significantly ($p < 0,05$) accumulated in adult leaves. Finally, neither Ni nor Mn or Co (Fig. 3D) are accumulated in *P. oceanica* leaves, and Mn and Ni level observed decreases are further significant ($p < 0,05$) in adult leaves. Mean contamination factor between days C0 and C1 (Mn, Ni and Co excluded) is 171.8 ± 478.8 for intermediate leaves (min. = 1.5 for Fe; max. = 1685.5 for Bi) and 33.6 ± 54.6 for adult leaves (min. = 1.2 for Fe and Cd; max. = 189.3 for Bi), as a result of the different uptake kinetics displayed by both leaf types (Table 5).

Rhizome accumulate none of the 15 chemicals during the 24 hours of the acute contamination (Table 6), and their concentrations remain mostly constant during this period, as shown for Zn, Cu and Ag in Fig. 4.

3.3.3. Trace element trapping

Posidonia oceanica density in the Calvi Bay, regularly monitored with a 25 x 40 cm random quadrat at 10 m depth, is 407 ± 141 shoots m^{-2} (n = 141). The mean annual dry weight of one *P. oceanica* shoot is 0,877 ± 0,494 g; the mean annual concentrations of the 15 studied TEs in *P. oceanica* shoots sampled at 10 m depth in the reference meadow of the

Calvi Bay is given in Table 4; TE contamination factors are listed in Table 5. By multiplying these 4 parameters, we can easily calculate the quantity of pollutants that 1 m^2 of *P. oceanica* shoots can trap (in $g.m^{-2}$; Table 5).

One m^2 of *P. oceanica* shoots traps a total of 124 g of the 15 studied TEs (min. = 0.015 g for Bi; max. = 34.7 g for Zn) at the end of the 5 days of moderate contamination, compared to the 85 g for the uncontaminated reference meadow. The total amount of the TEs trapped by *P. oceanica* contaminated at acute levels is 266 $g.m^{-2}$ (min. = 0.56 g for Co; max. = 62.9 g for Mo). TEs initially more abundant in *P. oceanica* adult leaves (87 $g.m^{-2}$ for adult leaves and 79 $g.m^{-2}$ for intermediate leaves) display a higher total content in intermediate leaves after both the moderate and acute contamination periods (132 and 324 $g.m^{-2}$ for intermediate leaves and 113 and 222 $g.m^{-2}$ for adult leaves, respectively).

3.4. *Posidonia oceanica* trace element loss kinetics

3.4.1. Moderate contamination

Rapid initial loss kinetics of As, Mo and Pb as well as Cr (Fig. 3B) and Mn following the 5 days of moderate contamination are properly described with the exponential or the logarithmic models, respectively. Co (Fig. 3D), Ni, Cu (Fig. 3A), Zn, Ag, Cd and Bi decrease continuously in shoots, following a simple linear model. *P. oceanica* shoots eliminate TEs rapidly enough to reach back their initial C1 concentrations within 2 (V) to 15 (Cr, Zn) days, except for the essential micronutrient Cu, and for Ag and Bi. These last 2 TEs show furthermore the highest contamination factors (4.6 and 6.1, respectively). Accumulated Fe (Fig. 3C) and Al are not eliminated; V essentially decreased during the short time interval between sampling days C5 and D0, which explains the absence of a significant loss kinetic ($p > 0,05$) of this element during the 15 consecutive decontamination days.

Intermediate leaves eliminate As, Mo and Pb more rapidly (exponential decrease) than adult leaves (logarithmic decrease). Linear Cr loss (Fig. 3B) in intermediate leaves is logarithmic in adult ones. Mn and Zn (logarithmic model) as well as Co (Fig. 3D), Ni, Cu (Fig. 3A), Cd and Bi (linear regression) levels significantly decrease in adult leaves, but not in intermediate ones (slopes of models are further always more elevated for adult leaves). As for integral shoots, accumulated Fe (Fig. 3C) and Al are not eliminated. The modeling of the Ag decrease is not significant ($p > 0,05$), and accumulated V was essentially lost during the short

time interval between sampling days C5 and D0. The proportion of remaining TEs after 15 days of depuration (D15) is 10 % higher for adult leaves (65 ± 23 %, ranging from 27% for Pb to 100 % for Al) than for intermediate leaves (55 ± 27 %, ranging from 14 % for Pb to 100 % for Al) when compared to their respective levels recorded at the end (C5) of the exposure period.

Rhizome TE concentrations do not evolve significantly ($p > 0,05$) during the decontamination period (Table 6), except for Cd. However, results of the linear modeling of the rhizome TE concentration evolution during the 20 days of the entire experiment (i.e. both contamination and decontamination periods together) show a significant ($p < 0.05$) little increase of Al, V, Mn, Cu, Cd and Bi levels over time (Table 6); Fe level increase is further very close to be significant ($p = 0,06$).

3.4.2. Acute contamination

Posidonia oceanica TE loss kinetic following the acute contamination is linear for Ag, but follow a rapid initial exponential decrease for As, V, Cr (Fig. 3B), Cu (Fig. 3A), Zn, Mo, Cd, Pb and Bi; for Cu, the reached plateau concentration is about 2,6 times higher than the initial (C0) concentration. *P. oceanica* shoots excrete As, V, Mo and Cd so rapidly that they reach back their initial contents within 2 decontamination days, while it takes 15 days for Pb. *P. oceanica* Mn (logarithmic model) and Ni (linear regression) contents significantly ($p < 0,05$) decrease while Co linear decrease is not significant (Fig. 3D). As for the moderate contamination, accumulated Al and Fe (Fig. 3c) are not significantly ($p > 0,05$) lost during the decontamination period.

Intermediate and adult leaves eliminate most TEs following the same model: a very rapid initial exponential decrease for As, V, Cr (Fig. 3B), Cu (Fig. 3A), Mo, Cd, Pb and Bi. Accumulated Fe (Fig. 3C) and Al are logarithmically eliminated by intermediate leaves only to reach back their initial (C0) concentrations. The continuous linear decrease of Ag in intermediate leaves is initially more rapid (logarithmic model) in adult leaves. Finally, leaves that did not accumulate Mn, Co (Fig. 3D) and Ni during the contamination period significantly eliminate Mn (both leaf types) and Co (intermediate leaves) during the decontamination period. The proportion of remaining TEs after 15 days of depuration in uncontaminated seawater was 7 % higher for adult leaves (39 ± 38%, ranging from 2% for As and Mo to 100% for Al) than for intermediate leaves (32 ± 33%, ranging from 1% for As and

Mo to 84% for Fe) when compared to their respective levels recorded at the end of the exposure period.

Rhizome TE concentrations do not evolve significantly ($p > 0,05$) during the decontamination period (Table 6), except for Cu (Fig. 4B) and Zn (Fig. 4A). Concentrations of Cu, Zn and Ag (Fig. 4C) also significantly ($p < 0,05$) increase during the 16 days of the entire experiment (i.e. both contamination and decontamination periods together). Al, Fe, Mn and Ni level increases are further very close to be significant ($0,07 \leq p \leq 0,09$; Table 6).

3.5. Trace element uptakes by epiphytes

Before contaminations (at T0), mean TE concentrations in epiphytes decrease in the following order: Al > Fe > Zn > Mn > V, Ni > As > Cu, Pb > Cd > Cr, Mo > Co > Ag > Bi (Table 4B). At the end of the contamination periods, the 15 TEs present different patterns of accumulation (compared to T0), depending on the contamination levels. Al, Fe, As, Mo (significant – $p < 0.05$) and V (non-significant – $p > 0.05$) are accumulated at acute contamination levels only. Cr, Cu, Pb, Bi (significant – $p < 0.05$) and Cd (non-significant – $p > 0.05$) are accumulated at both contamination levels, and epiphyte concentrations tend to be the highest at acute levels. Co, Ni and Zn are more accumulated (significant – $p < 0.05$) at moderate contamination levels than at acute levels. Finally, Ag concentrations are equally concentrated (significant – $p < 0.05$) in contaminated epiphytes, as for Mn (non-significant – $p > 0.05$).

4. Discussion

4.1. The Calvi Bay as a reference for the Northwestern Mediterranean

Mean concentrations of the dissolved bioavailable TEs measurable with DGTs are low to very low in the control *P. oceanica* bed (Table 2A) in comparison to other Mediterranean coastal areas (Table 1C). The water column of the Calvi Bay deserves the status of reference body of water for the Northwestern Mediterranean. This finding corroborates observations made by Luy et al. (2012) who monitored the chemicals contamination along the French

Mediterranean coasts using *P. oceanica* as bioindicator: shoot TE levels in the Calvi Bay were always among the lowest when compared to the other studied sites.

The seagrass meadow in front of the oceanographic station STARESO presents furthermore an overall good ecological status, with a low anthropization index of its water body (index defined as the sum of 7 impact factors affecting the seawater quality and/or the biotope quality: fish farming, industrial development, agriculture, tourism, fishing, commercial ports and urbanization; Gobert et al. 2009). This *P. oceanica* bed also presents criteria of "a good reference monitoring site", as defined in the SeagrassNet Monitoring Manual (Short et al. 2002): the meadow is representative of the location and is relatively homogeneous; it is located in a place which you can come back to and monitor again at regular intervals, and is removed from any large obvious impact (e.g. a marina, a dredge channel, or a sewage outfall).

TE concentrations in STARESO superficial sediments are similar to others uncontaminated *P. oceanica* meadows from the Northwestern Mediterranean, and are in the lowest range of values recorded for this basin (Schlacher-Hoenlinger and Schlacher 1998, Sanchiz et al. 2000, Tranchina et al. 2005, Lafabrie et al. 2007). As for its water column, STARESO sediments can be considered as a reference for numerous bioavailable trace metals (Cu, Zn, Pb, Cd, Ni) classically investigated in monitoring surveys within the Mediterranean. Co, Cu, Zn, Pb, Cd, Ni, Co, As, Cr and Ag concentrations in *P. oceanica* vegetated sediments are also low to very low when compared to the wide range of concentrations reported for other seagrass bed sediments (Lewis et al. 2007, Lewis and Devereux 2009).

4.2. *Posidonia oceanica* natural compartmentalization

Luy et al. (2012; ecotoxicological survey) and Sanz-Lázaro et al. (2012; TE cycling by *P. oceanica*) recently enlarged notoriously the number of TEs measured in *P. oceanica* to Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb, Bi, Ba, Cs, Ga, Li, Ni, Rb, Sr and Tl. These TEs, sometimes essential to the plant, may also be toxic and have been barely studied or not at all, contrary to Cd, Hg, Pb, Cu, Zn, Cr, Fe and Ni. The majority of these TEs are efficiently accumulated by *P. oceanica* (Luy et al. 2012, Sanz-Lázaro et al. 2012), and the plant may play a relevant role in their cycling in the Mediterranean (Sanz-Lázaro et al. 2012). The spatial variation of recorded levels along the Mediterranean coasts results either from specific

anthropogenic pressures or from a natural heterogeneity of the environmental facies (Luy et al. 2012, Sanz-Lázaro et al. 2012).

In the reference *P. oceanica* bed of the Calvi Bay, V, Cr, Mn, Co, As, Mo, Cd and Pb are preferentially accumulated in *P. oceanica* leaves, while rhizomes concentrate more Al, Fe, Ni, Cu, Zn, Ag and Bi (mean annual trends; Table 4A). This accumulation pattern within plant compartments can however vary for each TE between reference sites. For instance, the TE compartmentalization near STARESO is consistent with results obtained by Sanz-Lázaro et al. (2012) in the reference *P. oceanica* meadow of Sounion, Greece, for Cr, Mn, Co, Cd in leaves or Fe, Ag and Bi in rhizomes, but not for V, As, Pb (> in rhizomes), Cu and Zn (> in leaves) or Ni (≈ in both tissues). High levels of Pb in leaves, rhizomes and sediments (6.1 ± 1.6, 15.2 ± 7.5 and 120 $\mu g.g_{DW}^{-1}$, respectively) measured at Sounion result from the old mining activity of this area, as for Zn (98 $\mu g.g_{DW}^{-1}$) in sediments (Smith and Cronan 1975, Sanz-Lázaro et al. 2012). Natural Cr enrichment of sediments (37 $\mu g.g_{DW}^{-1}$) probably derived from the mafic rocks which occur throughout the Attic-Cyclades massif (Smith and Cronan 1975), and explains high levels recorded in leaves and rhizomes (5.5 ± 2.5 and 5.9 ± 2.0 $\mu g.g_{DW}^{-1}$, respectively; Sanz-Lázaro et al. 2012). In 5 sites located along the little anthropized coast of Favigna Island, Sicily (Italy), Campanella et al. (2001) systematically measured higher Cd and Zn levels in leaves and higher Cr and Pb levels in rhizomes, while similar Cu concentrations between tissues were higher in leaves (3 sites) or rhizomes (2 sites), depending on sites.

Sanchiz et al. (2000) monitored 17 sites along the Mediterranean Spanish coast and measured higher Cd and Zn concentrations in leaves, but lower to higher Pb levels in leaves compared to rhizomes. Very high Cd (25.0 $\mu g.g_{DW}^{-1}$) and Zn (703.5 $\mu g.g_{DW}^{-1}$) leaf accumulations recorded in the southernmost sampled location suggested a contamination of the water column, as no higher Cd or Zn contents in unsieved sediments were measured (mean concentrations estimated from graphs, contaminated sites excluded: 0.1, 17.0 and 12.0 $\mu g.g_{DW}^{-1}$ for Cd, Zn and Pb, respectively).TE distribution in *P. oceanica* above- and below-ground compartments can be inconsistent, even between or within uncontaminated areas, as summarized above. Such differences in TE accumulation among plant compartments are due to differences in the relative bioavailability of trace elements in the the water column and sediments (Malea et al. 2008).

4.3. TE uptake kinetics by *P. oceanica*

Levels of pollutants used in the 2 experimental mesocosms are realistic when compared to the literature (Table 1), and even concentrations measured in the mesocosm contaminated at acute levels are similar to highly polluted coastal areas colonized by seagrasses (e.g. Huelva Estuary – Spain, Tokyo Bay – Japan). In the experimental design purposed, with the injected amounts of pollutants used, the principal route of TE uptake is through the water column, as no contamination of superficial sediments was observed. *Posidonia oceanica in situ* contaminated respond specifically and quantitatively to the added chemicals; most dissolved TEs were accumulated efficiently by the shoots (and epiphytes), except for Mn and Ni at moderate levels and Mn, Co (Fig. 3D) and Ni at acute levels.

The advantage in using low added contaminant levels is that several TEs may be studied simultaneously, with little or no inter-element interference. In this way, there is some assurance that the uptake and loss kinetics of each of the tracers will not be disturbed by the presence of the other elements. This approach diminishes the biological variability occurring when separate experiments are driven for each TE and enhance the comparability of their response (Warnau et al. 1996). It also reduces the number of required mesocosms and facilitates their deployment *in situ*, which permits to contaminate and to monitor seagrasses in their natural habitat.

Neither antagonistic nor synergistic interactions between TEs were quantified in the present study. However, at least some antagonistic interactions occurred as Co, accumulated at moderate levels, was no further accumulated at acute levels. Noraho and Gaur (1995) reported that 100 µM of interacting cations and metals (Ca, Mg, K, Na, Cu, Fe, Ni and Zn) lowered the Cd accumulation efficiency of the duckweed *Lemna polyrhiza*, and Dhir and Srivastava (2011) observed that the capacity of trace metal removal by the free-floating weed *Salvinia natans* was influenced by the combination of metals present in the medium and by their initial concentration. The total concentration of TEs (V, As, Mo and Bi excluded, because not quantifiable with the DGT technique) dissolved in the moderate and acute mesocosms are 24.0 and 193.4 ppb (4.4 ppb in the control *P. oceanica* bed), respectively. These low multi-element levels, when compared to most toxicological studies focusing on 1 or a few metals, are similar to multi-element pollution levels recorded in some coastal areas of the Mediterranean colonized by *P. oceanica*, where antagonistic accumulation effects are then expected to occur.

The overall good incorporation of TEs at even low environmental levels was previously reported by Warnau et al. (1996). They contaminated *P. oceanica* shoots in 10 L laboratory tanks during 15 days with very low levels of radiolabelled Zn, Ag, Cd, Cs and Am, and observed a continuous linear uptake of Zn and Cd, while Ag followed a one-phase association uptake model. Ledent et al. (1992) contaminated *P. oceanica in situ* under Plexiglas bell with Cd at 6 concentrations (1, 5, 20, 50, 100 and 200 ppb); contaminations were performed during 5 days (20 days for the 5 ppb concentration). Except for the concentration 1 ppb, the Cd levels used in their experiment are very high and unrealistic compared to natural seawater concentrations (Table 1). However, *P. oceanica* leaves and epiphytes successfully accumulated linearly Cd even at the highest contamination levels, and the leaf-epiphyte complex reached a maximal concentration of 130 $\mu g.g_{DW}^{-1}$ after 5 days of exposure at 200 ppb.

Several studies have been carried out concerning the use of a wide diversity of magnoliophyta as bio-indicators of TE contamination (reviews in Pergent-Martini and Pergent 2000, Lewis and Devereux 2009, Luy et al. 2012). However, according to Rainbow and Phillips (1993), if these species integrate the quality of their environment, they generally respond belatedly, thus limiting their utilization for biomonitoring short term (daily to monthly) environmental variations. *P. oceanica* seems to be an exception to this rule at least for TEs. Indeed, our experiences on TE incorporation kinetics demonstrate the inverse, at least for TEs, as plants immediately and proportionally accumulate pollutants present in their environment within hours, from very low to high levels.

There are abundant data on concentrations of TEs in seagrass tissues (Ralph et al. 2006), but little is known about their physiological and ecological role under natural conditions (Romero et al. 2006), except for Fe. In carbonate-rich and Fe-poor sediments, Fe additions have been shown to stimulate seagrass growth (Duarte et al. 1995, Marbà et al. 2007). Fe addition can also enhance seagrass phosphorus uptake and nitrogen fixation, and protect the plant through binding the toxic sulphide ion (Wu et al. 2000, Chambers et al. 2001, Holmer et al. 2003, Marbà et al. 2007). Furthermore, Al and Fe uptake rates, loss kinetics and acropetal translocation processes are similar. Luy et al. (2012) observed a significant correlation (n=270) between Al and Fe levels in *P. oceanica* sampled along the Mediterranean French coast, and experimental results in terrestrial plants show synergistic accumulation and effects between both TEs (e.g. Yamamoto et al. 1997, Nguyen et al. 2005). Malea and Haritonidis (1996) studied the toxicity and uptake of Al by the seagrass *Halophila stipulacea*;

however, no synergistic study on Al and Fe uptake and toxicity exist for seagrasses, while some evidences suggest its existence for *P. oceanica*. So, The low level multielement addition experiments performed in the present study highlight some general aspects on essential and non-essential TE dynamics in seagrasses and open new routes of investigations in this poorly documented axis of research.

TE uptake kinetics differ between tissues. Contaminations factors are always higher for intermediate leaves than for adult leaves, except for Fe or Ag for the moderate or acute contamination, respectively (higher c.f. in adult leaves), and corroborate results of previous studies for Cd (Ledent et al. 1992, Warnau et al. 1996), Zn, Ag, Cd, Cs and Am (Warnau et al. 1996). The higher growth rate of intermediate leaves compared to adult leaves may explain this difference in accumulation efficiency, as suggested by Ledent et al. (1992). However, contamination factors of TEs (Co, Cu, Fe, Mn, Mo, Zn) essential and beneficial for the development of plants (Kapustka et al. 2004, Romero et al. 2006) differ little between Intermediate leaves and adult leaves, mainly when they are added at low levels for a longer period (i.e. moderate experiment design). *A contrario*, TEs with high contamination factors (e.g. As, Cr, Ag, Pb, Bi) are mostly non-essential, which suggest little or no regulation of their accumulation compared to the previous.

While *P. oceanica* accumulates chemicals, possible defence mechanisms are activated concurrently. For exemple, Ranvier et al. (2000) and Ferrat et al. (2002) measured an increase of the GST activities, a biotransformation enzyme implies in the metabolism of xenobiotics in plants (Ferrat et al. 2003), in *P. oceanica* tissues exposed to increasing Hg concentrations (from 0.01 to 1 ppb for 48 to 144 hours). Hamoutene et al. (1996) studied the accumulation of Cd by *P. oceanica* (levels in leaves and per mg of protein following subcellular fractionation) exposed to concentrations of 5, 10 and 20 ppb for 48h00, and the effect of these expositions on certain aspects of the plant xenobiotic metabolism (oxidative stress, cytochrome P-450 activity, EROD and GST enzymatic activities). The inhibition or induction phenomena of the *P. oceanica* enzymatic system when exposed to Cd provided to authors a number of indications concerning possible defence mechanisms in the plant. However, in our experimental designs, we can estimate that the low to very low levels of many pollutants added in mesocosms during the short periods of both contaminations were probably not sufficient to induce any protective mechanisms, and if some mechanisms were induced, they did not prevent the contamination of *P. oceanica* shoots.

Hamoutene et al. (1996) also observed that the major accumulated portion of Cd in the experimentally contaminated plant soluble fraction consisting essentially of cytosol and other soluble compounds. They also observed an increase in the percentage of Cd in the fraction containing the nucleus, mitochondria, chloroplasts and cell wall fragments with the increase of Cd concentrations, showing perhaps a progressive binding to the membranes. From their observations, we can suggest a pathway of chemical accumulation first from the seawater to the plant soluble fraction, then bound to the plant cellular membranes while chemical levels and exposure time increase. Such a "step by step" assimilation process could explain why we observed for Cd, Zn and Ag, accumulated at similar levels in both experiments, a slower decontamination kinetic after the 5 days of moderate contamination compared to the 24 hours of acute contamination (Table 5, Annex B); the longer the plant would be exposed to chemicals, the higher would be the pollutant sequestration by the plant cells.

For the moderate experimental design, the length of the contamination period was not long enough to reach a plateau, as most TEs displayed a linear or an exponential uptake. Only Fe, Co and Zn accumulation slowed down at the end of the contamination period to reach a plateau. *A contrario*, *P. oceanica* contaminated at acute levels accumulated TEs following a one phase association or a logarithmic model, except for Fe (exponential uptake), Mn, Co and Ni (no uptake). The election of mostly the one phase association or the logarithmic model follows from the experimental design of chemical injections (4 injections the first 12 hours, the 5^{th} injection lasting 12 more hours). When TE uptake kinetics are modeled for the first 12 hours only (chemicals injected regularly every 3 hours), most TEs are then accumulated linearly, except for As, V, Mo and Pb (results of AIC analysis); at acute levels, pollutant concentrations are mostly not saturating during the first 12 hours of contamination (from C0 to C0.5; see Fig. 3).

During the last 12 hours of the acute contamination (from C0.5 to C1; see Fig 3), kinetic uptakes started to bend, while pollutant levels remained relatively similar to the previous first 12 hours of the experiment. It appears that at least 2 components regulate the uptake kinetic of TEs by *P. oceanica* shoots: i) the concentration of the pollutant in the contaminated meadow and ii) the frequency of pollutant inputs. In other words, for a given level of coastal pollution, *P. oceanica* will accumulate faster and continuously chemicals if there are frequently added to the meadow; but once the pollutant inflow will slow down *P. oceanica* TE contents will stop increasing and will reach a plateau (logarithmic to one-phase association models) in balance with their new environmental pollution status.

4.4. TE loss kinetics by *P. oceanica*

Within 2 to 15 days, the major part of most TEs uptaken by *P. oceanica* shoots during the contamination period of both experiments is eliminated (Fig. 3, Annex B), which is consistent with the rapid TE loss kinetics reported by Ledent et al. (1992) and Warnau et al. (1996). This is particularly true for toxic non-essential TEs, which are not trapped by *P. oceanica* leaves, contrary to essential TE (mainly Cu (Fig. 3A), Fe (Fig. 3C) and Zn). The slower loss kinetics exhibited by leaves for essential micronutrients involves the existence of specific and efficient sequestration mechanisms within plant tissues.The higher proportion of remaining TEs in adult leaves than in intermediate leaves after 15 days of depuration in uncontaminated seawater, when compared to their respective levels recorded at the end of the exposure periods, appears to be due to the generally higher retention capacity of adult leaves compared with that of intermediate leaves (Warnau et al. 1996). Post-control shoots sampled in November 2009 and March 2010 had recovered their initial TE levels of June 2009 (Fig. 3, Annex B), the seasonal variability still observed being correlated to the plant natural physiological cycle.

When the non-contaminating environment was restored, the concentration of some TEs still little increased during 6 hours to 4 days (Co (Fig 3D), Cd and Bi in the moderate contamination; Fe (Fig. 3C), Ag and Al in the acute contamination). Ledent et al. (1992) also observed this phenomenon in Cd contaminated *P. oceanica* leaves. According to previous studies on Cd and ^{14}C transfers between seagrass above- and below-ground tissues (Brinkhuis et al. 1980, Libes and Boudouresque 1987), they suggested that the little Cd quantities accumulated in contaminated rhizomes could supply leaves by translocation processes during a limited time. We did not observe any decrease of Co, Cd, Bi, Fe, Ag or Al concentrations in rhizomes during the decontamination period of the experiments that could reveal their basipetal translocation to leaves; furthermore, Bi, Cd, Ag (Fig. 4C), Fe and Al levels increased in rhizomes during the overall experiments, meaning that basipetal translocation processes from leaves to rhizome dominated. Following these observations, assumptions made by Ledent et al. (1992) are not satisfying for the present study.

TEs biosorb on *P. oceanica* fibers, as shown for Cr by Ncibi et al. (2008) and Krika et al. (2012). Biosorption, even if more efficient at acidic pH, could also be relevant for many metal at naturel seawater pH, as shown for the seaweed *Sargassum* spp. or *Ulva fasciata* when exposed to Cd and Pb (Kumar and Kaladharan 2006, Nessim et al. 2011). TEs biosorbed on

external leaf surface, proportionally to the pollution level experimented, could later be absorbed within leaf tissues once the non-contaminating environment is restored, resulting in the little transitory TE concentration increase sometimes observed. Numbers of phytoremediation studies further focus on this sorption ability of macrophytes fibres for the removal of pollutants from the environment (Lytle and Lytle 2001, Gardea-Torresdey 2003, Rai 2009).

4.5. Trace element kinetics in rhizomes

Ledent et al. (1992) observed an increase of Cd levels in rhizomes after 5 days of *in situ* contamination, at Cd levels of 100 and 200 ppb only, equivalent to 4 and 7 times their initial natural Cd concentration (0.5 $\mu g.g_{DW}^{-1}$), a contamination factor much lower than for leaves or epiphytes. These authors suggested that the differences between the plant compartment responses towards Cd exposure were in relation with their respective physiological activity, as leaves and epiphytes productivity is considerably higher than rhizomes (1000 to 10000 $Kg.ha^{-1}.y^{-1}$ against 250 $Kg.ha^{-1}.y^{-1}$, respectively; Ledent et al. 1992). However, they did not follow Cd kinetics in rhizomes during the 2 decontamination days of their experiment, and they did not consider the basipetal translocation of TEs from leaves to rhizomes. Our results show that there was no significant evolution of TE concentrations in rhizomes during the contamination periods of both experiments. However, Cu, Zn and Cd significantly increased ($p<0.05$) during the 15 decontamination days following the moderate or acute contamination, and Al, V, Mn, Cu, Zn, Ag, Cd and Bi increased significantly ($p<0.05$) when considering the overall moderate or acute contamination experiment (Fig. 4, Annex B).

TE levels that increased in rhizomes during the decontamination periods of the experiments are either essential: Cu, Zn and Mn, or are naturally preferentially accumulated in this tissue: Al, Ag and Bi. Rhizomes are key organs for nutrient storage; this storage occurs during periods of high availability and low demand (winter). Stored nutrients then supply plant demands during the moment of maximum leaf growth (late spring) (Alcoverro et al. 2000, Invers et al. 2002, Romero et al. 2006). We experimentally supplied *P. oceanica* with essential micronutrients, and shoots responded by translocating part of the leaf accumulated metals to the rhizomes. It is more the imbalance between below- and above-ground tissues concentrations than the dissolved TE levels in the water column that regulate this acropetal

translocation, as rhizome TE concentrations increased continuously during the decontamination periods.

4.6. Trace element levels in epiphytes

Contrary to *P. oceanica* leaves and rhizomes, data on TE levels in epiphytes are scarce. Concentrations vary highly, ranging from 0.045 $\mu g.g_{DW}^{-1}$ for Bi to 778 $\mu g.g_{DW}^{-1}$ for Al (Table 4B). Levels of V, Cr, Fe, Zn, As, Pb and Bi are 4.5 ± 2.6 times higher in epiphytes than in *P. oceanica* leaves, and till 18.4 times higher for Al. *A contrario*, *P. oceanica* leaves concentrate more Mn, Co, Ni, Cu, Mo (mean ratio = 2.2 ± 0.7) and mainly Ag (ratio = 6.4) than epiphytes and Cd levels are similar between shoots and epiphytes. These observations are consistent with Sanz-Lázaro et al. (2012) for V, Cr, Fe, As, Pb and Ni but not for Zn (higher levels in leaves), Mn, Co and Cu (higher levels in epiphytes), and with Schlacher-Hoenlinger and Schlacher (1998) for Cu and Pb but not for Zn and Cd (higher level in leaves).

P. oceanica epiphytes, analysed at the end of both contaminations periods (Table 4B), accumulated Cr, Mn, Cu, Zn, Mo, Cd and Pb with a relative efficiency similar to shoots, as observed by comparing their respective concentration factors (c.f. of *P. oceanica* shoots are listed in Table 5A; c.f. of epiphytes – not shown – were calculated from concentrations reported in Table 4B). Furthermore, significantly ($p < 0,05$) lower Co, Ni and Zn accumulation measured in epiphytes exposed to the acute treatment when compared to the moderate exposure suggest that some antagonistic interactions occurred between TEs, as reported earlier for Co incorporation in *P. oceanica* shoots.

For As, Al, V, Fe, Ag and Bi, specific responses of *P. oceanica* shoots and epiphytes to pollutant exposures rely on their natural TE contents. As, V and mainly Al and Fe are naturally more abundant in epiphytes than in *P. oceanica* shoots (Table 4; Sanz-Lázaro et al. 2012). The low amounts of these 4 TEs injected in the moderate mesocosm (Table 2) were probably insufficient to observe any supplementary incorporation in epiphytes (Table 4B), while they were significantly ($p < 0,05$) accumulated in *P. oceanica* shoots (Table 5A); it further explains the lower incorporation efficiency of As and V by epiphytes at acute levels, compared to *P. oceanica* shoots. In the same way, the relative incorporation of Ag is more efficient in epiphytes (Warnau et al. 1996), this element being naturally 9 times less concentrated in epiphytes than in *P. oceanica* shoots; *a contrario*, the relative incorporation of

Bi is more efficient in *P. oceanica* shoots, this element being naturally 6 times less concentrated in *P. oceanica* shoots than in epiphytes. These observations therefore suggest a passive incorporation of most TEs linked to the external/internal concentration gradient. Also, in ecotoxicological surveys, it is not so much the absolute pollutant concentration in a bioindicator, but rather the relative difference between pollutant levels (i.e. contamination factors between stations, compared to reference conditions), that should condition the election of one specific species or tissue for a particular pollutant.

Conclusions

Posidonia oceanica in situ contaminated by a mix of 15 TEs (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Pb and Bi) at realistic environmental concentrations successfully accumulate dissolved chemicals from the water column. Depending on the plant compartment where the TEs are mainly accumulated and on their incorporation and loss dynamics, *P. oceanica* can act as a sink or a source of TEs and certainly plays a key role in the cycling of TEs in the coastal Mediterranean.

The experimental exposure of shoots to the multielement solution showed that antagonistic and synergistic interactions between TEs can already occur at levels reported in contaminated areas of the Mediterranean. Such interactions, as well as toxicity tests, should be further investigated to enhance our knowledge on *P. oceanica* ecology and ecotoxicology. The very rapid and proportional response of *P. oceanica* leaves to their environmental chemical status makes it undoubtedly an excellent biomonitor and suggests their routine use in regularly scheduled monitoring programs. Nevertheless, to by-pass *P. oceanica* leaves deciduous character and their capability to detoxify rapidly, long term accumulation recordings would also necessitate belowground tissues analyses.

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Annex A.

Trace element concentrations (mean ± SD, in µg.g^{-1} of dry weight) in intermediate and adult leaves of *P.oceanica* regularly sampled (June 2009) during the contamination phase ("C") at moderate levels and during the decontamination phase ("D"). Concentrations in post-controls sampled in Novembre (2009) and March (2010) are also given. *, ** and struck-through values represent concentrations < L_Q, < L_D and < L_C, respectively. Contamination times are given in days, from T0 to C5. Decontaminantion periods lasted 15 days.

Moderate contamination

Concentrations (µg.g^{-1}) Intermediate leaves (IL)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb		Bi
Mean																
IL-T0	2,18	28,4	11,45	0,243	43,5	47,3	1,86	27,2	5,3	75	2,18	0,53	2,32	0,74	**	0,005
IL-C1	0,80	7,9	2,79	0,357	29,0	43,0	2,81	39,4	9,8	129	1,30	2,77	3,50	3,30		0,092
IL-C2	0,71	5,1	2,08	0,434	27,8	39,8	2,72	34,6	11,1	146	1,22	3,97	3,84	4,68		0,156
IL-C3	1,26	12,7	6,50	0,716	30,7	43,3	3,31	37,8	14,9	177	1,23	6,95	4,14	6,46		0,264
IL-C4	1,32	11,6	3,54	0,897	35,1	46,2	3,83	40,6	16,1	216	1,42	7,52	5,10	9,88		0,373
IL-C5	2,55	20,0	9,51	1,164	34,7	55,0	3,58	31,1	24,7	233	2,87	13,36	6,22	15,44		0,677
IL-D0	1,25	34,5	2,39	0,747	39,4	35,7	2,98	29,9	17,9	211	2,00	7,39	4,49	15,58		0,380
IL-D1	1,64	20,3	2,82	0,794	33,3	53,1	3,54	31,3	21,8	251	2,53	12,78	5,71	12,38		0,578
IL-D2	1,06	12,4	1,39	0,834	29,2	42,6	4,20	37,7	23,8	288	1,19	12,41	6,77	13,89		0,636
IL-D3	1,13	19,1	2,15	0,756	37,6	38,4	3,60	41,6	23,9	266	1,43	11,21	5,80	10,82		0,521
IL-D5	0,69	18,1	1,97	0,490	32,1	29,2	3,05	35,0	21,0	238	1,21	9,74	5,21	7,86		0,495
IL-D7	0,94	16,1	2,63	0,553	31,3	45,8	3,09	31,0	25,5	247	3,30	13,60	6,13	7,57		0,631
IL-D9	0,72	32,2	2,31	0,435	39,8	29,7	2,95	34,0	18,7	224	1,42	8,94	4,18	3,59		0,332
IL-D15	1,01	27,1	4,27	0,285	33,3	33,9	2,20	23,8	14,5	206	2,66	7,05	3,30	2,17		0,195
November	0,63	16,3	0,65	0,159	28,5	15,0	0,23	11,4	15,6	37	0,63	1,28	1,41	0,33	**	0,004
March	1,90	26,9	3,54	0,134	44,0	37,9	1,70	30,2	11,0	86	1,50	0,74	2,82	0,57	**	0,005
SD																
IL-T0	0,54	8,5	2,11	0,043	4,4	5,3	0,27	4,3	0,5	11	0,58	0,08	0,27	0,10		0,002
IL-C1	0,18	2,0	0,98	0,047	2,5	1,8	0,24	1,4	0,7	15	0,09	0,19	0,31	0,85		0,018
IL-C2	0,19	2,3	0,60	0,051	4,3	4,9	0,51	3,4	1,4	8	0,03	0,35	0,25	0,97		0,023
IL-C3	0,22	3,7	1,97	0,005	2,1	1,8	0,08	0,1	2,2	4	0,09	1,78	0,14	0,22		0,037
IL-C4	0,09	3,1	0,84	0,155	5,4	4,5	0,33	2,5	4,0	25	0,15	2,64	0,27	1,21		0,078
IL-C5	0,12	5,2	1,86	0,219	3,7	1,5	0,32	0,4	5,5	19	0,49	3,13	1,02	4,63		0,234
IL-D0	0,44	18,5	0,93	0,293	10,3	10,0	0,93	4,7	5,8	68	0,37	3,30	1,36	9,98		0,193
IL-D1	0,64	3,4	0,29	0,109	2,7	7,2	0,23	0,7	3,3	23	1,26	2,03	1,34	3,69		0,174
IL-D2	0,07	0,6	0,07	0,053	1,4	1,0	0,34	0,5	3,3	13	0,11	2,23	0,68	2,03		0,063
IL-D3	0,02	6,6	0,72	0,050	9,1	2,2	0,30	2,6	0,4	11	0,17	0,17	0,61	1,52		0,067
IL-D5	0,04	3,4	0,23	0,054	0,8	6,2	0,46	5,0	3,5	12	0,04	2,64	0,82	1,82		0,114
IL-D7	0,02	1,3	0,00	0,065	1,8	4,5	0,31	1,6	3,7	6	0,06	2,05	0,07	0,11		0,065
IL-D9	0,26	4,6	0,62	0,243	10,7	10,0	0,90	0,7	6,0	57	0,15	4,45	0,73	1,15		0,186
IL-D15	0,11	2,1	0,73	0,023	1,7	4,5	0,07	2,8	3,9	6	1,05	1,51	0,29	0,16		0,017
November	0,23	7,6	0,41	0,158	12,9	7,6	0,11	5,2	5,4	11	0,34	0,51	0,75	0,18		0,002
March	0,35	5,3	2,25	0,057	9,5	5,5	0,33	2,0	4,6	9	0,30	0,14	0,30	0,06		0,001

Moderate contamination

Concentrations (µg.g^{-1}) Adult leaves (AL)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb		Bi
Mean																
AL-T0	2,14	48,6	9,14	0,352	46,8	50,7	2,70	31,2	4,9	92	2,31	0,30	2,44	1,09	*	0,008
AL-C1	1,08	15,7	3,14	0,369	32,4	48,0	3,21	36,5	6,8	144	1,34	1,92	3,39	2,64		0,065
AL-C2	1,14	18,8	2,80	0,495	35,2	52,8	3,75	37,6	7,6	180	1,48	2,57	3,74	3,47		0,100
AL-C3	1,28	23,3	3,71	0,680	42,0	51,7	3,75	38,9	10,2	164	1,42	4,27	3,71	4,40		0,152
AL-C4	1,86	24,8	3,49	0,766	39,5	49,2	3,78	38,0	11,0	175	1,49	4,98	3,75	5,82		0,210
AL-C5	2,08	27,5	7,67	0,862	40,6	52,9	3,72	33,7	14,9	152	2,78	6,81	3,21	6,86		0,240
AL-D0	1,45	51,3	3,91	0,846	44,1	45,5	3,55	31,2	15,7	174	2,44	7,05	4,04	11,28		0,304
AL-D1	1,45	26,9	2,80	0,748	36,2	50,6	3,63	32,4	19,1	182	2,08	9,49	3,93	6,21		0,305
AL-D2	1,01	23,7	1,76	0,756	35,9	43,4	3,77	35,1	15,8	195	1,46	8,12	4,46	6,71		0,344
AL-D3	1,20	36,8	2,16	0,836	46,7	46,6	3,82	37,2	16,8	184	1,46	8,93	3,93	5,43		0,288
AL-D5	0,87	32,7	2,43	0,579	42,7	31,6	2,89	31,8	12,3	151	1,45	5,88	3,42	3,42		0,213
AL-D7	0,74	47,0	2,95	0,483	52,6	35,3	2,19	23,4	16,4	138	2,97	8,19	3,37	2,59		0,201
AL-D9	0,57	31,2	2,23	0,351	37,7	24,7	2,21	24,6	12,7	123	1,50	5,69	3,12	1,97		0,181
AL-D15	1,07	38,5	5,81	0,420	39,3	33,6	2,41	23,5	12,4	132	3,15	5,19	2,43	1,83		0,104
November	0,90	55,6	2,57	0,219	53,9	33,9	0,91	15,3	11,1	43	1,60	0,94	1,79	0,88	*	0,009
March	1,95	49,7	5,46	0,177	44,5	41,8	2,13	29,7	8,9	78	1,57	0,51	2,37	0,85	**	0,006
SD																
AL-T0	0,49	25,3	1,58	0,061	9,9	5,6	0,49	8,5	0,6	14	0,87	0,05	0,38	0,23		0,004
AL-C1	0,26	5,2	0,99	0,043	3,7	5,5	0,44	2,9	0,3	20	0,10	0,05	0,18	0,35		0,009
AL-C2	0,10	5,8	0,40	0,046	2,9	5,8	0,40	2,3	0,6	17	0,23	0,21	0,13	0,28		0,009
AL-C3	0,18	4,3	0,82	0,035	1,9	3,3	0,28	2,0	0,7	7	0,18	0,42	0,19	0,52		0,008
AL-C4	0,31	8,5	0,60	0,077	3,4	4,0	0,38	4,2	2,1	16	0,06	1,29	0,20	0,89		0,023
AL-C5	1,12	12,1	4,29	0,548	7,0	19,2	0,91	3,2	9,4	39	1,45	5,75	0,38	4,68		0,200
AL-D0	0,51	27,6	2,58	0,238	9,7	10,9	0,73	5,6	3,4	27	0,58	2,09	0,78	3,13		0,080
AL-D1	0,61	9,7	1,47	0,076	2,1	5,1	0,46	1,3	0,1	6	0,51	0,10	0,37	1,49		0,058
AL-D2	0,03	1,6	0,25	0,091	3,6	5,8	0,24	3,0	1,0	5	0,06	0,92	0,27	0,68		0,020
AL-D3	0,22	11,7	0,46	0,131	3,8	0,4	0,13	0,9	0,6	5	0,13	0,60	0,31	0,80		0,033
AL-D5	0,27	3,3	0,49	0,098	6,9	10,6	1,04	7,3	1,1	36	0,21	1,16	0,87	1,43		0,069
AL-D7	0,09	24,1	0,42	0,098	22,4	6,8	0,50	3,1	2,2	29	0,55	1,90	0,60	1,06		0,104
AL-D9	0,28	9,5	0,65	0,192	3,1	8,8	0,99	6,2	2,7	48	0,36	2,42	0,55	0,92		0,102
AL-D15	0,11	3,0	1,63	0,141	1,5	10,6	0,96	4,7	2,7	35	1,27	2,08	0,35	0,53		0,042
November	0,07	11,5	1,49	0,083	20,1	10,4	0,32	2,0	3,3	2	0,42	0,32	0,22	0,32		0,004
March	0,54	10,2	3,01	0,042	8,1	4,8	0,18	3,0	3,2	7	0,17	0,16	0,06	0,10		0,001

Annex A *(Continued)*.

Trace element concentrations (mean ± SD, in µg.g^{-1} of dry weight) in shoots and rhizomes of *P. oceanica* regularly sampled (June 2009) during the contamination phase ("C") at moderate levels and during the decontamination phase ("D"). Concentrations in post-controls sampled in Novembre (2009) and March (2010) are also given. *, ** and struck-through values represent concentrations < L_Q, < L_D and < L_C, respectively. Contamination times are given in days, from T0 to C5. Decontaminantion periods lasted 15 days.

Moderate contamination

Concentrations (µg.g^{-1}) Shoots (S)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
Mean															
S-T0	2,16	39,3	10,18	0,304	45,1	49,3	2,33	29,3	5,1	84	2,27	0,40	2,38	0,93 *	0,007
S-C1	0,99	13,2	3,01	0,364	31,3	46,2	3,07	37,4	7,8	139	1,33	2,19	3,42	2,85	0,074
S-C2	1,02	13,4	2,60	0,478	32,4	48,3	3,41	36,7	8,7	169	1,41	3,05	3,74	3,80	0,118
S-C3	1,25	19,3	4,70	0,695	37,6	48,6	3,58	38,5	12,0	169	1,36	5,36	3,87	5,19	0,196
S-C4	1,60	18,4	3,49	0,809	36,9	47,4	3,78	38,9	13,0	191	1,48	5,91	4,32	7,46	0,274
S-C5	2,41	25,2	8,78	1,033	38,6	55,4	3,70	32,7	19,9	193	2,93	10,16	4,63	11,06	0,449
S-D0	1,41	44,0	3,46	0,829	42,4	42,7	3,43	31,0	16,8	194	2,31	7,34	4,37	13,62	0,351
S-D1	1,50	25,2	2,79	0,765	35,4	51,1	3,60	32,0	20,0	204	2,19	10,54	4,50	8,15	0,391
S-D2	1,03	19,7	1,62	0,784	33,6	43,3	3,94	36,1	18,7	229	1,36	9,64	5,32	9,39	0,452
S-D3	1,21	29,5	2,15	0,820	43,6	43,9	3,77	38,5	19,3	214	1,41	9,81	4,63	7,62	0,381
S-D5	0,81	29,3	2,34	0,554	39,5	30,1	2,86	31,8	14,2	169	1,42	6,60	3,83	4,52	0,276
S-D7	0,83	40,1	2,88	0,504	49,0	38,6	2,48	26,3	19,8	176	3,06	10,02	4,36	4,40	0,352
S-D9	0,58	31,8	2,19	0,354	38,4	25,3	2,29	25,9	13,8	140	1,46	6,21	3,29	2,21	0,203
S-D15	1,06	36,5	5,36	0,384	38,2	32,4	2,27	23,0	13,0	141	3,12	5,50	2,57	1,82	0,118
November	0,85	46,4	2,55	0,222	41,9	33,5	0,88	14,0	11,2	43	1,53	0,92	1,70	0,89 *	0,009
March	1,92	37,0	4,43	0,153	44,7	39,6	1,89	29,9	10,1	82	1,54	0,65	2,63	0,69 **	0,005
SD															
S-T0	0,51	15,2	1,56	0,046	6,8	4,5	0,33	6,2	0,5	11	0,76	0,06	0,32	0,13	0,002
S-C1	0,23	3,9	0,96	0,042	3,2	3,7	0,35	2,4	0,4	17	0,08	0,06	0,20	0,52	0,012
S-C2	0,13	2,5	0,21	0,011	1,6	2,7	0,16	1,6	0,4	21	0,19	0,22	0,07	0,06	0,005
S-C3	0,05	4,2	0,93	0,023	1,0	3,1	0,20	1,3	1,5	6	0,10	0,96	0,10	0,24	0,021
S-C4	0,14	4,6	0,71	0,067	3,4	4,2	0,37	3,6	1,8	17	0,06	1,29	0,26	0,65	0,013
S-C5	0,39	3,7	2,60	0,330	1,8	9,2	0,59	1,6	6,1	19	0,85	3,57	0,38	3,67	0,170
S-D0	0,36	21,7	1,69	0,157	8,1	7,5	0,46	4,1	2,9	36	0,44	1,76	0,78	4,98	0,082
S-D1	0,60	7,6	1,09	0,076	0,6	4,4	0,39	1,0	1,1	12	0,69	0,66	0,59	2,07	0,090
S-D2	0,01	1,7	0,12	0,075	3,3	3,5	0,07	1,8	1,7	14	0,08	1,30	0,55	1,61	0,053
S-D3	0,18	4,3	0,58	0,126	4,5	1,9	0,19	0,6	1,5	23	0,08	0,10	0,75	1,29	0,067
S-D5	0,20	5,1	0,47	0,089	3,3	8,8	0,89	6,1	1,1	36	0,18	1,25	0,90	1,73	0,081
S-D7	0,04	27,8	0,31	0,062	22,9	7,3	0,57	3,3	3,9	53	0,35	3,07	1,25	2,20	0,208
S-D9	0,28	5,8	0,62	0,199	0,7	8,9	1,00	6,0	3,7	59	0,28	3,02	0,69	1,17	0,133
S-D15	0,06	3,7	1,06	0,096	1,2	8,4	0,77	4,3	2,5	42	1,25	1,72	0,35	0,49	0,042
November	0,09	12,0	1,05	0,058	8,0	4,8	0,10	1,8	2,7	1	0,14	0,23	0,29	0,06	0,002
March	0,45	5,5	2,02	0,050	7,4	4,7	0,24	2,2	3,9	8	0,23	0,13	0,20	0,07	0,001

Moderate contamination

Concentrations (µg.g^{-1}) Rhizomes (Rz)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
Mean															
Rz-T0	1,02	51,9	0,65	0,132	40,6	3,4	0,17	33,4	6,4	66	1,28	5,29	1,16	0,16 *	0,004
Rz-C1	0,89	61,2	0,40	0,154	51,8	3,1	0,14	23,0	5,2	49	0,79	3,64	1,15	0,21 *	0,004
Rz-C2	0,61	24,9	0,24	0,051	24,3	2,1	0,10	24,0	6,0	65	0,68	4,86	1,31	0,19 **	0,001
Rz-C3	0,56	77,1	0,61	0,093	36,3	3,0	0,12	21,5	6,0	62	0,94	5,30	1,22	0,16 *	0,002
Rz-C4	0,37	9,5	0,13 *	0,035	13,7	2,1	0,09	20,2	5,6	56	0,30	4,40	1,11 *	0,06	~~0,001~~
Rz-C5	0,55	34,1	0,14	0,092	27,1	2,5	0,09	11,7	5,8	39	0,28	4,38	1,05	0,11 *	0,004
Rz-D0	0,62	42,1	0,48	0,102	31,7	3,2	0,14	23,6	6,7	53	1,14	5,64	1,15	0,24 *	0,003
Rz-D1	0,54	87,6	0,29	0,110	52,4	3,4	0,13	17,0	6,8	41	0,35	3,52	1,12	0,18 *	0,005
Rz-D2	1,27	66,7	1,22	0,217	53,2	3,8	0,15	14,4	7,2	49	1,92	5,30	1,30	0,26 *	0,004
Rz-D3	0,76	85,6	0,95	0,107	43,4	2,2	0,12	29,2	7,1	43	0,79	3,39	1,24	0,16 *	0,003
Rz-D5	0,84	175,3	0,98	0,200	110,1	4,7	0,20	34,6	10,2	66	1,69	4,10	1,40	0,35 *	0,005
Rz-D7	0,48	129,6	0,33	0,127	58,2	3,5	0,13	26,9	9,6	64	0,43	7,37	1,48	0,22	0,009
Rz-D9	0,52	92,1	0,35	0,105	56,0	3,8	0,11	21,5	7,6	52	0,75	4,78	1,22	0,15 *	0,004
Rz-D15	0,71	59,3	1,16	0,091	42,5	4,1	0,15	19,3	8,1	58	0,93	5,65	1,30	0,14	0,007
November	0,38	29,1	0,22	0,052	25,9	2,4	0,10	33,2	8,4	44	0,45	2,58	1,21	0,07 **	0,002
March	0,91	132,1	0,85	0,156	71,3	3,8	0,13	28,6	7,8	61	1,11	4,02	1,29	0,27 *	0,005
SD															
Rz-T0	0,52	33,2	0,49	0,086	25,7	1,6	0,09	16,2	3,0	25	1,31	3,50	0,27	0,09	0,003
Rz-C1	0,12	22,1	0,31	0,073	31,6	0,6	0,01	2,6	0,2	7	0,44	0,67	0,05	0,12	0,002
Rz-C2	0,24	11,0	0,20	0,016	7,1	0,1	0,02	7,9	0,8	15	0,35	2,05	0,08	0,09	0,000
Rz-C3	0,21	48,1	0,57	0,069	18,6	0,9	0,00	3,1	0,6	15	0,61	2,15	0,09	0,09	0,002
Rz-C4	0,03	3,5	0,06	0,007	1,5	0,3	0,01	0,9	0,0	1	0,04	0,54	0,04	0,01	0,000
Rz-C5	0,08	8,4	0,03	0,015	5,7	0,7	0,01	4,3	0,6	4	0,07	0,20	0,15	0,04	0,002
Rz-D0	0,26	19,3	0,51	0,056	16,1	0,9	0,05	12,0	1,7	20	1,08	1,95	0,24	0,11	0,001
Rz-D1	0,16	48,0	0,03	0,018	18,6	0,5	0,01	2,2	0,5	3	0,02	0,62	0,08	0,02	0,001
Rz-D2	1,22	18,8	1,53	0,211	15,5	0,7	0,04	11,1	1,7	11	2,29	2,34	0,07	0,22	0,003
Rz-D3	0,18	5,1	1,01	0,043	14,9	0,2	0,01	19,1	2,9	9	0,48	0,37	0,23	0,06	-
Rz-D5	0,47	153,5	1,13	0,188	105,0	1,9	0,10	4,4	3,6	13	1,40	0,83	0,14	0,33	0,005
Rz-D7	0,08	81,8	0,12	0,048	24,4	0,8	0,01	4,7	0,9	6	0,10	2,33	0,17	0,10	0,007
Rz-D9	0,03	41,4	0,18	0,040	33,5	1,0	0,02	7,9	0,8	9	0,43	1,25	0,16	0,03	0,003
Rz-D15	0,23	111,8	0,71	0,097	34,1	0,8	0,01	4,7	0,4	21	0,53	1,32	0,22	0,21	0,006
November	0,11	11,5	0,15	0,023	6,1	0,2	0,03	10,2	3,0	11	0,18	0,85	0,28	0,04	0,001
March	0,20	34,1	0,62	0,026	12,4	0,8	0,02	2,5	2,6	8	0,37	1,04	0,11	0,07	0,001

Annex A *(Continued)*.

Trace element concentrations (mean ± SD, in µg.g^{-1} of dry weight) in intermediate and adult leaves of *P. oceanica* regularly sampled (June 2009) during the contamination phase ("C") at acute levels and during the decontamination phase ("D"). Concentrations in post-controls sampled in Novembre (2009) and March (2010) are also given. *, ** and struck-through values represent concentrations < L_Q, < L_D and < L_C, respectively. Contamination times are given in days, from T0 to C1. Decontaminantion periods lasted 15 days.

Acute contamination

Concentrations (µg.g^{-1}) Intermediate leaves (IL)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
Mean															
IL-T0	1,51	19,7	4,24	0,197	42,4	45,5	2,43	28,1	6,1	77	1,95	0,66	2,76	0,86	~~0,002~~
IL-C0,125	7,43	25,5	14,77	0,606	41,8	40,3	1,94	26,5	11,9	97	46,05	3,28	3,29	10,74	0,322
IL-C0,25	1,87	21,3	4,28	0,767	40,5	48,9	2,75	28,3	7,7	116	1,92	1,87	3,50	2,88	0,064
IL-C0,375	277,43	25,9	54,96	2,635	55,5	36,9	2,28	24,8	24,7	137	989,27	11,79	6,51	67,82	3,903
IL-C0,5	46,13	25,9	17,40	3,287	42,9	39,7	2,44	31,0	39,2	156	32,48	12,67	5,45	38,72	1,113
IL-C1 = IL-D0	191,49	46,6	45,02	4,214	62,0	41,9	2,42	27,1	44,3	140	227,34	9,28	4,46	62,85	3,528
IL-D0,5	53,44	51,9	8,60	2,583	61,9	35,1	2,53	32,9	42,1	119	68,36	13,05	4,11	23,27	1,754
IL-D1	12,93	38,7	4,61	2,536	57,1	42,4	2,39	26,8	25,4	105	9,66	11,95	3,30	10,89	0,452
IL-D2	1,61	42,6	1,74	1,654	58,0	38,8	2,90	36,1	21,8	133	1,27	9,78	3,81	6,55	0,183
IL-D4	58,05	19,5	3,91	1,567	43,5	34,1	2,39	29,6	24,7	107	34,13	10,79	3,20	13,83	0,894
IL-D6	3,91	18,7	1,29	0,931	37,1	30,5	2,15	28,8	18,2	99	1,46	6,45	3,09	2,81	0,270
IL-D8	110,85	18,9	2,79	1,498	35,4	29,5	1,86	28,8	29,7	107	4,04	15,48	3,52	6,33	0,987
IL-D15	1,64	25,7	3,50	0,541	52,1	48,6	1,99	22,8	14,4	112	2,07	3,24	3,31	2,06	0,095
November	1,03	27,6	1,82	0,183	40,2	30,3	0,85	16,3	15,8	54	1,33	1,58	1,94	0,77 *	0,015
March	1,83	38,6	6,29	0,128	42,7	29,1	1,30	24,9	13,3	70	1,67	1,24	2,59	0,63 *	0,008
SD															
IL-T0	0,12	9,1	0,39	0,024	4,1	5,6	0,23	2,5	0,4	7	0,85	0,08	0,19	0,08	0,000
IL-C0,125	2,95	11,5	9,76	0,116	6,4	2,9	0,07	1,9	2,1	11	23,78	2,03	0,25	4,30	0,177
IL-C0,25	0,08	4,4	0,45	0,165	3,7	1,2	0,46	1,0	1,2	9	0,25	0,64	0,24	0,61	0,022
IL-C0,375	263,69	5,5	39,76	0,101	5,5	7,6	0,55	5,6	5,4	15	895,42	0,36	1,43	43,74	3,394
IL-C0,5	49,54	8,3	10,42	0,362	4,3	5,8	0,21	1,8	19,3	4	19,83	1,20	1,21	25,78	1,075
IL-C1 = IL-D0	219,92	15,2	36,45	0,612	14,9	10,4	0,28	5,9	8,5	10	224,26	2,67	0,91	47,96	2,910
IL-D0,5	37,44	23,1	4,99	0,467	12,4	1,1	0,16	1,8	13,7	8	101,35	1,65	0,58	3,82	0,694
IL-D1	18,46	24,1	2,53	0,350	18,1	5,3	0,40	2,6	4,7	16	5,52	1,40	0,61	4,07	0,359
IL-D2	1,15	41,1	0,90	0,376	32,5	3,6	0,55	3,9	0,5	14	0,10	0,31	0,34	1,42	0,024
IL-D4	96,07	5,7	3,62	0,425	3,2	6,1	0,43	6,6	8,0	16	55,24	2,24	0,59	17,30	1,245
IL-D6	5,70	5,6	0,92	0,908	10,0	7,4	0,60	9,1	14,7	27	0,83	5,86	1,13	2,53	0,394
IL-D8	190,52	2,9	1,62	0,443	5,2	8,8	0,71	2,4	7,8	12	5,34	4,72	1,04	4,31	1,334
IL-D15	0,28	8,3	1,16	0,372	6,8	6,9	0,24	3,2	11,0	15	0,36	2,87	0,86	0,90	0,119
November	0,37	13,7	1,48	0,120	9,0	10,5	0,54	3,6	7,1	6	0,24	0,47	0,63	0,28	0,018
March	0,20	13,6	1,53	0,012	4,9	2,3	0,22	2,9	2,9	6	0,41	0,39	0,35	0,09	0,002

Acute contamination

Concentrations (µg.g^{-1}) Adult leaves (AL)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
Mean															
AL-T0	1,54	34,3	3,67	0,311	56,2	56,9	3,34	37,0	6,3	105	2,03	0,36	2,66	1,35	0,009
AL-C0,125	17,53	33,1	28,99	1,016	52,2	50,0	2,95	32,8	13,9	129	195,22	6,05	3,22	38,81	0,444
AL-C0,25	2,00	34,9	7,41	0,853	50,4	55,1	3,60	36,4	7,6	130	2,41	1,19	2,60	3,01	0,059
AL-C0,375	35,51	63,3	18,22	2,115	54,6	47,8	3,08	33,4	19,3	127	301,42	6,56	3,55	31,19	0,577
AL-C0,5	27,70	73,9	24,62	2,935	56,1	50,6	3,67	38,3	20,7	153	267,73	8,26	3,36	21,02	1,683
AL-C1 = AL-D0	95,99	62,2	30,33	3,821	68,8	45,5	2,94	28,2	33,4	132	146,64	9,20	3,28	30,44	1,746
AL-D0,5	87,96	82,9	13,97	3,210	77,6	38,4	2,92	30,3	28,4	126	63,45	10,64	2,93	28,04	2,227
AL-D1	19,18	42,6	7,36	2,543	60,5	46,0	3,40	33,5	21,5	128	16,60	10,23	2,60	14,07	0,484
AL-D2	2,18	62,3	2,32	1,628	61,1	36,5	2,97	31,5	16,3	120	1,49	6,27	2,72	4,83	0,142
AL-D4	7,68	57,4	3,80	1,572	62,6	40,2	3,29	36,1	14,2	138	2,39	6,39	2,80	4,44	0,266
AL-D6	32,23	44,7	2,66	1,408	55,5	33,5	2,78	28,6	15,9	130	2,31	6,68	2,80	5,41	0,672
AL-D8	1,39	56,9	2,59	1,471	60,9	37,7	2,89	32,0	16,3	125	1,19	7,12	2,90	3,35	0,172
AL-D15	2,10	61,9	4,67	0,755	64,8	44,5	2,45	22,6	9,2	110	2,33	3,44	2,44	2,31	0,069
November	0,73	30,1	2,25	0,204	41,9	38,6	1,16	12,9	12,2	51	1,94	1,54	1,61	1,18 *	0,009
March	2,07	53,2	8,31	0,169	47,9	30,2	1,68	22,3	9,6	64	1,78	0,73	2,03	0,92 *	0,011
SD															
AL-T0	0,01	12,4	0,78	0,065	6,4	4,3	0,48	2,2	0,7	12	0,90	0,09	0,34	0,18	0,003
AL-C0,125	24,87	6,6	16,45	0,174	5,8	4,8	0,29	1,3	5,0	13	310,10	7,54	0,56	56,81	0,429
AL-C0,25	0,05	14,3	1,31	0,148	6,1	1,9	0,17	1,5	1,3	11	0,39	0,27	0,15	0,97	0,027
AL-C0,375	55,47	5,9	18,78	0,532	8,6	1,9	0,20	1,6	5,2	6	413,23	2,25	0,92	35,40	0,781
AL-C0,5	43,86	14,3	22,69	0,403	5,6	4,6	0,34	3,3	1,7	6	421,04	3,05	0,57	13,55	2,559
AL-C1 = AL-D0	120,61	29,2	33,12	1,194	17,6	9,8	0,54	4,7	10,3	16	186,33	2,90	0,45	26,95	1,863
AL-D0,5	104,82	41,6	13,80	1,784	24,9	2,3	0,11	1,3	12,4	8	74,79	4,66	0,17	28,03	2,499
AL-D1	21,80	8,8	1,94	0,353	14,1	7,3	0,88	7,9	1,6	29	11,10	3,95	0,32	5,07	0,306
AL-D2	1,38	9,1	0,69	0,324	6,7	2,0	0,29	1,3	3,7	10	0,13	2,05	0,18	1,44	0,054
AL-D4	10,36	16,6	0,94	0,226	5,9	1,8	0,07	1,1	2,7	5	1,33	3,26	0,10	0,86	0,164
AL-D6	53,52	18,0	1,69	0,706	9,2	3,6	0,27	1,3	5,6	21	1,84	3,23	0,31	4,58	1,011
AL-D8	0,47	12,9	0,85	0,789	14,5	3,6	0,29	2,4	7,5	1	0,09	4,21	0,12	1,80	0,136
AL-D15	0,29	15,0	1,37	0,460	5,6	5,4	0,22	1,9	4,2	13	0,50	3,83	0,28	0,91	0,068
November	0,06	11,1	1,39	0,079	6,3	7,5	0,36	0,3	2,6	7	0,45	0,31	0,25	0,35	0,003
March	0,23	12,4	1,27	0,011	6,3	1,1	0,18	2,0	0,6	7	0,21	0,07	0,16	0,09	0,005

Annex A *(Continued)*.

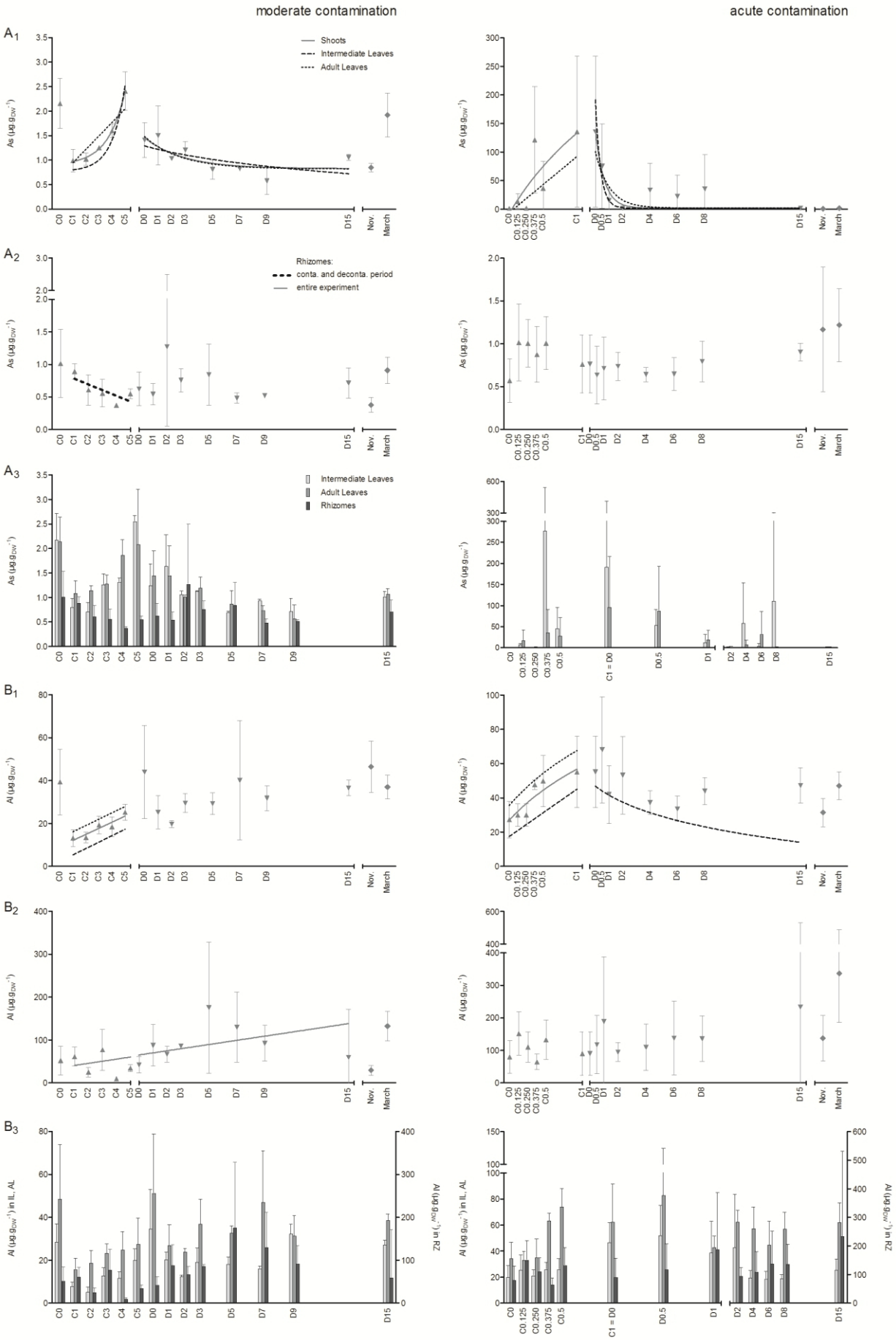
Trace element concentrations (mean ± SD, in µg.g^{-1} of dry weight) in shoots and rhizomes of *P. oceanica* regularly sampled (June 2009) during the contamination phase ("C") at acute levels and during the decontamination phase ("D"). Concentrations in post-controls sampled in Novembre (2009) and March (2010) are also given. *, ** and struck-through values represent concentrations < L_Q, < L_D and < L_C, respectively. Contamination times are given in days, from T0 to C1. Decontaminantion periods lasted 15 days.

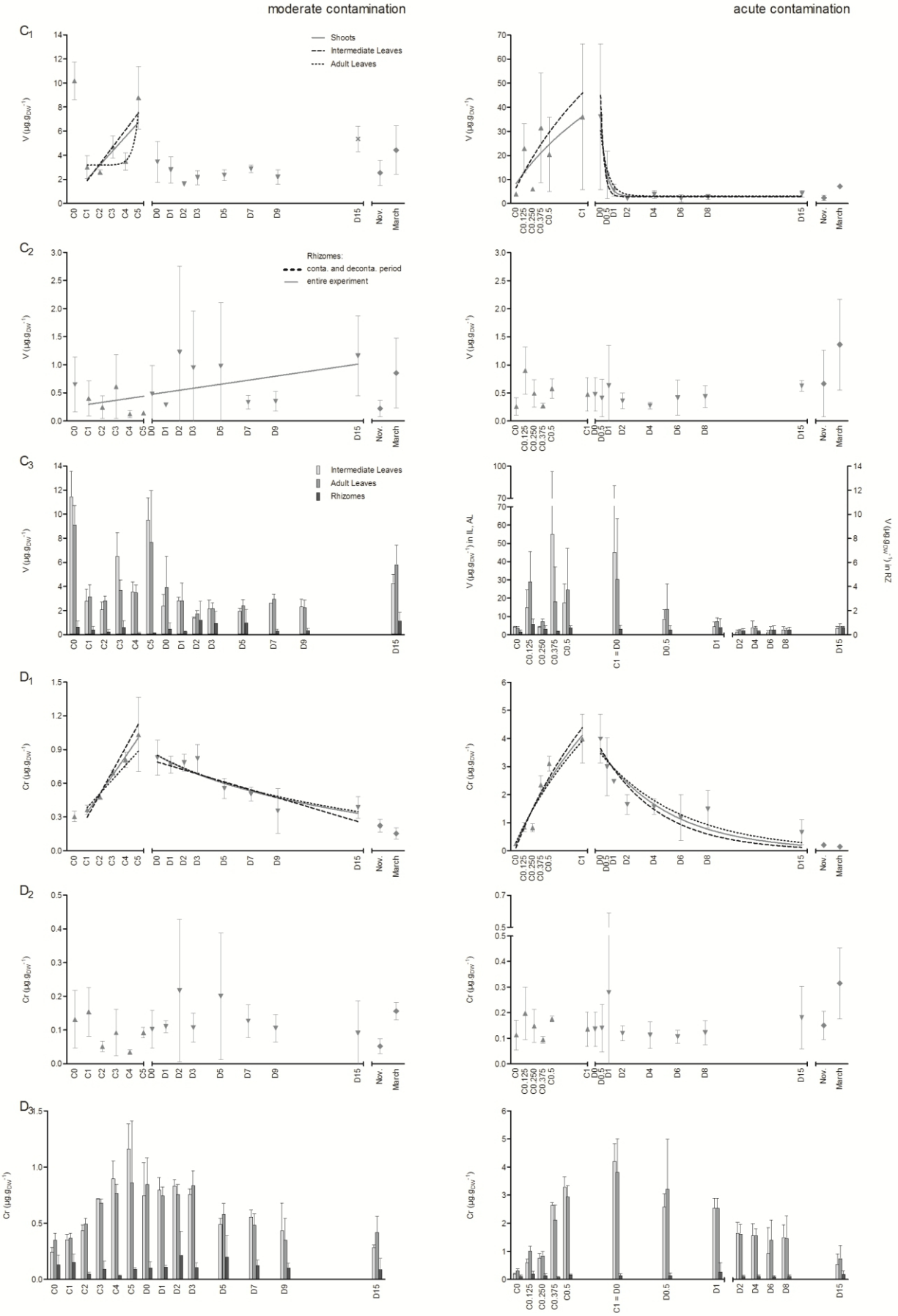
Acute contamination

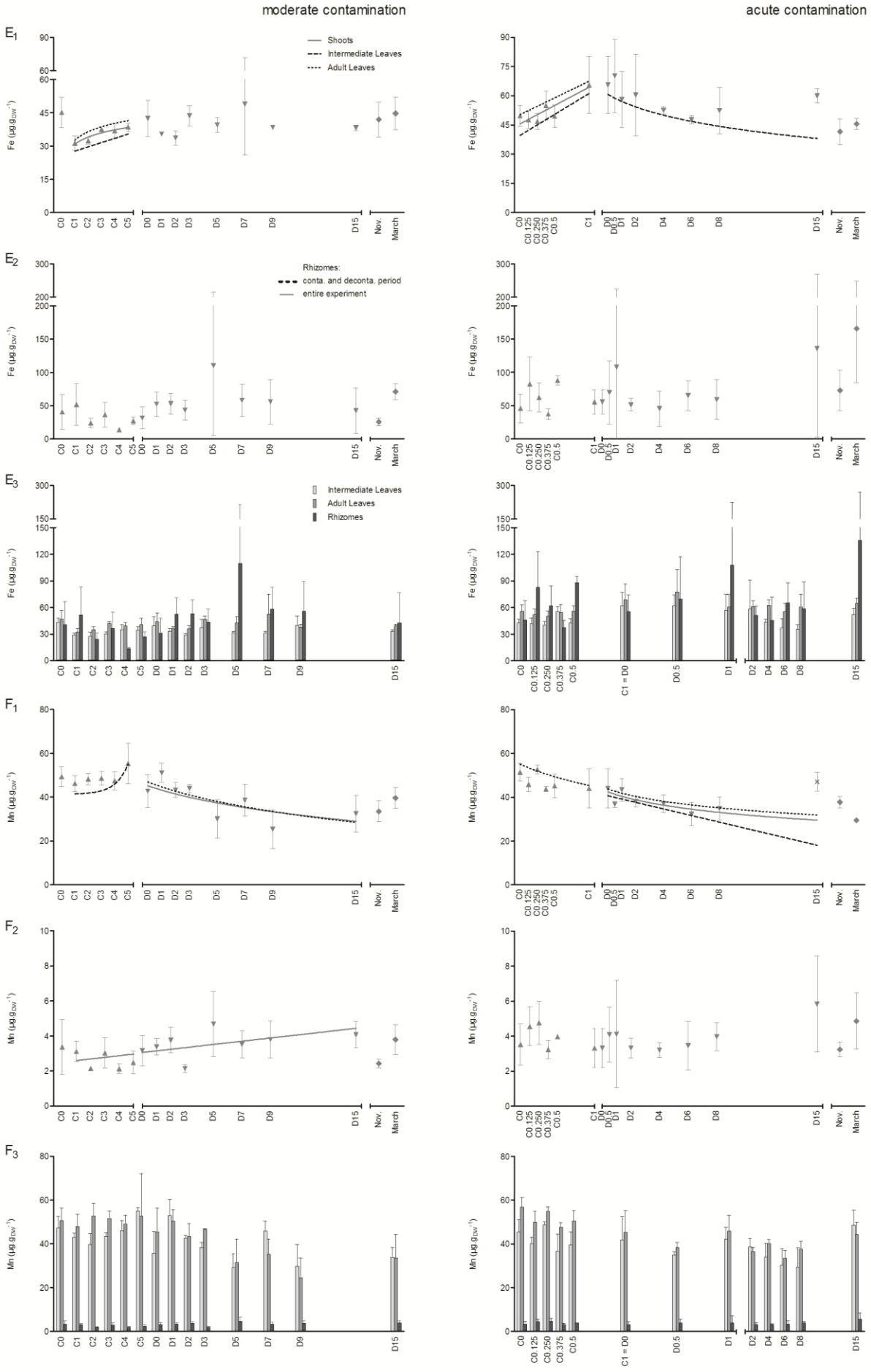
Concentrations (µg.g^{-1}) Shoots (S)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb		Bi
Mean																
S-T0	1,53	27,3	3,95	0,257	49,6	51,5	2,90	32,7	6,2	92	1,99	0,50	2,70	1,12	**	0,006
S-C0,125	13,57	29,9	22,88	0,845	47,6	45,9	2,52	30,1	13,1	115	135,17	4,91	3,25	27,47		0,392
S-C0,25	1,94	30,0	6,00	0,820	46,9	52,8	3,29	33,2	7,7	124	2,22	1,50	2,97	2,93		0,061
S-C0,375	121,41	47,6	31,48	2,341	55,0	43,8	2,78	30,1	21,5	132	539,37	8,79	4,72	44,51		1,771
S-C0,5	36,65	49,9	20,37	3,105	49,6	45,2	3,08	34,8	30,5	155	138,40	10,36	4,38	30,11		1,351
S-C1 = S-D0	135,80	55,1	36,04	3,987	65,6	44,0	2,73	27,8	37,9	136	178,97	9,22	3,76	43,70		2,437
S-D0,5	75,53	68,0	11,90	2,996	70,2	36,8	2,74	31,4	35,3	123	65,64	12,19	3,46	26,99		2,089
S-D1	15,49	41,9	5,55	2,471	58,1	43,4	2,75	29,0	23,3	112	12,47	11,29	3,01	11,89		0,449
S-D2	1,89	53,2	2,06	1,649	60,4	38,1	2,98	34,4	18,9	128	1,37	8,07	3,27	5,64		0,161
S-D4	33,71	37,3	3,82	1,566	52,4	37,1	2,82	32,8	19,8	122	18,79	8,77	3,03	9,31		0,588
S-D6	22,37	33,5	2,10	1,181	47,6	32,3	2,51	28,9	16,5	115	1,99	6,41	2,91	4,36		0,528
S-D8	35,70	44,0	2,64	1,483	52,3	34,9	2,54	30,9	20,9	119	2,08	9,97	3,13	4,35		0,431
S-D15	1,93	47,2	4,08	0,660	59,9	47,1	2,27	22,8	11,1	111	2,21	3,42	2,82	2,22		0,078
November	0,86	31,4	2,34	0,208	41,5	37,7	1,16	13,7	12,5	53	1,81	1,53	1,65	1,11	*	0,011
March	1,96	47,0	7,19	0,149	45,5	29,5	1,49	23,6	11,3	67	1,72	0,96	2,30	0,77	*	0,009
SD																
S-T0	0,06	10,6	0,40	0,041	5,3	4,0	0,29	2,0	0,2	9	0,88	0,04	0,23	0,11		0,002
S-C0,125	13,58	6,6	10,24	0,162	4,5	3,3	0,19	0,5	3,7	10	194,04	4,30	0,43	34,08		0,250
S-C0,25	0,02	6,6	0,34	0,141	4,1	2,1	0,16	0,5	1,3	8	0,39	0,52	0,32	0,78		0,025
S-C0,375	93,31	2,8	22,85	0,332	7,4	1,2	0,17	2,3	5,2	4	422,83	1,34	0,69	35,07		1,292
S-C0,5	47,48	14,9	15,50	0,268	5,7	5,5	0,17	1,4	11,6	3	196,34	1,10	0,39	20,70		1,757
S-C1 = S-D0	132,35	20,8	30,27	0,868	14,8	8,9	0,34	4,6	7,5	11	192,82	2,78	0,62	34,83		1,974
S-D0,5	73,90	31,0	9,83	1,041	18,9	1,4	0,04	0,8	8,8	6	81,01	1,78	0,26	17,07		1,698
S-D1	20,93	16,8	2,36	0,078	14,4	5,2	0,30	3,5	2,3	12	8,61	2,57	0,50	4,30		0,331
S-D2	1,15	22,6	0,76	0,352	20,8	2,5	0,36	2,7	2,5	12	0,09	1,19	0,16	1,35		0,036
S-D4	47,23	6,8	1,66	0,275	1,9	4,0	0,20	3,6	3,2	6	28,09	1,69	0,38	8,85		0,584
S-D6	37,03	7,4	1,44	0,828	2,1	5,3	0,41	5,1	9,0	22	1,52	4,28	0,65	3,98		0,802
S-D8	60,04	7,8	1,10	0,670	12,0	5,3	0,42	2,2	7,4	4	1,70	4,19	0,47	2,57		0,505
S-D15	0,20	10,2	1,40	0,442	3,7	4,3	0,08	2,3	6,5	14	0,41	3,46	0,44	0,90		0,086
November	0,18	8,4	1,04	0,067	6,5	2,7	0,08	0,9	1,7	5	0,20	0,36	0,13	0,16		0,004
March	0,11	8,1	0,75	0,003	2,8	1,0	0,23	2,1	1,4	6	0,30	0,14	0,20	0,08		0,003

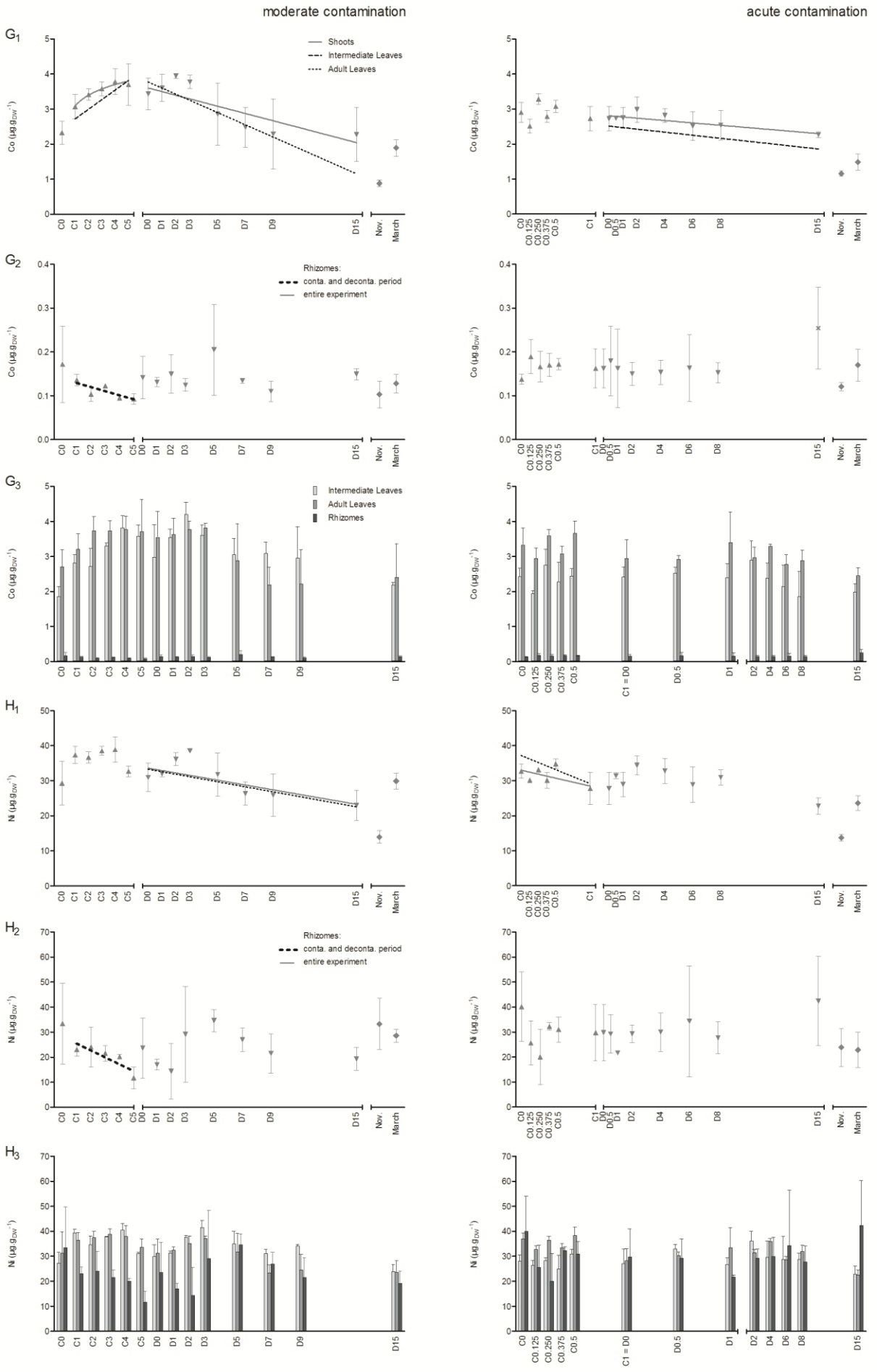
Acute contamination

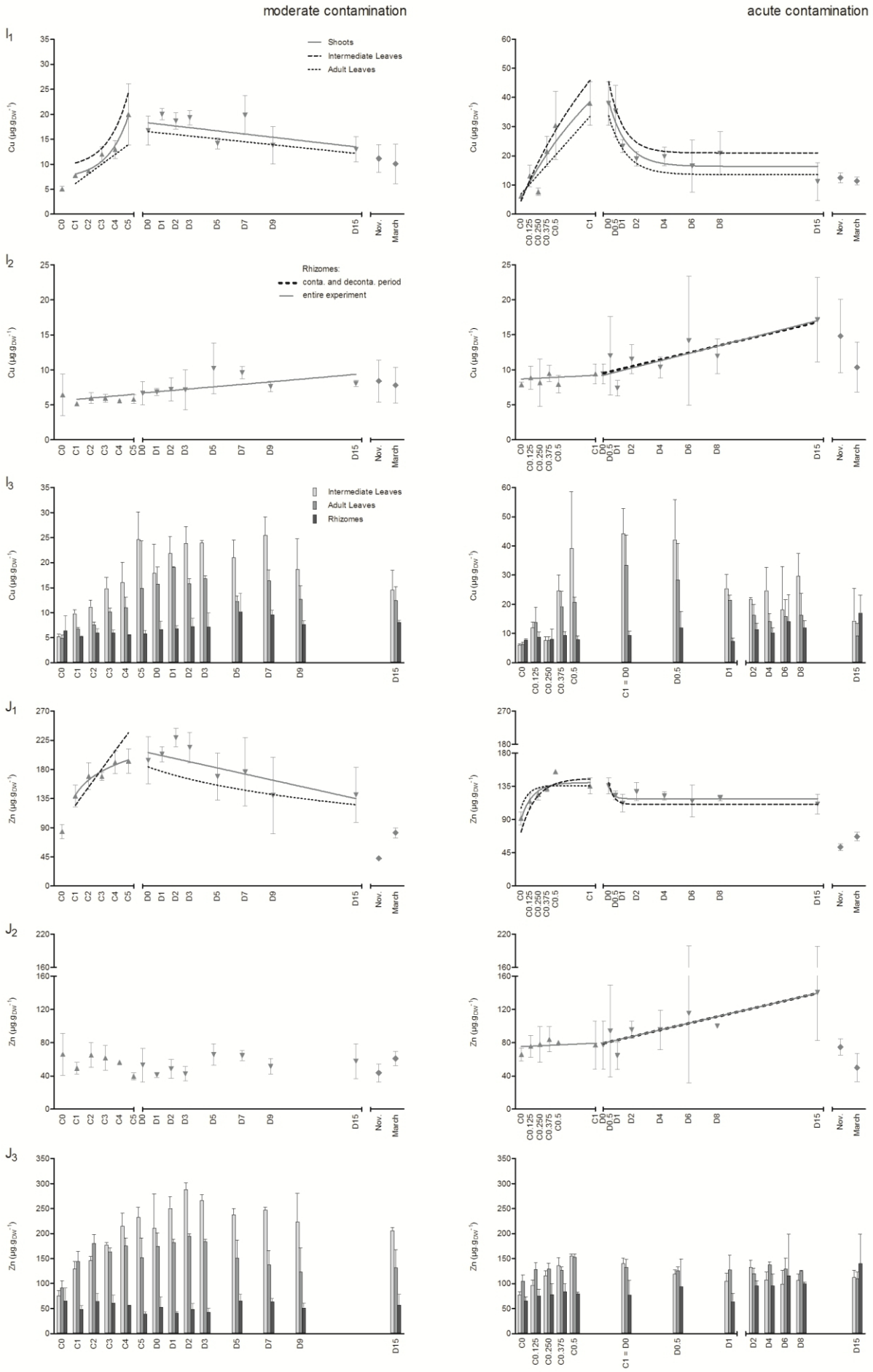
Concentrations (µg.g^{-1}) Rhizomes (Rz)	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb		Bi
Mean																
Rz-T0	0,57	80,0	0,26	0,113	45,8	3,5	0,14	40,1	7,9	66	0,68	4,48	1,23	0,16		0,030
Rz-C0,125	1,01	151,6	0,90	0,198	82,8	4,6	0,19	25,6	8,9	76	1,95	6,06	1,67	0,35		0,009
Rz-C0,25	1,00	110,5	0,50	0,148	62,3	4,8	0,17	20,1	8,2	78	1,21	5,47	1,61	0,23		0,007
Rz-C0,375	0,88	64,6	0,27	0,094	37,4	3,2	0,17	32,3	9,5	84	1,54	5,92	1,63	0,17	*	0,004
Rz-C0,5	1,01	133,0	0,58	0,175	88,0	4,0	0,17	31,0	7,9	80	1,27	4,76	1,43	0,31		0,007
Rz-C1 = Rz-D0	0,76	90,3	0,48	0,136	55,6	3,3	0,16	29,8	9,4	77	3,21	5,58	1,40	0,28		0,007
Rz-D0,5	0,64	117,5	0,41	0,140	69,8	4,1	0,18	29,2	12,0	94	0,87	5,98	1,42	0,29		0,007
Rz-D1	0,71	188,0	0,63	0,279	108,1	4,1	0,16	21,7	7,4	64	0,78	4,84	1,33	0,57		0,017
Rz-D2	0,74	94,9	0,36	0,119	51,3	3,3	0,15	29,3	11,5	95	1,18	7,17	1,96	0,21		0,006
Rz-D4	0,64	109,5	0,27	0,112	45,6	3,2	0,15	30,0	10,4	95	0,88	7,05	1,53	0,17	*	0,004
Rz-D6	0,65	137,9	0,42	0,106	65,2	3,5	0,16	34,3	14,1	115	1,50	6,76	1,53	0,21	*	0,005
Rz-D8	0,79	135,9	0,44	0,122	59,0	4,0	0,15	27,7	11,9	100	1,75	7,06	1,79	0,24		0,006
Rz-D15	0,90	233,1	0,63	0,181	136,0	5,8	0,25	42,4	17,1	141	1,01	8,14	1,49	0,30		0,007
November	1,17	137,3	0,67	0,150	72,8	3,3	0,12	23,9	14,8	75	1,35	6,31	1,53	0,28	*	0,005
March	1,22	336,9	1,36	0,315	166,2	4,9	0,17	22,9	10,3	50	3,04	5,89	1,19	0,50		0,012
SD																
Rz-T0	0,25	50,3	0,16	0,058	21,6	1,2	0,01	13,9	0,4	8	0,45	0,87	0,07	0,11		0,043
Rz-C0,125	0,45	66,9	0,42	0,103	40,1	1,1	0,04	8,7	1,7	13	0,84	1,41	0,16	0,17		0,002
Rz-C0,25	0,28	47,1	0,24	0,065	21,9	1,2	0,04	11,1	3,4	21	0,39	3,04	0,30	0,15		0,002
Rz-C0,375	0,33	24,1	0,05	0,014	8,0	0,5	0,03	1,5	1,2	15	0,25	1,96	0,21	0,05		0,000
Rz-C0,5	0,31	60,7	0,17	0,011	7,0	0,0	0,01	5,0	1,3	2	0,62	0,56	0,12	0,01		0,000
Rz-C1 = Rz-D0	0,34	66,6	0,30	0,067	18,5	1,1	0,04	11,2	1,4	29	4,87	3,07	0,17	0,18		0,004
Rz-D0,5	0,34	89,6	0,33	0,093	47,5	1,6	0,08	7,7	5,6	55	0,48	4,59	0,19	0,25		0,004
Rz-D1	0,37	199,5	0,72	0,312	115,6	3,1	0,09	0,8	1,1	16	0,68	1,56	0,21	0,73		0,021
Rz-D2	0,16	29,3	0,14	0,029	9,9	0,6	0,03	3,6	2,0	10	0,63	1,58	0,22	0,09		0,001
Rz-D4	0,08	70,9	0,06	0,052	26,2	0,4	0,03	7,7	1,5	24	0,42	2,36	0,15	0,06		0,001
Rz-D6	0,19	113,8	0,31	0,025	22,7	1,4	0,08	22,1	9,2	84	0,82	5,66	0,24	0,08		0,002
Rz-D8	0,24	69,4	0,20	0,047	30,0	0,8	0,02	6,3	2,5	2	1,22	0,64	0,10	0,08		0,003
Rz-D15	0,10	299,3	0,10	0,122	133,7	2,7	0,09	17,8	6,0	58	0,18	2,74	0,18	0,18		0,007
November	0,73	70,2	0,59	0,056	30,2	0,4	0,01	7,7	5,3	10	1,09	1,10	0,19	0,16		0,001
March	0,43	150,9	0,81	0,138	81,7	1,6	0,04	7,1	3,6	17	1,55	1,44	0,28	0,18		0,003

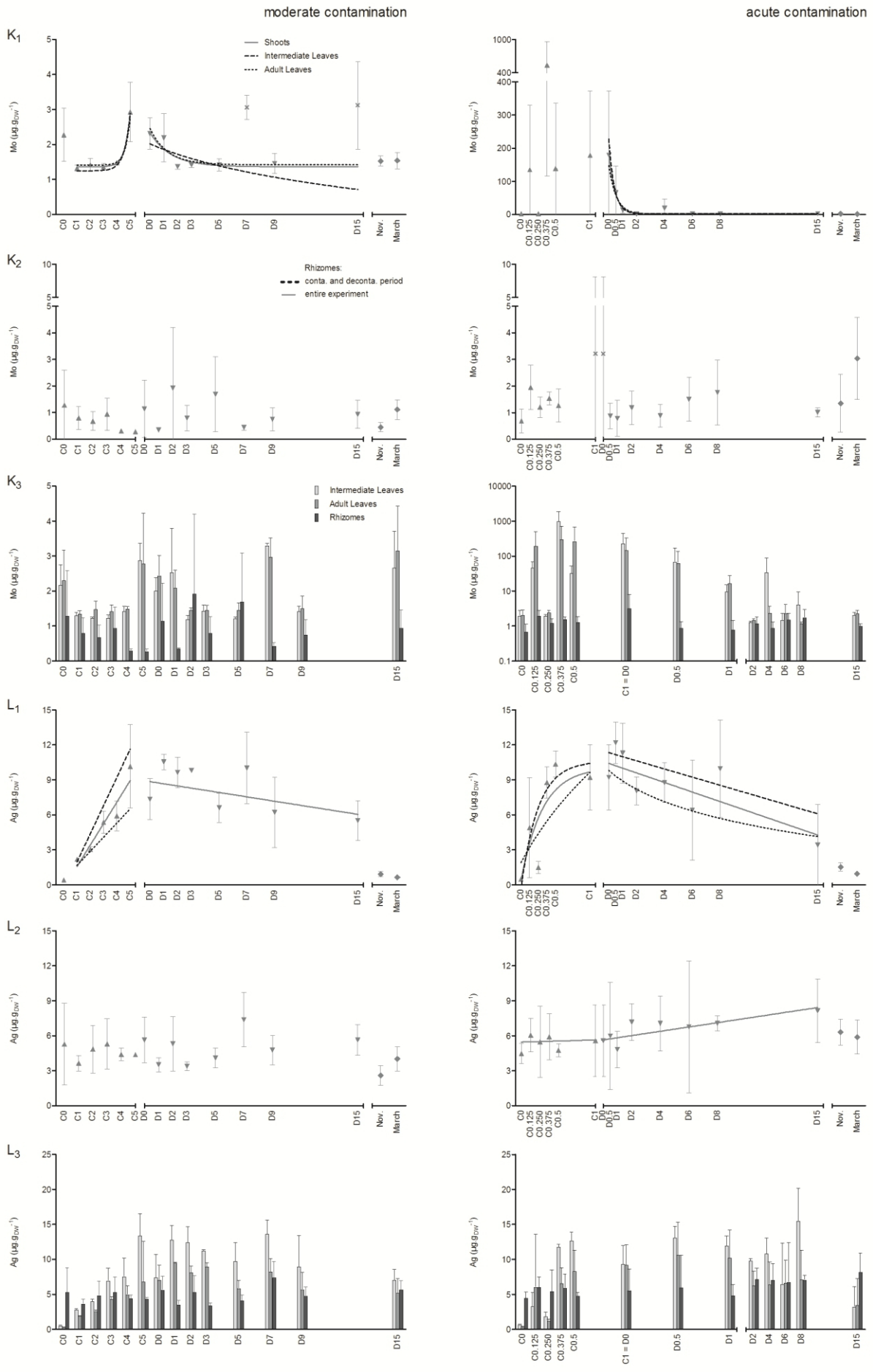

moderate contamination
acute contamination
A1
A2
A3
B1
B2
B3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
As (µg g$_{DW}^{-1}$)
Al (µg g$_{DW}^{-1}$)
Al (µg g$_{DW}^{-1}$) in IL, AL
Al (µg g$_{DW}^{-1}$) in RZ
C0
C1
C2
C3
C4
C5
D0
D1
D2
D3
D5
D7
D9
D15
Nov.
March
C0.125
C0.250
C0.375
C0.5
D0.5
D4
D6
D8
C1 = D0

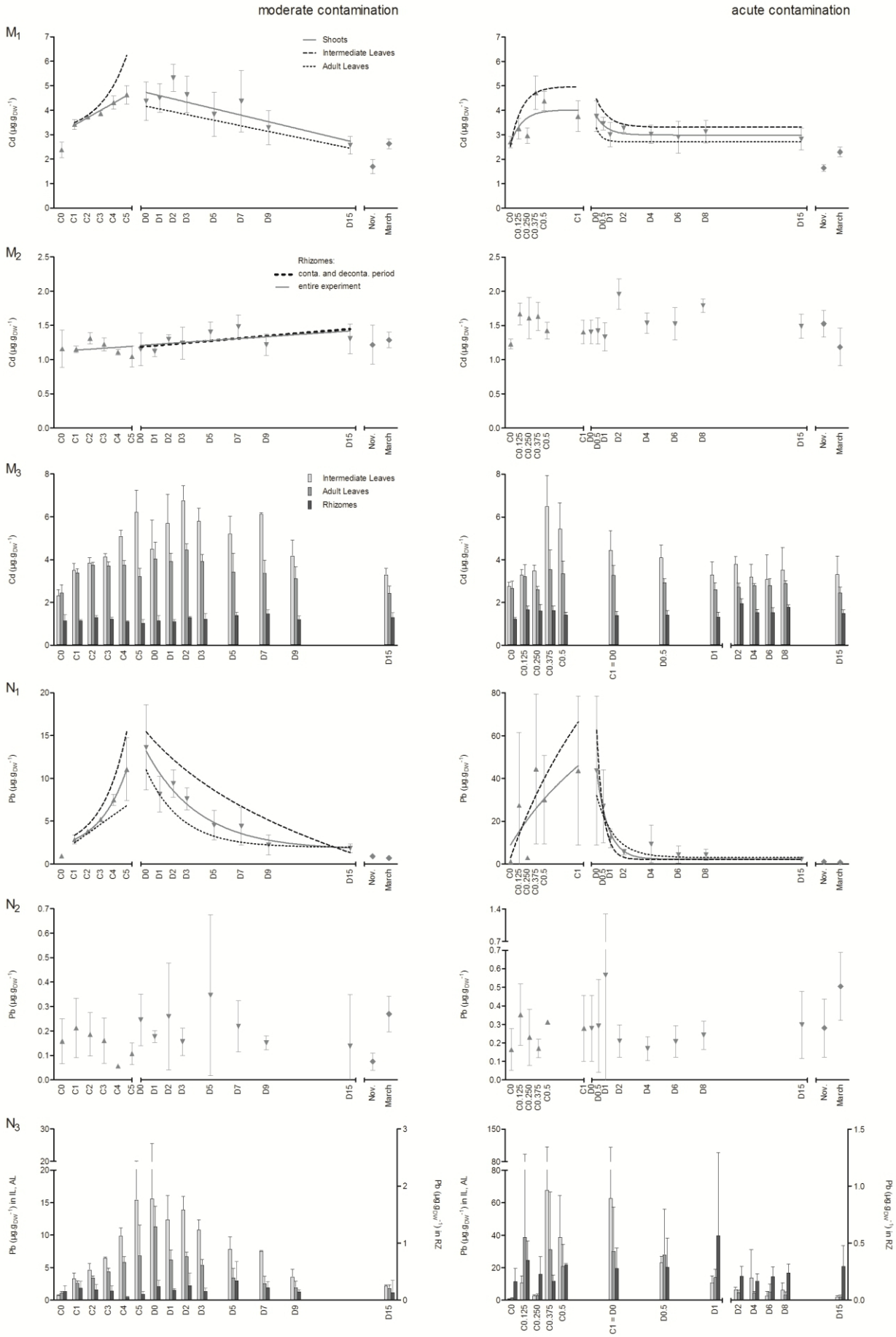

moderate contamination
acute contamination
C1
C2
C3
D1
D2
D3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
V (µg gDW-1)
Cr (µg gDW-1)
V (µg gDW-1) in IL, AL
V (µg gDW-1) in RZ
C0
C0.125
C0.250
C0.375
C0.5
C1
C1 = D0
D0.5
D1
D2
D4
D6
D8
D15
Nov.
March

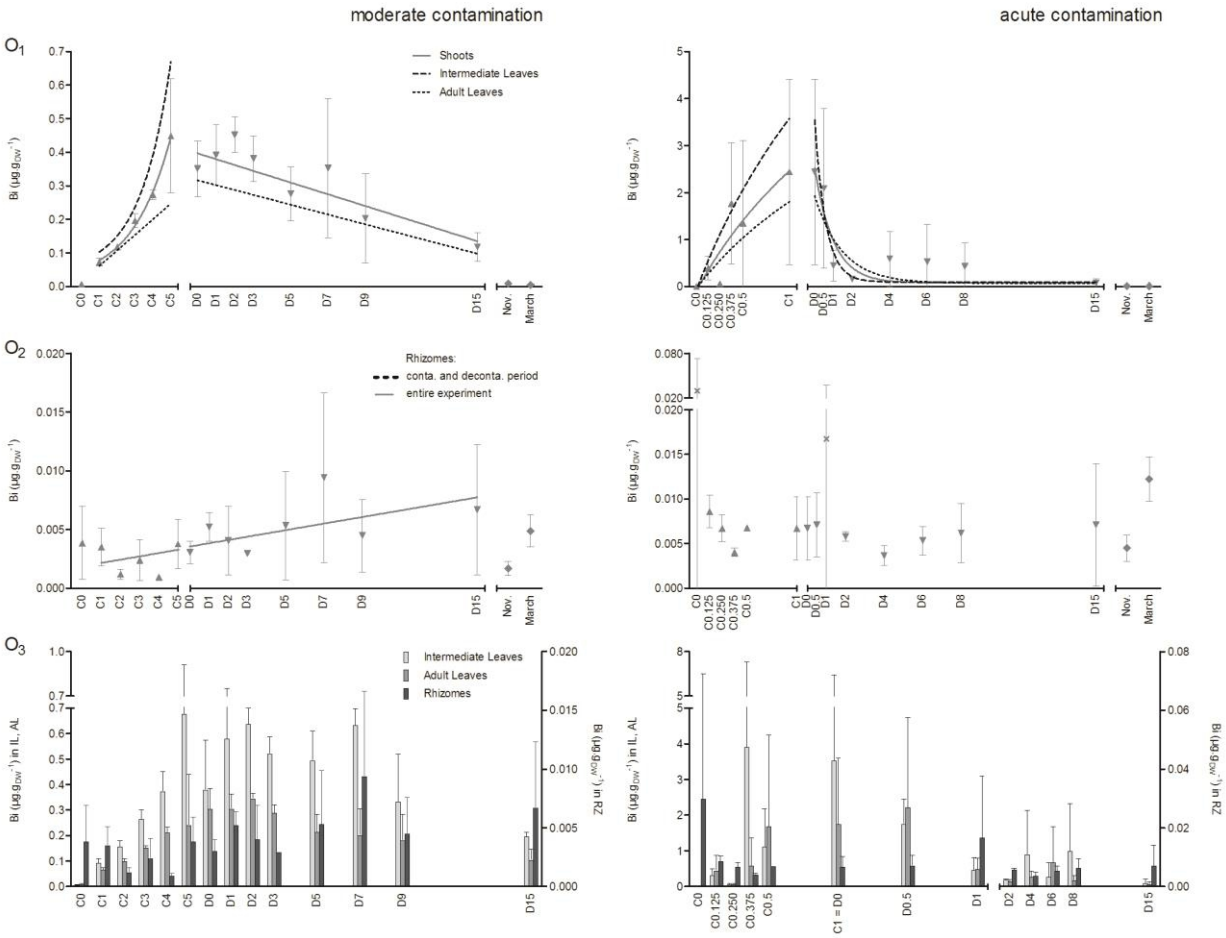

moderate contamination
acute contamination
E1
E2
E3
F1
F2
F3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
Fe (µg g$_{DW}^{-1}$)
Mn (µg g$_{DW}^{-1}$)
C0
C1
C2
C3
C4
C5
D0
D1
D2
D3
D5
D7
D9
D15
Nov.
March
C0.125
C0.250
C0.375
C0.5
D0.5
D4
D6
D8
C1 = D0


moderate contamination
acute contamination
G1
G2
G3
H1
H2
H3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
Co (µg gDW-1)
Ni (µg gDW-1)
C0
C1
C2
C3
C4
C5
D0
D1
D2
D3
D5
D7
D9
D15
Nov.
March
C0.125
C0.250
C0.375
C0.5
C1 = D0
D0.5
D4
D6
D8


moderate contamination
acute contamination
I1
I2
I3
J1
J2
J3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
Cu (µg gDW-1)
Zn (µg gDW-1)
C0
C1
C2
C3
C4
C5
D0
D1
D2
D3
D5
D7
D9
D15
Nov.
March
C0.125
C0.250
C0.375
C0.5
D0.5
D4
D6
D8
C1 = D0


moderate contamination
acute contamination
K1
K2
K3
L1
L2
L3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
Mo (µg.gDW-1)
Ag (µg.gDW-1)
C0
C1
C2
C3
C4
C5
D0
D1
D2
D3
D5
D7
D9
D15
Nov.
March
C0.125
C0.250
C0.375
C0.5
D0.5
D4
D6
D8
C1 = D0


moderate contamination
acute contamination
M1
M2
M3
N1
N2
N3
Shoots
Intermediate Leaves
Adult Leaves
Rhizomes:
conta. and deconta. period
entire experiment
Rhizomes
Cd (µg g$_{DW}^{-1}$)
Pb (µg g$_{DW}^{-1}$)
Pb (µg g$_{DW}^{-1}$) in IL, AL
Pb (µg g$_{DW}^{-1}$) in RZ
C0
C1
C2
C3
C4
C5
C0.125
C0.250
C0.375
C0.5
C1 = D0
D0
D0.5
D1
D2
D3
D5
D7
D9
D15
D4
D6
D8
Nov.
March



Annex B.

Uptake and loss kinetic models of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A_1-O_1) in *Posidonia oceanica* shoots (full grey line), intermediate leaves (IL, dashed black line) and adult leaves (AL, dotted black line) contaminated at moderate (left) or acute (right) levels. For clarity purpose, only concentrations calculated for shoots were indicated on kinetic graphs (symbols significance: upward triangle = uptake; downward triangle = loss; trapezium = November and March post-controls; cross = excluded from analysis).

Kinetics of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A_2-O_2) in rhizomes of *Posidonia oceanica* shoots contaminated at moderate (left) or acute (right) levels. The dotted thick black lines represent distinct linear kinetics observed during the contamination and decontamination periods, respectively; the continuous grey thin line models linear kinetics of the evolution of TE concentrations in rhizomes during the entire experiments (i.e. contamination and decontamination periods together).

Histograms of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A_3-O_3) tissue compartmentalizations between intermediate leaves (light grey bar), adult leaves (medium grey bar) and rhizomes (dark grey bar) during the contamination and decontamination periods at moderate (left) or acute (right) levels.

On the temporal X-axis, contamination periods C0 to C5 (moderate level) or C0 to C1 (acute level) and decontamination periods D0 to D15 (both contaminations) are given in days. TE concentrations are expressed in µg.g^{-1} of dry weight. Double bars symbolize standard deviations. Legends of kinetics and tissue compartmentalizations are only given on the 3 upper left graphs (moderate contamination) for clarity purpose, and are the same for the other graphs.

Chapter 4

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Influence of the physiology on the bioaccumulation of TEs by *Mytilus galloprovincialis*

The influence of body size and compartment, sex and reproductive status on the bioaccumulation of 19 trace elements by *Mytilus galloprovincialis*.

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Abstract

Numerous trace elements (TEs) of previous little concern can be considered as potential pollutants of the environment, their mining productions and industrial uses increasing worldwide. Their monitoring can be achieved through the use of bioindicator species, such as *Mytilus spp.*, widely used to monitor the chemical pollution of coastal ecosystems mainly by Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V. Levels of these 9 TEs, as well as levels of Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi, little or not surveyed in mussel watch programs, were analyzed by DRC ICP-MS in rope-grown Mediterranean mussels, *Mytilus galloprovincialis*.

Mussels efficiently accumulate the 19 studied trace elements (TEs). Bioaccumulation process is driven by numerous mutually dependent biological parameters such as the body size (length and flesh weight) and the tissue compartmentalization, the sex and the reproductive status; their respective influence still appear to be sometimes ambiguous. TE bioaccumulation is a power function of mussel soft body dry weight; total contents linearly increase with the shell length. These 2 models less fit in with small-size individuals, as they overall accumulate more chemicals, with a high variability. However, as small or large size mussels only represent about 5 % of individuals grown on commercial ropes, they are of minor importance for monitoring purposes. The influence of gametogenesis in determining female body higher TE concentrations prior to spawn cannot be neglected and varies depending on the element. TEs are preferentially accumulated in the hepatopancreas, except for Zn, Se, Cd and Mo which are more concentrated in gills. Gametogenesis does not influence the TE tissue distribution, but likely dilute their concentrations as a direct consequence of massive reproductive tissue production.

The results from the present study underline the potential use of *Mytilus galloprovincialis* in the biomonitoring of numerous little studied TEs and give some insight into the decisive role played by some relevant biological parameters in the bioaccumulation process of the 19 investigated TEs by rope-grown mussels.

Keywords: trace elements, *Mytilus galloprovincialis*, ICP-MS, Mediterranean Sea, morphometry, sex, body compartmentalization.

1. Introduction

In the ocean, trace element (TE) biogeochemical balance varies spatially and with depth depending on water masses physicochemical parameters (salinity, pH, temperature, etc.) and biological processes (Bruland and Lohan 2003). This balance may be dramatically modified at local, regional or global scales in all the abiotic and biotic compartments of coastal and estuarine waters due to anthropogenic activities such as urbanization, industry, agriculture or mining (Laubier 2005, Fernandez et al. 2007, Benedicto et al. 2011). If high Ag concentrations are usually associated with local urban sewage discharges (Lopez y Royo et al. 2009, Luy et al. 2012), inflows through major river outfalls (e.g. river Seine, France) might impact stations located several miles from these outfalls, as shown by Chiffoleau et al. (2005) who monitored Ag distribution in mussels and oysters along the French coasts. The evidence of long-range transports of metals (Cd, Ni and Cu) was also shown using bivalves along the Venezuelan coast near the outfall of the Tuy River (Jaffe et al. 1995). Atmospheric and oceanic currents can transfer chemicals far from their emission sources, as traced for radionuclides: controlled discharges to the British and the French coastal zones of man-made radionuclides from nuclear reprocessing facilities undergo long-distance transports, from European emission sources to the Arctic Ocean (Dahlgaard 1995). Oil and coal consumption are the dominant mechanism for anthropogenic mobilization of V (Hope 2008). If on a global scale, owing to the world ocean large mass and the observed variability in V concentrations, relative changes are unlikely to be discernible (Hope 2008), anthropogenic inputs at smaller scales and in the locality of specific sources have been observed worldwide (Amiard et al. 2004, Wang and Wilhelmy 2009).

Since the mid-70^{ies}, when Goldberg (1975) proposed the mussel-watch concept to record the quality of marine waters, mussels from the genus *Mytilus* have been used worldwide in biomonitoring surveys (Andral et al. 2004, Kimbrough et al. 2008). To biomonitor the extend and the importance of the coastal pollution by trace elements, some researchers resort to indigenous populations of wild or cultivated mussels (passive biomonitoring), while others rely upon transplanting individuals from a reference site (active biosurveillance). This active biomonitoring using caged raft mussels allow to bypass the natural variability of internal biological factors (size, sex, sexual maturity, reproduction stages, seasonal growth cycles) governing the metal uptake and depuration processes by mussels (Casas and Bacher 2006, Casas et al. 2008), and to solve the scarcity of mussel stocks along certain coasts (Andral et al. 2004). Mussel watch programs historically focus on a limited number of metals such as Pb, Zn or Cu. Nowadays development of very sensitive equipment (GFAAS, ICP-MS, NAA etc.) allow scientists and environment managers to measure some TEs found at very low environmental levels (e.g. Be; Hsu et al. 2004). In parallel, recent technological developments lead to an increase of mining extractions and industrial refinements of TEs of previous little concern (e.g. Sb; Filella et al. 2002). Environmental quantification of certain less studied potential pollutants is henceforth now possible and relevant (Luy et al. 2012).

The first purpose of this paper is to measure levels of numerous TEs that were little or never studied in the Mediterranean mussel *Mytilus galloprovincialis* (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi), in addition to TEs classically investigated in this species (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V). As the influence of mussel size on soft tissue TE levels still appears to be ambiguous (Przytarska et al. 2010), the second objective of this study is to model the effect of mussel length and flesh weight on the concentrations and contents of the 19 investigated TEs. Knowing that the accumulation of reserves during the sexual dormancy and their subsequent mobilization during gonadal development influence TE levels and body compartmentalization in *Mytilus spp.* (Cossa 1989), the last objective of this work is to study the importance of these physiological processes in *M. galloprovincialis*.

2. Material and methods

Only materials in ceramic, plastic and glass were used for sample treatment and storage. All materials were previously decontaminated in HCl 5% of analytical grade for a minimum of 48h. Powder-free nitril gloves were used, and dissection material and lab benches were systematically cleaned with HCl 2% of analytical grade.

2.1. Biological material

The species used was *Mytilus galloprovincialis* Lamarck. Rope-grown mussels were purchased from the shelfish farm of the Diane pond, East Corsica (42°07'45.00''N, 9°31'01.00"E), in March 2010 – after spawning – and February 2011 – before spawning. Mussels were carefully detached from the ropes with a ceramic scalpel. Forty large size (70-80 mm shell length) mussels purchased in March 2010 (n = 20) and February 2011 (n = 20) were used for tissue compartmentalization analysis after and before spawning, respectively. Seventy four supplementary mussels purchased in February 2011 were used to model the relationships between mussel morphometry (dry weight – from 0.17 to 3.36 g – or shell length – from 43.40 to 86.41 mm) and TE levels, and to study the differences between male and female TE accumulations prior to spawn. Collected samples were stored at -28°C.

2.2. Sample preparation

In the laboratory, mussels were measured with an electronic calliper (0.01 mm). Soft tissues (byssus excluded) were carefully removed from the shell with a ceramic scalpel. For the tissue compartmentalization study, mussels were dissected and tissues were sorted and recorded as follow: gills (G), hepatopancreas (H), mantle (M) and remaining soft tissues (ST). All samples (tissues or entire individuals) were freeze dried (BenchTop 3L, VirTis Company Inc.) and weighed. Samples weighing more than 300 mg were further ground with liquid nitrogen in an agate mortar and then re-lyophilized to eliminate condensed ambient water vapour. Dried powders and unground samples were mineralized with HNO_3 and H_2O_2 (suprapure grade, Merck KGaA) in teflon bombs using a closed microwave digestion labstation (Ethos D, Milestone Inc.). Mineralisats were diluted to an appropriate volume of 50

cm^3. Mussel shells were oven dried (48h at 60°C) and weighed to calculate the individual condition index.

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.). This instrument requires the selection of an appropriate reaction gas to overcome spectral overlaps (Olesik and Jones 2006): no reaction gas in standard mode (for ^{9}Be, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{75}As, ^{208}Pb and ^{209}Bi and) and NH_3 (for ^{27}Al, ^{51}V, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn and ^{78}Se) in DRC modes. Analytical accuracy was checked by analysing Certified Reference Materials (Table 1): BCR 278 (mussel tissue) from the Belgian Institute for Reference Materials and Measurements, DOLT-3 (dogfish liver) and DORM-2 (dogfish muscle) from the National Research Council of Canada, and NIST 1566b (oyster tissue), NIST 1577c (bovine liver) and NIST 2976 (mussel tissue) from the American National Institute of Standards and Technology. For each element, detection decision (LC), detection limit (LD) and quantification limit (LQ) were calculated according to Currie (1999) or Grinzaid et al. (1977), depending on their specific blank distribution (Table 1). Data were analysed as TE concentrations or total contents on a dry weight basis and are expressed in $\mu g.g^{-1}_{DW}$ or in µg, respectively.

2.4 Mathematical and statistical analysis

TEs measured in the 4 body compartments of each mussel were added to calculate individual TE total contents, and balanced by their respective dry weight to calculate individual TE concentrations.

	Al	V	Fe	Cr	Mn	Co	Ni	Cu	Zn	Se
CRM values										
BCR 278	*70.1 ± 1.1*		133 ± 4	0.80 ± 0,08	7.3 ± 0,2	*0.366 ± .088*	*1.10 ± 0.10*	9.60 ± 0,16	76 ± 2	1.66 ± 0,04
DOLT-3	*25*		1484 ± 57	*3.5*			2.72 ± 0,35	31.2 ± 1,0	86.6 ± 2,4	7.06 ± 0,48
DORM-2	10.9 ± 1,7		142 ± 10	34.7 ± 5,5	3.66 ± 0,34	0.182 ± 0,031	19.4 ± 3,1	2.34 ± 0,16	25.6 ± 2,3	1.40 ± 0,09
NIST 1566b	197.2 ± 6,0	0.577 ± 0,023	205.8 ± 6,8		18.5 ± 0,2	0.371 ± 0,009	1.04 ± 0,09	71.6 ± 1,6	1424 ± 46	2.06 ± 0,15
NIST 1577c		0.00817 ± 0,00066	197.94 ± 0,65	0.053 ± 0,014	10.46 ± 0,47	0.300 ± 0,018	0.0445 ± 0,0092	275.2 ± 4,6	181.1 ± 1,0	2.031 ± 0,045
NIST 2976	*134 ± 34*		171.0 ± 4,9	*0.50 ± 0.16*	*33 ± 2*	*0.61 ± 0.02*	*0.93 ± 0.12*	4.02 ± 0,33	137 ± 13	1.80 ± 0,15
Our values										
BCR 278 (n = 3)	56,0 ± 2,7	0,430 ± 0,023	128 ± 2	0,59 ± 0,02	7,6 ± 0,0	0,346 ± 0,005	0,88 ± 0,03	9,17 ± 0,07	76 ± 4	1,66 ± 0,09
DOLT-3 (n = 6)	29 ± 1	0,351 ± 0,008	1562 ± 29	3,5 ± 0,4	10,7 ± 0,3	0,368 ± 0,064	2,86 ± 0,27	33,4 ± 0,9	96,9 ± 2,4	7,57 ± 0,30
DORM-2 (n = 8)	9,8 ± 1,5	0,077 ± 0,014	102 ± 16	18,0 ± 4,3	2,38 ± 0,45	0,109 ± 0,023	9,7 ± 2,5	2,01 ± 0,10	25,0 ± 1,4	1,49 ± 0,06
NIST 1566b (n = 10)	137,5 ± 22,3	0,531 ± 0,030	201,4 ± 7,8	0,28 ± 0,07	18,5 ± 0,5	0,363 ± 0,011	0,95 ± 0,06	70,5 ± 1,8	1376 ± 55	2,16 ± 0,07
NIST 1577c (n = 10)	1,1 ± 0,3	0,00874 ± 0,00064	199,65 ± 4,36	0,051 ± 0,003	10,29 ± 0,15	0,304 ± 0,003	0,0420 ± 0,0112	275,6 ± 5,2	181,9 ± 3,4	2,063 ± 0,061
NIST 2976 (n = 10)	148 ± 9	0,737 ± 0,018	179,6 ± 5,7	0,35 ± 0,04	38 ± 1	0,64 ± 0,03	0,83 ± 0,10	3,98 ± 0,16	148 ± 4	2,12 ± 0,05
Limits										
LC	0,1318 ± 0,0654	0,0007 ± 0,0004	0,0487 ± 0,0312	0,0035 ± 0,0020	0,0029 ± 0,0014	0,0010 ± 0,0004	0,0261 ± 0,0112	0,0084 ± 0,0110	0,0320 ± 0,0188	0,0783 ± 0,0353
LD	0,2587 ± 0,1283	0,0014 ± 0,0009	0,0955 ± 0,0613	0,0069 ± 0,0039	0,0056 ± 0,0027	0,0020 ± 0,0009	0,0513 ± 0,0220	0,0165 ± 0,0216	0,0626 ± 0,0369	0,1536 ± 0,0694
LQ	0,7445 ± 0,3691	0,0041 ± 0,0025	0,2740 ± 0,1770	0,0198 ± 0,0113	0,0161 ± 0,0079	0,0058 ± 0,0025	0,1475 ± 0,0633	0,0470 ± 0,0615	0,1782 ± 0,1049	0,4420 ± 0,1996

	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi
CRM values									
BCR 278	*0.118 ± 0.006*	0.34 ± 0,02			5.9 ± 0,2	*0.35 ± 0.03*		1.91 ± 0,04	
DOLT-3	1.20 ± 0,07	19.4 ± 0,6	*0.4*		10.2 ± 0,5			0.319 ± 0,045	
DORM-2	0.041 ± 0,013	0.043 ± 0,008	*0.023*		18.0 ± 1,1			0.065 ± 0,007	
NIST 1566b	0.666 ± 0,009	2.48 ± 0,08	*0.031 ± 0,008*	*0.011 ± 0.002*	7.65 ± 0,65			0.308 ± 0,009	
NIST 1577c	0.0059 ± 0,0016	0.0970 ± 0,0014		*0.00313 ± 0.00031*	0.0196 ± 0,0014	3.30 ± 0,13		0.0628 ± 0,0010	
NIST 2976	*0.011 ± 0.005*	0.82 ± 0,16	*0.096 ± 0.039*		13.3 ± 1,8			1.19 ± 0,18	
Our values									
BCR 278 (n = 3)	0,153 ± 0,003	0,32 ± 0,00	0,137 ± 0,005	0,009 ± 0,0004	6,1 ± 0,0	0,32 ± 0,01	0,0034 ± 0,0009	1,74 ± 0,02	0,0400 ± 0,0004
DOLT-3 (n = 6)	1,27 ± 0,02	19,6 ± 0,3	0,5 ± 0,023	0,016 ± 0,001	9,9 ± 0,3	3,62 ± 0,25	0,0014 ± 0,0006	0,292 ± 0,018	0,0404 ± 0,0008
DORM-2 (n = 8)	0,037 ± 0,002	0,046 ± 0,002	0,030 ± 0,005	0,027 ± 0,001	18,5 ± 0,5	0,17 ± 0,05	0,0005 ± 0,0006	0,054 ± 0,010	0,0026 ± 0,0004
NIST 1566b (n = 10)	0,648 ± 0,007	2,45 ± 0,03	0,022 ± 0,006	0,009 ± 0,001	7,69 ± 0,14	0,19 ± 0,01	0,0088 ± 0,0013	0,290 ± 0,004	0,0073 ± 0,0005
NIST 1577c (n = 10)	0,0061 ± 0,0003	0,1004 ± 0,0022	0,010 ± 0,002	0,00327 ± 0,00079	0,0248 ± 0,0030	3,57 ± 0,05	0,0003 ± 0,0005	0,0627 ± 0,009	0,0005 ± 0,0004
NIST 2976 (n = 10)	0,010 ± 0,001	0,86 ± 0,02	0,101 ± 0,022	0,011 ± 0,0005	14,7 ± 0,3	0,52 ± 0,01	0,0042 ± 0,0009	1,16 ± 0,03	0,0082 ± 0,0006
Limits									
LC	0,0007 ± 0,0004	0,0006 ± 0,0003	0,0065 ± 0,0043	0,0017 ± 0,0012	0,0054 ± 0,0032	0,0051 ± 0,0031	0,0028 ± 0,0018	0,0032 ± 0,0029	0,0006 ± 0,0004
LD	0,0013 ± 0,0008	0,0012 ± 0,0005	0,0126 ± 0,0084	0,0033 ± 0,0023	0,0106 ± 0,0063	0,0100 ± 0,0061	0,0056 ± 0,0035	0,0064 ± 0,0057	0,0011 ± 0,0007
LQ	0,0038 ± 0,0023	0,0034 ± 0,0015	0,0356 ± 0,0237	0,0093 ± 0,0066	0,0304 ± 0,0182	0,0280 ± 0,0172	0,0093 ± 0,0058	0,0183 ± 0,0165	0,0030 ± 0,0018

Table 1. Evaluation of analytical accuracy (mean ± SD) through Certified Reference Materials (CRMs), and values of detection decisions (LC), detection limits (LD) and quantification limits (LQ) of the analytical method used. Italic values represent indicative values; n represents the number of replicates.

One-way analysis of variance (ANOVA) followed by Tukey HSD pairwise comparison test of means with equal n's ($p < 0.05$) were performed, after testing for homogeneity of variances (Levene test) on raw or log-transformed data, to emphasize significant differences between mean TE levels measured in the 4 body compartments and entire individuals (for each reproductive status, respectively). Non-parametric analysis of variance (Kruskal–Wallis test) was performed when assumptions prior to ANOVAs (normality and/or homoscedasticity) were not encountered, followed by Dunn pairwise comparison test of means ($p < 0.05$). An equivalent statistical procedure (for unequal n's) was performed to compare TE levels between mussel size classes.

TE levels recorded in each body compartment and in entire individuals were then compared between reproductive status, using Student pairwise comparison test of means with equal n's ($p < 0.05$), after testing for homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric pairwise comparison test of means (test U of Mann-Whitney, $p < 0.05$) was performed when assumptions prior to Student test (normality and/or homoscedasticity) were not encountered. An equivalent statistical procedure (for unequal n's) was performed to compare TE levels between males and females before spawning.

The effect of mussel size on TE levels was modelled using a power function, according to previous studies (Lobel et al. 1992, Saavedra et al. 2004, Mubiana et al. 2006): TE concentration (M, in $\mu g.g^{-1}_{DW}$) is a power function of mussel soft tissue dry weight (W), and total TE content (M, in µg) is a power function of mussel shell length (L):

$M = aW^b$ (1) and $M = aL^b$ (2)

The relationships of equations (1) and (2) were double logarithmically transformed to yield 2 linear functions:

$\log_{10}M = \log_{10}a + b\log_{10}W$ (3) and $\log_{10}M = \log_{10}a + b\log_{10}L$ (4)

The effect of mussel size on TE levels was also modelled using a linear regression model, TE concentrations and total TE contents being a linear function of mussel soft tissue dry weight (W) or shell length (L), respectively:

$M = bW + a$ (5) and $M = bL + a$ (6)

b is the slope of linear functions (3-6); $\log_{10}a$ and a are the Y-intercepts. To compare the relative goodness of fit of the two models (power function or linear regression), an Akaike information criterion (AIC) analysis was performed. The AIC expresses the probability that

each model is correct, with the probabilities summing to 100 %. Similar analyses were performed on mussels separated into size-classes or segregated according to their sex. The effect of mussel size on TE levels (separated or not into size-classes, or segregated by sex) was also modelled for individuals longer than 55 mm only.

Individual condition index (CI = the ratio of dry flesh weight to dry shell weight) was calculated according to Andral et al. (2004), to ensure that this physiological index did not statistically evolve with mussel size. There was no significant linear relation ($p = 0.7818$) between CI and shell length. Mussel gamete maturation coupled together with the important food availability in late winter – early spring planktonic bloom results in the relatively high mean CI (0.226 ± 0.053) calculated (Casas 2005). The mean CI measured after spawning on additional mussels, from mid-March to-mid June 2011, was 0.111 ± 0.39, a value close to the reference CI 0.124 calculated by Andral et al. (2004) within the framework of the biointegrator network (RINBIO) along the French Mediterranean coasts. As mussels showed the same physiological status independently of their size, they could be all used for modelling the effect of mussel size on TE levels.

Mathematical and statistical analyses were performed with STATISTICA 9 (StatSoft Inc.), GraphPad Prism 5 (GraphPad Software, Inc) and Microsoft Office Excel 2007 (Microsoft Office Enterprise 2007) softwares.

3. Results and Discussion

3.1. TE concentrations

Cr, Ni, Cu, Zn, Cd, As and Pb concentrations in rope-grown mussels from the Diane pond (all size together) are within the range of values measured in the Mediterranean (Tables 2,3; concentrations in the paragraphs bellow are expressed in $\mu g.g^{-1}_{DW}$), notably by the French mussel watch programs (RINBIO 2001, Andral et al. 2004, RNO 2006, Benedicto et al. 2011). For example, Cd (0.374 ± 0.131), Pb (0.336 ± 0.192) and Ni (1.41 ± 0.54) concentrations are very low to moderate when compared to 123 sites in the Western Mediterranean basin (mean levels for Cd = 1.33, Pb = 1.40, Ni = 1.10) surveyed with caged mussels between years 2004 and 2006 (Benedicto et al. 2011).

	Reference	Studied area	Species	Al	V	Fe	Cr	Mn
1.	Benedicto et al. 2011	Western Mediterranean bassin (123)	*M. galloprovincialis*					
2.	RINBIO 2001, Andral et al. 2004	French Mediterranean coasts (93)	*M. galloprovincialis*				1	
3.	Bartolomé et al. 2010	Atlantic coast of northwestern Spain (10)	*M. galloprovincialis*		1.7-7.1		2.6-6.1	5.6-55.3
4.	Desideri et al. 2009	Central Adriatic Sea (7)	*M. galloprovincialis*			45-755		73-83
5.	Çevik et al. 2008	Eastern Black Sea (5)	*M. galloprovincialis*			1150–4030	1–3	41–59
6.	Favretto et al. 1997	Muggia Bay, north Adriatic Sea (1)	*M. galloprovincialis* *			15.5-64.8		0.70-2.42
7.	this study	Diane pond, east Corsica (1)	*M. galloprovincialis* *	39-877	2.3-12.5	66-656	0.19-2.17	3.4-20.6
8.	RNO 2006	French Med. and English Channel-Atlantic coasts (83)	*Mytilus* spp.		0.43-15.4		0.12-9.21	
9.	Giltrap et al. 2012	Irish coasts (4)	*M. edulis*	40-201	0.95-1.59	30-261	0.71-1.51	4.6-13.4
10.	Gobert et al. 1992	Belgian coast (4)	*M. edulis*			166-247	0.9-1.2	
11.	Brooks and Rumsby 1965	Tasman Bay, New Zealand (1)	*M. edulis aoteanus* *		2-8	960-2640	9-24	12-38
12.	Watling and Watling 1976	Saldanha Bay, South Africa (3)	*C. meridionalis*					9-11
13.	Giltrap et al. 2012	Irish coasts (4)	*C. gigas*	81-148	0.88-1.29	175-254	0.79-1.70	21.3-47.1
14.	Hsu et al. 2004	Chigu Lagoon, southwestern Taiwan (1)	*C. gigas*					
15.	Watling and Watling 1976	Langebaan Lagoon, South Africa (1)	*C. gigas*					12
16.	Brooks and Rumsby 1965	Tasman Bay, New Zealand (1)	*O. sinuata* *		2-4	630-750	2-6	1-11
17.	Brooks and Rumsby 1965	Tasman Bay, New Zealand (1)	*P. novae-zelandiae* *		5-14	1140-6900	3-23	12-306
18.	Papadopoulu 1973 in Eisler 2010	Greek coasts	*A. noae*					

	Co	Ni	Cu	Zn	Se	Ag	Cd	Sn	Sb	As	Mo	Be	Pb	Bi
1.		0.1–3.4					0.46–2.89						0.5–8.3	
2.		2	2.9-9.2	116-203			0.1-5.9			20			0.5-5.4	
3.	0.4-69.3	0.8-15.4	6.9-59.9	192-301	5.4-9.3		0.4-2.3	0.1-1.4		14-32			1.1-13.3	
4.		1.3-7.6	18-156	61-190			0.6-1.0	0.6-3.9					2.0-9.0	
5.		1–6	90–260	180–630			2–4						5–21	
6.	0.06-0.53	0.41-2.30	0.77-2.23	6.9-29.6			0.12-0.38						0.48-1.79	
7.	0.37-1.37	0.71-3.39	2.8-7.4	35-224	1.5-4.3	0.005-0.033	0.21-1.03	0.015-0.089	0.007-0.028	17-46	4.8-33.1	0.005-0.037	0.14-1.10	0.005-0.021
8.		0.45-8.41	3.8-67.0	36-409		0.01-7.75	0.17-10.00						0.1-27.7	
9.	0.54-0.88	0.88-1.56	5.8-14.1	118-251			0.67-1.77		0.25-0.39	6.7-17.4	1.00-2.64		1.01-4.24	
10.			5.6-6.2	107-142			0.5-1.2						0.7-2.3	
11.		1-17	5-11	50-180		0.1-0.3	<10				0.1-1.0		3-25	
12.	2-3	2-3	7-14	73-113			1-8						2-5	5-6
13.	0.46-0.52	0.81-1.58	21-282	673-1466			1.14-1.49		0.27-0.28	9.5-20.7	0.83-1.85		0.74-1.65	
14.												0.0247		
15.	1	1	33	424			9						1	4
16.		1-3	21-53	850-1500		4.5-7.3	10-43				0.1-0.4		6-14	
17.		2-17	2-14	195-368		0.2-2.3	210-299				0.1-2.3		10-23	
18.											88.0			

Table 2. TE mean concentrations, ranges of mean concentrations or ranges of individual (*) concentrations ($\mu g.g^{-1}_{DW}$) in *M. galloprovincialis* and other mollusc species. Number between brackets represent number of sampled stations for each study, respectively (not available for ref. 18).

TE	mean	median	TE	mean	median
Al	200 ± 150	152	Ag	0,0123 ± 0,0054	0,0111
V	5,35 ± 2,02	5,07	Cd	0,374 ± 0,131	0,336
Fe	177 ± 97	149	Sn	0,0318 ± 0,0167	0,0259
Cr	0,554 ± 0,320	0,459	Sb	0,0126 ± 0,0042	0,0115
Mn	9,86 ± 3,87	9,19	As	31,2 ± 6,1	31,3
Co	0,634 ± 0,205	0,589	Mo	17,1 ± 5,8	17,1
Ni	1,41 ± 0,54	1,28	Be	0,0135 ± 0,0056	0,0122
Cu	4,82 ± 1,50	4,03	Pb	0,336 ± 0,192	0,279
Zn	72,6 ± 33,6	64,3	Bi	0,0087 ± 0,0032	0,0078
Se	2,70 ± 0,78	2,45			

Table 3. Trace element concentrations in mussels (mean ± SD and median, in $\mu g.g^{-1}_{DW}$; n = 74).

Cr (0.554 ± 0.320), Cd and Pb are in the low range of concentrations when compared to the 97 mussel cages immersed in year 2000 along the French Mediterranean coasts (mean levels for Cr = 1, Cd = 0.9, Pb = 1 and As = 20, Ni = 2, Cu = 4.1) and in the intermediate range for As (31.2 ± 6.1), Ni (1.41 ± 0.54) and Cu (4.82 ± 1.50); Zn (72.6 ± 33.6) concentrations are half the mean stable value measured in this area (Zn = 148.3) (RINBIO 2001, Andral et al. 2004).

Cr, Ni, Cu, Zn, Cd, As and Pb concentrations measured in the present study are within accepted values for a relatively clean area and are similar to levels previously reported for mussels from the Diane pond (RINBIO 2001, Andral et al. 2004, RNO 2006). Concentrations of Cd and Pb are furthermore under the maximal values of 1 $\mu g.g^{-1}$ of bivalve mollusc fresh weight fixed by the European Legislation EC n° 466-2001 (2001). Ag (0.0123 ± 0.0054) mean concentration is also similar to mussel (*Mytilus sp.*) Ag levels from the cleanest stations of 83 sites sampled along the English Channel-Atlantic and Mediterranean French coasts (range of Ag levels = 0.01-7.75; RNO 2006).

V mean concentration (5.35 ± 2.02) is high compared to the range of available data for the Diane pond (0.92-3.62; http://www.ifremer.fr/envlit/) and to the median values for the English Channel-Atlantic (1.62) and Mediterranean (1.40) French coasts in general (RNO 2006). It can result from the combine effect of the pond confinement, the important aquaculture activity with regard to the pond size, and the inputs trough the 3 small rivers draining part of the 69 km^2 catchment basin of Bravona.

Available data from the literature for Fe, Mn, Co, Se and Sn in mollusc bivalves are more scarce. Results show that *Mytilus galloprovincialis* efficiently accumulates these TEs.

Bartolomé et al. (2010) notably studied the seasonal and pluriannual variations of Co, Mn, Se and Sn levels in wild *M. galloprovincialis* from the Atlantic coast of northwestern Spain (median concentrations for Co = 0.8, Mn = 21.6, Se = 8.1 and Sn = 0.4). Concentrations measured in the Diane pond (Co = 0.634 ± 0.205, Mn = 9.86 ± 3.87, Se = 2.70 ± 0.78 and Sn = 0.0318 ± 0.0167) are similar or lower than levels reported by these authors. Moreover, mean Sn (1.3) and Mn (77.5) concentrations measured in wild and raft *M. galloprovincialis* from the central Adriatic Sea are about 40 and 9 times higher than in the Diane pond, respectively (Desideri et al. 2009). *A contrario*, mean levels of Mn (1.29) and Co (0.27) measured in raft *M. galloprovincialis* from the Muggia Bay, north Adriatic Sea, are lower than in the Diane pond (Favretto et al. 1997). Fe concentrations (177 ± 97) are similar to mean levels recorded in *Mytilus spp.* from the Belgian coast (217; Gobert et al. 1992) and the central Adriatic Sea (199; Desideri et al. 2009), but lower than in the contaminated eastern Black Sea (2462; Çevik et al. 2008).

For Al, Sb, Mo, Be and Bi, fewer data are published; furthermore, at least 3 of these TEs (Sb, Mo and Bi) were identified as coastal pollutants (Luy et al. 2012). Giltrap et al. (2012) recently monitored Al and Sb in Irish coastal waters with caged mussels *Mytilus edulis* (mean levels for Al = 118, Sb = 0.28) and oysters *Crassostrea gigas* (mean levels for Al = 117, Sb = 0.32). Concentrations reported by these authors are lower than mean Al level (200 ± 150) measured in the Diane pond; this terrigenous TE is more concentrated and bioavailable in this semi-enclosed salt pond catching streaming waters from the basin of Bravona. Sb levels (0.0126 ± 0.0042) are *a contrario* very bellow concentrations measured by these authors. Be contents in marine organisms are poorly documented mainly because concentrations are often bellow detection limits of instrumental techniques employed. Nonetheless, Be concentrations in raft mussels from the Diane pond (0.0135 ± 0.0056) are similar to levels measured by Hsu et al. (2004) in the oyster *C. Gigas* (0.0247) harvested from Chigu Lagoon (southwestern Taiwan). Mo concentration (17.1 ± 5.8) is in-between the low levels measured by Brooks and Rumsby (1965) in three bivalves (mean Mo concentrations in the mussel *Mytilus edulis aoteanus* = 0.6, in the oyster *Ostrea sinuata* = 0.3, in the scallop *Pecten novae-zelandiae* = 0.9) collected in the Tasman Bay (New Zealand) and the higher concentration reported by Papadopoulu (1973) for the bivalve *Arca noae* (88.0) from Greek coastal waters (in Eisler 2010). Finally, the only available Bi levels in bivalves reported by Watling and Watling (1976) for the mussel *Choromytilus meridionalis* (5.0 to 6.0) and the

oyster *C. gigas* (4.0) are high when compared to Bi levels in mussels from the Diane pond (0.0087 ± 0.0032).

3.2. TE concentrations or total contents as a function of mussel weight or shell length

Tissue levels of the 19 studied TEs increase when the mussel shell length increases; their levels decrease when the mussel flesh dry weight increases (slope *b* in Table 4.A). This is consistent with previous studies modelling the effect of mussel morphometry on TE accumulation (e.g. Lobel et al. 1991, Martincic et al. 1992). Significant regressions were found for all TEs ($p<0.05$), except for the relationship between weight and Mo concentration in the double log transformed power model. Mean goodness of fit was higher for relationships between the mussel shell length and TE contents (mean r^2 = 0.564 or 0.553 for linear regression or power function model, respectively); linear equations deviate less often from the trend of data when modelling the effect of the mussel dry weight on TE levels (4 significant deviations – $p<0.05$ – for Mo, Cu and As, both models together). Akaike information criterions (AICs) show that the power function better describes relationships between the mussel dry weight and TE concentrations (mean AIC = 64.24 %), while the linear regression better describes relationships between the mussel shell length and TE contents (mean AIC = 69.95 %). The example for Sb given in Fig. 1 properly illustrates these statistical considerations.

These results are inconsistent with observations made by Saavedra et al. (2004) who elected the power function to model the effect of raft *M. galloprovincialis* size, ranging from 52 to 87 mm, on TE contents. In order to work with similar datasets, both models were run again for mussels longer than 55 mm only (Table 4.B). Mean calculated AICs are then equal to 48.27 % ± 4.14 and 51.73 % ± 4.14 for linear and power relationships between TE contents and mussel shell length, respectively. As the difference in likelihood (i.e. similar mean AICs) is very small, one may conclude that both models are correct, as did Saavedra et al. (2004).

A.	relationships between TE concentrations and mussel soft tissue dry weight - all mussels												relationships between TE contents and mussel shell length - all mussels											
	linear regressions						double log transformed power functions						linear regressions						double log transformed power functions					
	b	*a*	r^2	p	dev.	AIC	*b*	log10*a*	r^2	p	dev.	AIC	*b*	*a*	r^2	p	dev.	AIC	*b*	log10*a*	r^2	p	dev.	AIC
length weight	13,02	41,74	0,781	<0.001	n.s.	93,72%	0,2888	1,752	0,764	<0.001	n.s.	6,28%	0,06	-2,134	0,781	<0.001	n.s.	92,16%	2,278	-3,903	0,766	<0.001	n.s.	7,84%
Al	-92,47	355,3	0,263	<0.001	n.s.	<0,01%	-0,6721	2,3490	0,475	<0.001	n.s.	>99,99%	6,788	-158,9	0,273	<0.001	n.s.	37,89%	1,734	-0,7034	0,282	<0.001	n.s.	62,11%
V	-1,029	7,076	0,180	<0.001	n.s.	94,85%	-0,1720	0,7519	0,113	<0.01	n.s.	5,15%	0,232	-6,475	0,560	<0.001	n.s.	88,76%	1,669	-2,099	0,534	<0.001	n.s.	11,24%
Fe	-64	285,1	0,300	<0.001	n.s.	<0,01%	-0,5314	2,2980	0,507	<0.001	n.s.	>99,99%	6,959	-188	0,520	<0.001	n.s.	40,93%	1,812	-0,874	0,525	<0.001	n.s.	59,07%
Cr	-0,2046	0,8983	0,283	<0.001	n.s.	<0,01%	-0,5647	-0,2079	0,514	<0.001	n.s.	>99,99%	0,02239	-0,6321	0,520	<0.001	n.s.	34,62%	1,900	-3,541	0,528	<0.001	n.s.	65,38%
Mn	-3,055	14,99	0,430	<0.001	n.s.	72,89%	-0,3359	1,0350	0,414	<0.001	n.s.	27,11%	0,3317	-6,611	0,425	<0.001	n.s.	67,03%	1,385	-1,34	0,413	<0.001	n.s.	32,97%
Co	-0,1116	0,822	0,206	<0.001	n.s.	13,12%	-0,2295	-0,1678	0,245	<0.001	n.s.	86,88%	0,03401	-1,174	0,820	<0.001	s.	97,18%	2,176	-3,946	0,801	<0.001	s.	2,82%
Ni	-0,4289	2,136	0,436	<0.001	n.s.	0,16%	-0,3750	0,1940	0,527	<0.001	n.s.	99,84%	0,0591	-1,675	0,752	<0.001	n.s.	87,57%	1,788	-2,914	0,739	<0.001	n.s.	12,43%
Cu	-0,8148	6,195	0,205	<0.001	n.s.	96,69%	-0,1540	0,7051	0,129	<0.01	s.	3,31%	0,1974	-5,002	0,443	<0.001	n.s.	76,62%	1,557	-1,934	0,425	<0.001	n.s.	23,38%
Zn	-12,6	93,76	0,097	<0.01	n.s.	22,23%	-0,2404	1,8920	0,127	<0.01	n.s.	77,77%	3,95	-137,9	0,578	<0.001	n.s.	65,17%	2,246	-2,016	0,571	<0.001	n.s.	34,83%
Se	-0,5591	3,645	0,356	<0.001	n.s.	90,27%	-0,2243	0,4619	0,316	<0.001	n.s.	9,73%	0,1145	-3,116	0,548	<0.001	n.s.	79,45%	1,685	-2,427	0,531	<0.001	n.s.	20,55%
Ag	-0,00384	0,01872	0,348	<0.001	n.s.	76,21%	-0,3307	-1,8700	0,327	<0.001	n.s.	23,79%	0,00041	-0,00812	0,376	<0.001	n.s.	66,52%	1,362	-4,204	0,365	<0.001	n.s.	33,48%
Cd	-0,05853	0,472	0,138	<0.01	n.s.	2,75%	-0,2458	-0,3965	0,217	<0.001	n.s.	97,25%	0,01943	-0,6478	0,751	<0.001	n.s.	99,12%	2,004	-3,856	0,718	<0.001	s.	0,88%
Sn	-0,00879	0,04654	0,191	<0.001	n.s.	0,31%	-0,4105	-1,4540	0,308	<0.001	n.s.	99,69%	0,00138	-0,0404	0,374	<0.001	n.s.	60,03%	1,836	-4,646	0,367	<0.001	n.s.	39,97%
Sb	-0,00308	0,01781	0,368	<0.001	n.s.	2,73%	-0,3023	-1,8610	0,426	<0.001	n.s.	97,27%	0,00058	-0,01802	0,772	<0.001	n.s.	92,35%	1,923	-5,199	0,756	<0.001	s.	7,65%
As	-2,95	36,13	0,162	<0.001	n.s.	98,48%	-0,0690	1,5040	0,062	<0.05	s.	1,52%	1,528	-46,84	0,697	<0.001	n.s.	96,25%	1,844	-1,633	0,669	<0.001	n.s.	3,75%
Mo	-2,006	20,49	0,083	<0.05	s.	93,09%	-0,0586	1,2420	0,016	n.s.	s.	6,91%	0,7283	-18,92	0,466	<0.001	s.	88,66%	1,500	-1,27	0,435	<0.001	s.	11,34%
Be	-0,00296	0,01846	0,191	<0.001	n.s.	0,03%	-0,3601	-1,8300	0,350	<0.001	n.s.	99,97%	0,00069	-0,02305	0,646	<0.001	n.s.	51,21%	2,181	-5,638	0,646	<0.001	n.s.	48,79%
Pb	-0,07656	0,4648	0,111	<0.01	n.s.	12,10%	-0,3218	-0,4352	0,157	<0.001	n.s.	87,90%	0,01971	-0,7414	0,521	<0.001	n.s.	6,00%	2,809	-5,398	0,556	<0.001	n.s.	94,00%
Bi	-0,00206	0,01215	0,294	<0.001	n.s.	3,41%	-0,3060	-2,0240	0,355	<0.001	n.s.	96,59%	0,00038	-0,01113	0,672	<0.001	s.	93,65%	1,764	-5,069	0,648	<0.001	s.	6,35%
mean			0,244			35,76%			0,294			64,24%			0,564			69,95%			0,553			30,05%
SD			0,109			42,51%			0,161			42,51%			0,153			26,03%			0,148			26,03%
median			0,206			12,10%			0,316			87,90%			0,548			76,62%			0,534			23,38%

B.	relationships between TE concentrations and mussel soft tissue dry weight - mussels > 55 mm												relationships between TE contents and mussel shell length - mussels > 55 mm											
	linear regressions						double log transformed power functions						linear regressions						double log transformed power functions					
	b	*a*	r^2	p	dev.	AIC	*b*	log10*a*	r^2	p	dev.	AIC	*b*	*a*	r^2	p	dev.	AIC	*b*	log10*a*	r^2	p	dev.	AIC
length weight	9,389	50,32	0,529	<0.001	n.s.	95,67%	0,2549	1,767	0,473	<0.001	n.s.	4,33%	0,06	-1,874	0,529	<0.001	n.s.	40,04%	1,927	-3,245	0,536	<0.001	n.s.	59,96%
Al	-38,36	235,4	0,088	<0.05	n.s.	42,49%	-0,4652	2,3250	0,098	<0.05	n.s.	57,51%	9,146	-329,6	0,211	<0.001	n.s.	43,54%	2,146	-1,476	0,218	<0.001	n.s.	56,46%
V	-1,807	8,875	0,383	<0.001	n.s.	0,05%	-0,7429	0,9145	0,531	<0.001	n.s.	99,95%	0,1358	0,4536	0,154	<0.01	n.s.	49,92%	0,949	-0,7531	0,155	<0.01	n.s.	50,08%
Fe	-36,16	224,3	0,181	<0.01	n.s.	28,08%	-0,4681	2,3070	0,208	<0.001	n.s.	71,92%	7,615	-235,8	0,336	<0.001	n.s.	44,10%	1,829	-0,9055	0,342	<0.001	n.s.	55,90%
Cr	-0,1114	0,6956	0,165	<0.01	n.s.	23,32%	-0,4790	-0,1971	0,200	<0.001	n.s.	76,68%	0,02581	-0,8803	0,359	<0.001	n.s.	42,88%	1,988	-3,707	0,365	<0.001	n.s.	57,12%
Mn	-3,028	14,99	0,340	<0.001	n.s.	39,82%	-0,6062	1,1110	0,350	<0.001	n.s.	60,18%	0,1805	4,275	0,078	<0.05	n.s.	50,13%	0,751	-0,1562	0,078	<0.05	n.s.	49,87%
Co	-0,1628	0,9477	0,308	<0.001	n.s.	1,66%	-0,5504	-0,0597	0,403	<0.001	n.s.	98,34%	0,03209	-1,036	0,597	<0.001	n.s.	49,64%	1,842	-3,319	0,597	<0.001	n.s.	50,36%
Ni	-0,4227	2,139	0,406	<0.001	n.s.	0,16%	-0,6776	0,2897	0,530	<0.001	n.s.	99,84%	0,05366	-1,286	0,494	<0.001	n.s.	51,40%	1,507	-2,39	0,493	<0.001	n.s.	48,60%
Cu	-1,099	6,818	0,222	<0.001	s.	45,66%	-0,4328	0,7808	0,227	<0.001	n.s.	54,34%	0,1121	1,123	0,093	<0.05	n.s.	49,87%	0,871	-0,6545	0,093	<0.05	n.s.	50,13%
Zn	-17,46	105,7	0,115	<0.05	n.s.	26,28%	-0,5108	1,9860	0,148	<0.01	n.s.	73,72%	3,95	-138,1	0,317	<0.001	n.s.	47,38%	2,002	-1,559	0,320	<0.001	n.s.	52,62%
Se	-0,6361	3,818	0,291	<0.001	s.	23,47%	-0,4753	0,5330	0,321	<0.001	n.s.	76,53%	0,08499	-1,007	0,208	<0.001	n.s.	49,76%	1,207	-1,534	0,208	<0.001	n.s.	50,24%
Ag	-0,00412	0,01948	0,355	<0.001	n.s.	2,37%	-0,7277	-1,7600	0,436	<0.001	n.s.	97,63%	0,00018	0,00851	0,042	n.s.	n.s.	50,11%	0,599	-2,78	0,042	n.s.	n.s.	49,89%
Cd	-0,09407	0,56	0,356	<0.001	n.s.	4,89%	-0,5057	-0,2946	0,422	<0.001	n.s.	95,11%	0,01446	-0,2898	0,388	<0.001	n.s.	50,22%	1,394	-2,715	0,388	<0.001	n.s.	49,78%
Sn	-0,00598	0,04064	0,082	<0.05	n.s.	33,90%	-0,4416	-1,4240	0,104	<0.05	n.s.	66,10%	0,00129	-0,03351	0,150	<0.01	n.s.	51,19%	1,557	-4,123	0,148	<0.01	n.s.	48,81%
Sb	-0,00333	0,01855	0,359	<0.001	n.s.	2,59%	-0,5657	-1,7750	0,438	<0.001	n.s.	97,41%	0,00053	-0,01426	0,517	<0.001	n.s.	51,82%	1,604	-4,601	0,516	<0.001	n.s.	48,18%
As	-5,407	41,68	0,339	<0.001	n.s.	29,66%	-0,3313	1,5790	0,359	<0.001	n.s.	70,34%	1,095	-15,57	0,322	<0.001	n.s.	50,05%	1,252	-0,5245	0,322	<0.001	n.s.	49,95%
Mo	-6,253	30,17	0,458	<0.001	n.s.	7,82%	-0,6608	1,4210	0,504	<0.001	n.s.	92,18%	0,1684	21,42	0,023	n.s.	n.s.	48,73%	0,363	0,8522	0,025	n.s.	n.s.	51,27%
Be	-0,00159	0,01555	0,072	<0.05	n.s.	50,10%	-0,2382	-1,8420	0,072	<0.05	n.s.	49,90%	0,00074	-0,0271	0,432	<0.001	n.s.	51,34%	2,059	-5,409	0,430	<0.001	n.s.	48,66%
Pb	-0,07764	0,4725	0,089	<0.05	n.s.	24,45%	-0,5389	-0,3550	0,126	<0.01	n.s.	75,55%	0,02636	-1,221	0,437	<0.001	n.s.	35,13%	3,003	-5,764	0,449	<0.001	n.s.	64,87%
Bi	-0,00277	0,01387	0,394	<0.001	n.s.	0,55%	-0,6761	-1,9010	0,498	<0.001	n.s.	99,45%	0,00027	-0,00286	0,294	<0.001	n.s.	49,92%	1,179	-3,976	0,294	<0.001	n.s.	50,08%
mean			0,263			20,39%			0,315			79,61%			0,287			48,27%			0,289			51,73%
SD			0,129			17,31%			0,160			17,31%			0,169			4,14%			0,170			4,14%
median			0,308			23,47%			0,350			76,53%			0,317			49,92%			0,320			50,08%

Table 4. Comparison of linear regressions and double log transformed power functions modelling relationships between trace element concentrations and mussel soft tissue dry weight and relationships between total trace element contents and mussel shell length: **A.** all mussels together (n = 74); **B.** mussels longer than 55 mm (n = 55). *b* is the slope of linear functions; *a* and $\log_{10}a$ are the Y-intercepts. Fitting parameters are also indicated (r^2; p-levels; deviation (dev.) from the model; AIC). s = significant, n.s. = non-significant.

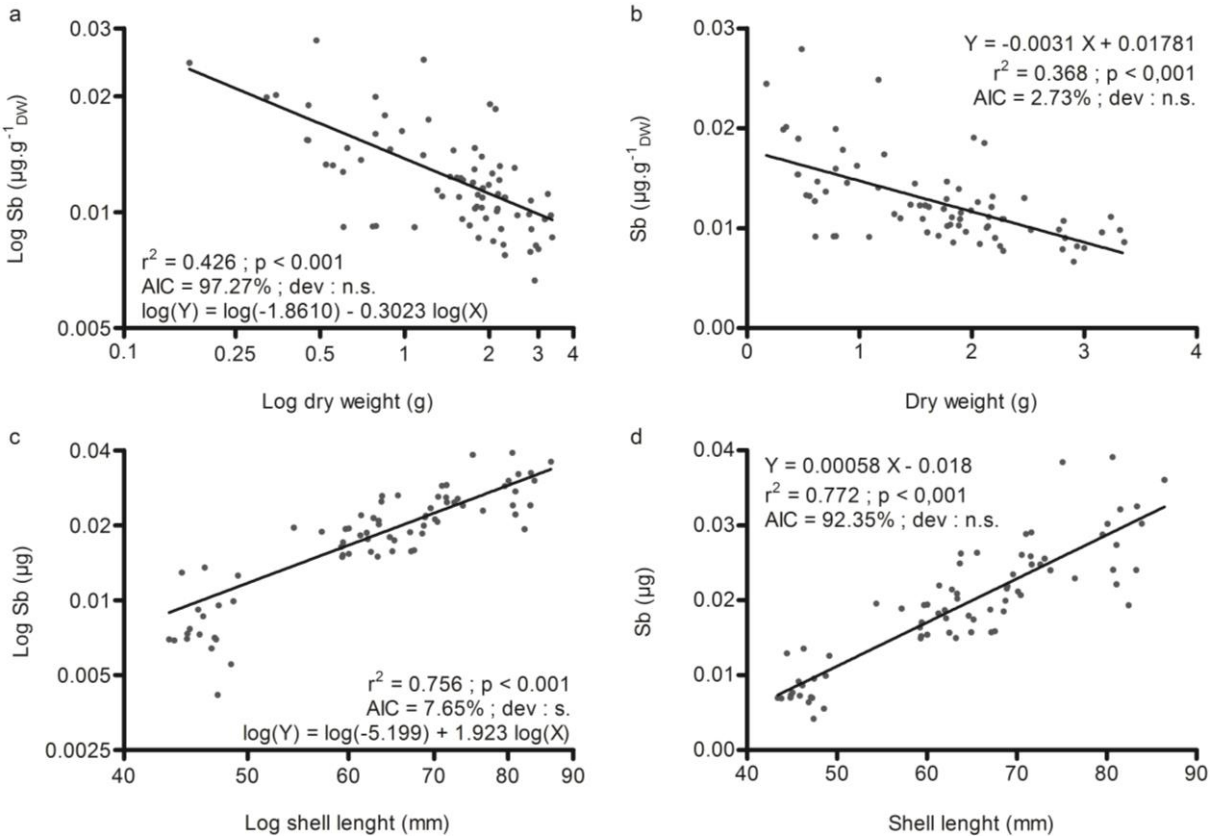

Fig. 1. Double log transformed power functions and linear regressions modelling relationships between a-b) the mussel soft tissue dry weight and Sb concentrations ($\mu g.g^{-1}_{DW}$) and between c-d) the mussel shell length and Sb total contents (µg), all individuals together. Linear equations and their corresponding fitting parameters (r^2; p-levels; deviation (dev.) from the model: s = significant, n.s. = non-significant; AIC) are reported on graphs.

The 7 relationships between V, Mn, Cu, Se, Ag, As and Mo concentrations and the mussel dry weight, previously better modelled by linear regressions, are now better modelled by power functions, in agreement with Lobel et al. (1989, 1991) and Mubiana et al. (2006). The example given in Fig. 2 for Bi properly illustrates these statistical considerations. Overall fitting parameters of power models are improved: the mean AIC increases from 64.24 % ± 42.51 to 79.61 % ± 17.31, and the mean r^2 from 0.294 ± 0.161 to 0.315 ± 0.160. It can be conclude that small-size rope-grown *M. galloprovincialis* have an antagonist effect on the modelling of relationships between the mussel-size and TE levels: they drive to elect linear functions to model relationships between the mussel shell length and TE contents, but diminish the significance of power functions modelling relationships between the mussel dry weight and some TE concentrations.

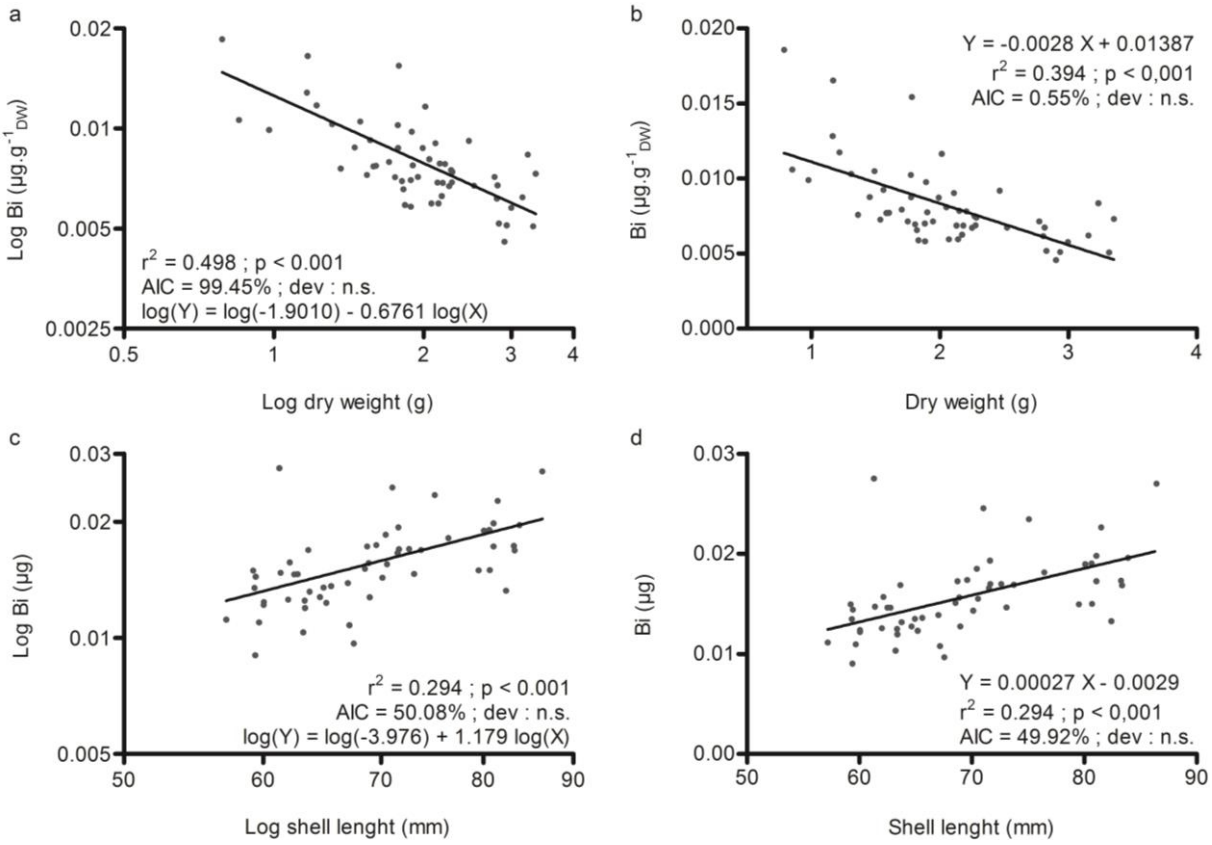

Fig. 2. Double log transformed power functions and linear regressions modelling relationships between (a-b) the mussel soft tissue dry weight and Bi concentrations ($\mu g.g^{-1}_{DW}$) and between (c-d) the mussel shell length and Bi total contents (µg), for individuals longer than 55 mm. Linear equations and their corresponding fitting parameters (r^2; p-levels; deviation (dev.) from the model: s = significant, n.s. = non-significant; AIC) are reported on graphs.

3.3. TE concentrations as a function of shell length

Concentrations of 14 out of the 19 studied TEs (Al, V, Fe, Cr, Mn, Ni, Cu, Se, Se, Ag, Cd, Sn, Sb, Bi) are correlated with the shell length ($p < 0.05$; Table 5). This is partially inconsistent with results obtained by Saavedra et al. (2004). These authors modelled the relationship between trace metal concentrations and shell length for mussels measuring from 52 to 87 mm, and separated into 4 size-classes; they observed no significant difference between Cd, Pb, Cr, Ni, As, Cu and Zn concentrations at the 4 size-classes mean lengths.

	p-values of lin. regr.		mean concentrations by size-classe							
	all ind.	> 55 mm	cl. 1: 43-54 mm		cl. 2: 55-64 mm		cl. 3: 65-74 mm		cl. 4: 75-87 mm	
Al	**0,0003**	0,9637	a	323 ± 223	ab	162 ± 63	b	150 ± 95	ab	160 ± 88
V	**0,0310**	**0,0229**	ab	5,80 ± 2,50	a	5,67 ± 1,84	ab	5,43 ± 1,67	b	4,11 ± 1,68
Fe	**0,0003**	0,6747	a	255 ± 146	ab	156 ± 43	b	148 ± 63	b	146 ± 55
Cr	**0,0007**	0,9116		0,803 ± 0,489		0,477 ± 0,146		0,462 ± 0,197		0,465 ± 0,181
Mn	**< 0,0001**	**0,0081**	a	12,89 ± 4,03	b	9,84 ± 2,84	b	9,07 ± 3,62	b	6,88 ± 2,51
Co	0,2192	0,8275		0,688 ± 0,255		0,605 ± 0,135		0,626 ± 0,235		0,616 ± 0,173
Ni	**0,0002**	0,3083	a	1,81 ± 0,66	b	1,30 ± 0,37	b	1,34 ± 0,50	b	1,16 ± 0,34
Cu	**0,0004**	**0,0063**	a	5,55 ± 1,40	a	4,86 ± 1,47	a	4,98 ± 1,55	b	3,56 ± 0,69
Zn	0,4092	0,9809		79,7 ± 37,8		66,2 ± 22,2		75,7 ± 43,8		67,9 ± 25,2
Se	**< 0,0001**	**0,0307**	a	3,24 ± 0,66	ab	2,64 ± 0,76	b	2,64 ± 0,81	b	2,17 ± 0,48
Ag	**< 0,0001**	**0,0062**	a	0,0157 ± 0,0068	a	0,0124 ± 0,0040	ab	0,0116 ± 0,0048	b	0,0083 ± 0,0027
Cd	0,2599	0,1196		0,390 ± 0,200		0,389 ± 0,100		0,357 ± 0,089		0,352 ± 0,111
Sn	**0,0048**	0,4958		0,0413 ± 0,0222		0,0282 ± 0,0117		0,0312 ± 0,0170		0,0248 ± 0,0073
Sb	**0,0026**	0,4752		0,0152 ± 0,0052		0,0119 ± 0,0028		0,0119 ± 0,0040		0,0113 ± 0,0038
As	**0,0096**	**0,0048**	a	32,7 ± 6,8	ab	31,7 ± 5,5	ab	32,3 ± 5,9	b	26,9 ± 4,5
Mo	0,1741	**0,0002**	ab	16,3 ± 6,0	a	19,5 ± 4,9	a	18,8 ± 5,6	b	12,3 ± 4,2
Be	**0,0173**	0,5205		0,0169 ± 0,0085		0,0122 ± 0,0030		0,0121 ± 0,0042		0,0128 ± 0,0040
Pb	0,4056	0,1768		0,400 ± 0,253		0,268 ± 0,138		0,324 ± 0,185		0,369 ± 0,154
Bi	**0,0064**	0,0673		0,0100 ± 0,0039		0,0089 ± 0,0032		0,0082 ± 0,0026		0,0073 ± 0,0021

Table 5. Left part of the Table: p-values of linear regressions modelling relationships between the shell length and trace element concentrations, all individuals together (n = 74) or limited to mussels longer than 55 mm (n = 55). Rest of the Table: trace element concentrations by size-classes (mean ± SD; n = 19, 21, 20 and 14 for size-classes cl. 1, 2, 3 and 4, respectively). Letters represent significant differences ($p < 0.05$) between size-classes.

When the linear regression model is run again for mussels longer than 55 mm, excluding small-size individuals, only 7 TEs (V, Mn, Cu, Se, Ag, As and Mo) still depend ($p < 0.05$) on the shell length instead of 14 TEs ($p < 0.05$; Table 5). The 74 mussels are furthermore ranged into 4 size-classes (43-54, 55-64, 65-74 and 75-87 mm). Mean TE concentrations calculated by size-class are always higher for small-size mussels (43-55 mm), except for Mo, As and Cd; standard deviations (SD) are also generally larger (Table 5). Henceforth, if small-size individuals accumulate proportionally more chemicals, they also present an important inter-individual variability. The example given in Fig. 3 for Cr properly illustrates these results, as the significant linear relationship ($p = 0.0007$, Fig. 3a) existing between the mussel shell length and Cr concentrations disappears ($p = 0.9116$, Fig. 3b) when mussels smaller than 55 mm are not taken into account. Small-size mussels were found inside the raft, close to the fixing rope, mixed with broken shells, covered by bigger individuals. Their shell also presented sometimes deformities due to the confinement. These unfavourable

conditions, slowing down their growth, led to the higher TE levels and to the bigger variability observed within this size-class.

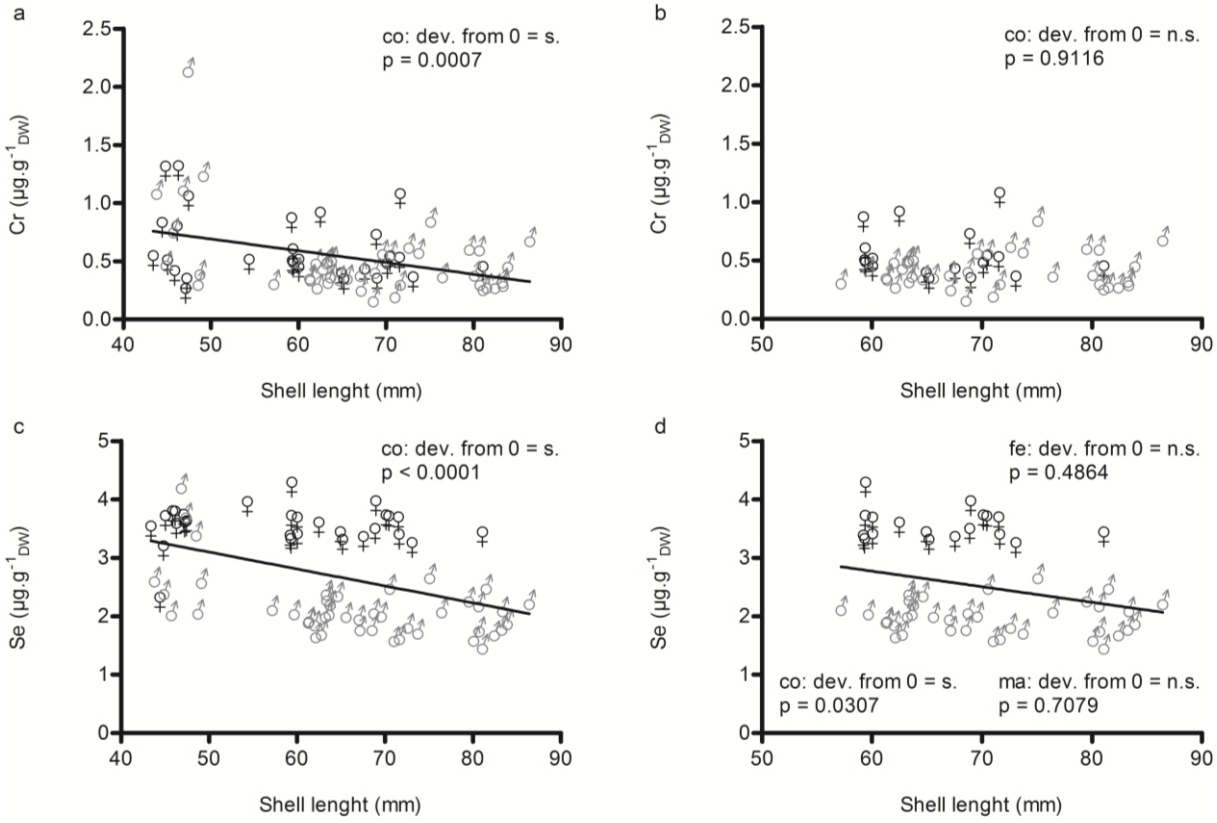

Fig. 3. Linear regressions modelling the relationship between the mussel shell length and Cr or Se concentrations ($\mu g.g^{-1}_{DW}$), all mussels together (a,c) or limited to individuals longer than 55 mm (b, d). ♀ and ♂ symbolize females and males, respectively. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to ♀ and ♂ for Cr, only significant ($p < 0.05$) when considering all individuals (a), underlines the determining effect played by small-size mussels. The regression common (co.) to ♀ and ♂ for Se, significant ($p < 0.05$) when considering all individuals (c) or only mussels longer than 55 mm (d), underlines the difference of Se accumulation linked to sex prior to spawn, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$).

Results show that for mid- to large-size *M. galloprovincialis* grown on ropes, the size does not influence body concentrations of most TEs. As their culture start synchronically and accordingly, all mussels on a rope have the same age (Saavedra et al. 2004), but may differ in size. Then, when sorting mussels for monitoring purposes, care will be taken not to use small- (restrained growth) but also large-size (fast growing) mussels, these individuals being not representative of the rope population. This consideration has to be minimized, as small (< 50

mm) and large (> 80 mm) mussels only represent around 5% of total individuals on a 10 kg rope.

3.4. TE concentrations as a function of sex

Even when considering only mussels from mid- to large size-classes, V, Mn, Cu, Se, Ag, As and Mo concentrations are still correlated ($0.05 < p < 0.0001$) to the shell length (Table 5). This observation results from the unequal accumulation of certain TEs between males and females during their gametogenesis, as shown for Se in Fig. 3: the significant relationship between Se concentrations and the mussel shell length partly relies on small-size individuals (Fig. 3c), but this effect is overlapped by differences between male and female Se accumulation prior to spawn (Fig. 3d). The comparison of relationships between TE concentrations and mussel soft tissue dry weight (individuals > 55mm), each sex apart, also states that no similar curve is shared between sexes for V, Mn, Cu, Se (Fig. 3d), Ag, As and Mo (statistics not shown).

In *Mytilus spp*, it is possible to tell the sex of individuals by the colour of their gonads (pink to orange for female and creamy-white to yellow for males, Mikhailov et al. 1995). After spawning, this sex segregation based on mantle colour is not possible anymore, as mantle becomes translucent, and sex determination necessitates more complex methods such as microscopic or histological examinations, or biochemical analysis (Mikhailov et al. 1995, Torrado and Mikhailov 1998).

On the basis of this colour pattern, the 74 mussels sampled in February 2011 were segregated according to their sex. Mean TE concentrations in females and males soft tissues are reported in Table 6. Concentrations differ significantly for most TEs ($p < 0.05$) and are higher in females (from 3% for Al up to 74% for Cu), except for Be. This is consistent with observations made by Lobel et al. (1991) for Mn, Cu, As and Se measured in wild *Mytilus edulis* from Newfoundland sampled in summer. Watling and Watling (1976) measured higher concentrations in wild females *Choromytilus meridionalis* from South Africa for Zn, Cu, Mn and Fe, similar in both sexes for Fe, Cd, Ag, Cr, Co and Ni, and slightly higher in males for Pb, and Bi. After the main breeding period, no difference was found between sexes (Orren et al. 1980), suggesting a reproduction-related metal accumulation. In wild *Perna perna* from the Gulf of Aden collected in summer, Cd, Cu, Mn, Pb and Zn were more concentrated in

females than in males, and inversely for Fe (Sokolowski et al. 2004). Hellou et al. (2003) collected male and female *M. edulis* in Nova Scotia, prior and during the spawning period (between April and July), and measured systematically higher concentrations of Cd, Pb, Hg, Ag, As, Cr, Cu, Sn and Zn in female soft tissues.

TE	females		males
Al	204 ± 143		197 ± 156
V	6,55 ± 2,40	*	4,57 ± 1,24
Fe	186 ± 89		172 ± 102
Cr	0,581 ± 0,288		0,537 ± 0,341
Mn	12,18 ± 3,48	*	8,36 ± 3,37
Co	0,707 ± 0,215	*	0,587 ± 0,185
Ni	1,68 ± 0,59	*	1,24 ± 0,43
Cu	6,50 ± 0,67	*	3,74 ± 0,61
Zn	86,3 ± 36,4	*	63,7 ± 28,8
Se	3,48 ± 0,34	*	2,21 ± 0,54

TE	females		males
Ag	0,0151 ± 0,0064	*	0,0104 ± 0,0038
Cd	0,397 ± 0,106	*	0,358 ± 0,144
Sn	0,0323 ± 0,0160		0,0314 ± 0,0174
Sb	0,0140 ± 0,0048	*	0,0118 ± 0,0036
As	36,3 ± 4,3	*	27,8 ± 4,6
Mo	20,7 ± 5,8	*	14,8 ± 4,5
Be	0,0127 ± 0,0056		0,0140 ± 0,0057
Pb	0,378 ± 0,211		0,309 ± 0,175
Bi	0,0097 ± 0,0034	*	0,0080 ± 0,0028

Table 6. Comparison between female (n = 29) and male (n = 45) trace element concentrations before spawning (mean ± SD, in $\mu g.g^{-1}_{DW}$). * represent significant differences ($p < 0.05$) between sexes.

The influence of gametogenesis in determining body TE concentrations cannot be neglected, even if its role is not clear yet and needs further studies. We can reasonably suggest that the closest mussels are from spawning, the most differences in TE concentrations rise. Mussels sampled for this study in mid-February were close to spawn: the mean Condition Index (CI) of individuals from the same ropes used for monitoring purposes decreased by half 2 to 4 weeks later (data not shown). This advanced reproductive status probably lead to the systematic higher TE concentrations measured in females for 18 of the 19 TEs.

Sokolowski et al. (2004), from observations made by Lobel and Wright (1982) and Pieters et al. (1980) on *M. edulis*, suggested that the formation of reserves during the prespawning period, more pronounced in females, could favour the faster accumulation of TEs in this sex. However, Suárez et al. (2005) showed that the contribution of the reproductive tissue to the total body weight of raft *M. galloprovincialis* was systematically higher in males all year round, this difference being even more pronounced in winter and spring, during gametogenesis (Suárez et al. 2005). The hypothesis suggested by Sokolowski et al. (2004) must then be partially rejected, at least in the case of raft *M. galloprovincialis*, since the contribution of the female reproductive tissues to their total body weight does not exceed that in males. Some evidences suggest that differences between males and females TE levels

prior to spawn could rely on a functional role played by metallothionein (MTs), as already suggested by Latouche and Mix (1981) in the early 80^{ies}. Akberali et al. (1985, in Earnshaw et al. 1986) experimentally showed that Cu and Zn uptake was higher into sperm than into eggs of *M. edulis*. Fitzpatrick et al. (2008) observed no influence of increasing Cu concentrations on *M. trossulus* egg viability and fertilization rates, while sperm motility and fertilization rates decreased, and Meistertzheim et al. (2009) recently measured a more important increase of MT concentrations in females gonads of *C. gigas* than in males during gametogenesis. The hypothetical role played by MTs in *M. galloprovincialis* gametogenesis cannot be neglected and need further investigations.

3.5. TE accumulation and tissue compartmentalization

All mussels analysed for tissue compartmentalization measured between 70 and 80 mm. Mean body and gonad dry weights of mussel sampled in February 2011 (prior spawning) are 0.981 and 0.178 g, respectively, and mean body and gonad dry weights of mussels sampled in March 2010 (after spawning) are 0.740 and 0.136 g, respectively. Gonad weights differ by 31 %; this is similar to the 33 % whole body weight difference between both reproductive statuses. In the genus *Mytilus*, up to 40 % of soft tissue weight can be lost during spawning, which shows the importance of gametogenesis in their physiology (Cossa 1989). TEs can be divided into 4 groups, depending on their concentrations and contents before and after spawning (Fig 4 and Table 7).

The first group is made up of Al, Fe, Cr (Fig. 4a,b), Mn, Ni, Sn, Mo, Be and Bi: they are less concentrated and less abundant prior to spawn, due to the combined effect of a tissue dilution during the gametogenesis body weight increase and an environmental diminution of available TEs between the two sampled years. This second effect, not quantified, seems to be the most important. A second group is made up of V and Ag (Fig. 4c,d), more concentrated and more abundant prior to spawn. These 2 TEs are sufficiently abundant in 2011 to mask the dilution effect due to gametogenesis. The Diane pond is a small size salt pond, highly influenced by the runoff of freshwater from the catchment basin of Bravona, bringing terrigenous TEs such as Al or Fe, and by the discontinuous connectivity with the open sea through a small “grau”, a channel silted up part of the year (Longere et al. 1972). These particular physical characteristics influence the physicochemical properties of the water

masses, and so the bioavailability of TEs. Data compiled from 2 mussel caging campaigns in spring 2010 and 2011 in the Calvi Bay, North-West Corsica, with mussels from the same ropes that the ones used in this study confirm this confinement hypothesis. TE concentrations were similar between the 2 monitored years in the Calvi Bay (data not shown), even for TE showing differences in this study as important as for Al or Fe. Se (Fig. 4e,f), Cd, Sb, As and Pb form a third group, showing similar concentrations but contents a little higher prior to spawn. These 5 TEs, more accumulated in mussel tissues in 2011, display similar concentrations with mussels having spawned as they are diluted during gametogenesis. Finally, the fourth and surely more interesting group is made up of Co, Cu (Fig. 4g,h) and Zn: these 3 essentials TEs display lower concentrations prior to spawn and similar contents at both physiological statuses. The evident role of MTs in Zn and Cu regulation probably account for stable contents observed between years.

TEs are preferentially concentrated in the hepatopancreas, except for Zn, Se, Cd and Mo which are more concentrated in gills (Fig. 4 and Table 7). When expressed as total contents in each body compartment, the 19 investigated TEs are systematically more accumulated in the hepatopancreas. Since the hepatopancreas preferentially accumulates most of the 19 studied TEs, this particular organ should be privileged in monitoring surveys, as previously suggested by many authors for classically investigated trace metals (e.g. Adami et al. 2002, Gupta and Singh 2011). TE distributions between tissues before and after spawning display the same patterns. During the prespawning period, mussels accumulate reserves (Pieters et al. 1980): glycogen is largely accumulated and stored in the mantle, and proteins and lipids in the non-mantle tissues (Gabbot and Bayne 1973 in Gabbott 1975). TE accumulation is mainly limited to the hepatopancreas, kidney and gills (Lobel and Wright 1982, Cossa 1989; this study); if mantle can also take up soluble TEs, it has to be considered as a transitional tissue, TEs being rapidly transported to nongonadal tissues (Lobel and Wright 1982). Energy for vitellogenesis, the final stage of gametogenesis, is supplied from the glycogen reserves, from lipid reserves stored in adipogranular cells in the mantle, and from freshly ingested food material (Newell 1989). Following the conversion of the stored glycogen to the lipid reserve of developing eggs (Latouche and Mix 1981), a movement of nutrients from the digestive gland to the mantle occurred (Gabbott 1975), leading to a consecutive redistribution of TEs within mussel tissues. So, the similar pattern of TE

distribution between tissues before and after spawning is closely related to the mussel reproductive cycle.

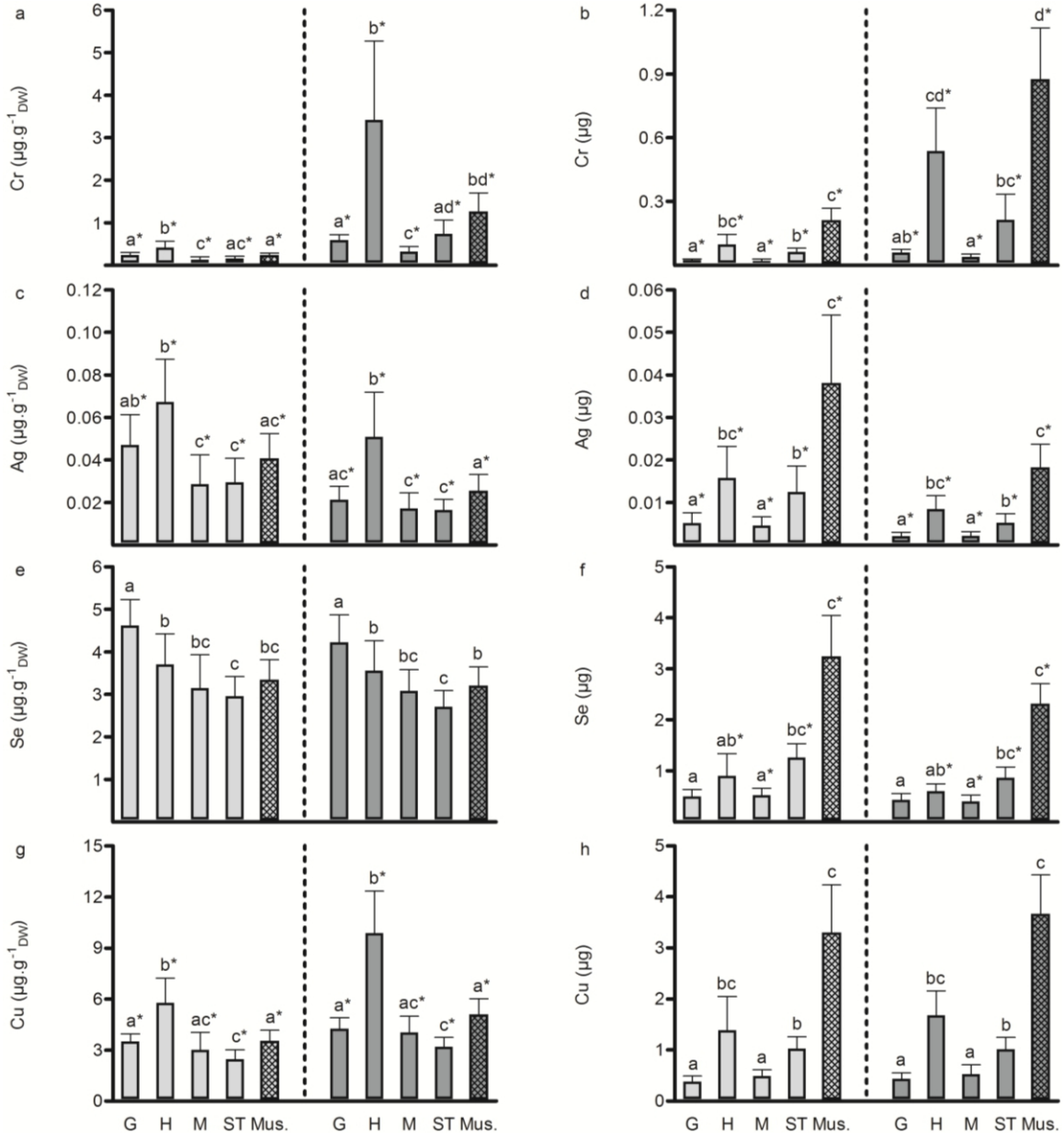

Fig. 4. a-b) Cr, c-d) Ag, e-f) Se and g-h) Cu levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ – 4 left graphs) or total contents (µg – 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status (*i.e.* multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e.* pairwise comparison tests of means), respectively.

Table 7. Dry weight (DW) of the 4 body compartments and the whole individuals (mean ± SD, in g), and corresponding trace element concentrations (mean ± SD, in $\mu g.g^{-1}_{DW}$), before (below) or after (next page) spawning, respectively. Letters represent significant differences ($p < 0.05$) between the 4 body compartments and the whole individuals of a same gametogenic status (*i.e* . multiple comparison test of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (*i.e* . pairwise comparison test of means), respectively.

before spawning		gills		hepatopancreas		mantle		remaining soft tissues		whole mussel
DW		0,110 ± 0,031		0,259 ± 0,136		0,178 ± 0,066		0,435 ± 0,135		0,981 ± 0,348
Al	a*	8,8 ± 7,2	b*	40,3 ± 27,7	c*	3,7 ± 2,4	a*	8,5 ± 6,8	d*	14,9 ± 7,3
V	ad*	1,8 ± 0,3	b*	11,6 ± 5,3	c	0,8 ± 0,3	ac*	1,0 ± 0,3	bd*	3,7 ± 1,3
Fe	a*	50 ± 11	b*	125 ± 46	c*	33 ± 7	c*	30 ± 8	ab*	57 ± 15
Cr	a*	0,239 ± 0,060	b*	0,420 ± 0,140	c*	0,131 ± 0,071	ac*	0,158 ± 0,056	a*	0,227 ± 0,060
Mn	a*	2,68 ± 1,41	a*	2,54 ± 0,75	a*	2,18 ± 1,04	a*	2,39 ± 1,07	a*	2,45 ± 0,98
Co	ab	1,01 ± 0,26	b*	1,44 ± 0,63	c*	0,37 ± 0,17	c*	0,46 ± 0,13	a*	0,75 ± 0,22
Ni	a*	1,19 ± 0,45	a*	2,64 ± 1,38	b*	0,46 ± 0,28	b*	0,55 ± 0,23	a*	1,12 ± 0,45
Cu	a*	3,52 ± 0,43	b*	5,79 ± 1,43	ac*	3,02 ± 1,03	c*	2,48 ± 0,54	a*	3,51 ± 0,65
Zn	a	113 ± 42	ab*	79 ± 25	b*	61 ± 13	b	65 ± 18	ab	74 ± 20
Se	a	4,62 ± 0,61	b	3,71 ± 0,72	bc	3,15 ± 0,78	c	2,96 ± 0,46	bc	3,33 ± 0,48
Ag	ab*	0,0471 ± 0,0142	b*	0,0672 ± 0,0202	c*	0,0286 ± 0,0137	c*	0,0295 ± 0,0113	ac*	0,0405 ± 0,0119
Cd	a	1,09 ± 0,24	a	0,91 ± 0,26	b*	0,49 ± 0,17	bc	0,61 ± 0,13	c	0,72 ± 0,16
Sn	ac*	0,0193 ± 0,0035	b*	0,0618 ± 0,0237	cd*	0,0132 ± 0,0054	d*	0,0127 ± 0,0034	ab*	0,0252 ± 0,0057
Sb	a*	0,0213 ± 0,0024	b	0,0551 ± 0,0156	c*	0,0123 ± 0,0049	c	0,0102 ± 0,0022	a	0,0229 ± 0,0043
As	a*	20,5 ± 2,6	b*	32,9 ± 4,5	c*	18,2 ± 2,0	d*	15,2 ± 1,4	a*	20,7 ± 2,0
Mo	a*	13,8 ± 5,0	a*	10,7 ± 4,1	b*	4,5 ± 1,5	b*	4,2 ± 0,9	c*	7,1 ± 2,2
Be	ab	0,0145 ± 0,0078	b*	0,0250 ± 0,0093	c*	0,0041 ± 0,0021	ac*	0,0082 ± 0,0035	a*	0,0125 ± 0,0042
Pb	ab*	1,74 ± 0,39	b	2,38 ± 1,16	c*	0,74 ± 0,24	cd*	0,98 ± 0,28	ad	1,32 ± 0,34
Bi	a	0,0168 ± 0,0037	b*	0,0419 ± 0,0126	c*	0,0064 ± 0,0032	c*	0,0076 ± 0,0024	a*	0,0170 ± 0,0038

Table 7. ***Continued***

after spawning	gills		hepatopancreas		mantle		remaining soft tissues		whole mussel	
DW		0,102 ± 0,025		0,176 ± 0,054		0,136 ± 0,052		0,326 ± 0,091		0,740 ± 0,182
Al	a*	136,9 ± 68,4	b*	1493,2 ± 774,4	c*	39,2 ± 21,8	a*	187,6 ± 110,3	d*	468,4 ± 190,2
V	a*	1,2 ± 0,2	b*	6,6 ± 2,5	c	0,6 ± 0,2	c*	0,9 ± 0,3	a*	2,2 ± 0,6
Fe	ac*	153 ± 35	b*	1061 ± 563	c*	89 ± 23	a*	180 ± 66	b*	361 ± 130
Cr	a*	0,599 ± 0,119	b*	3,421 ± 1,851	c*	0,324 ± 0,117	ad*	0,742 ± 0,325	bd*	1,254 ± 0,449
Mn	a*	7,41 ± 1,75	b*	23,44 ± 8,97	a*	6,45 ± 1,83	ac*	8,46 ± 3,10	c*	11,38 ± 3,80
Co	a	1,06 ± 0,28	b*	2,53 ± 0,99	c*	0,62 ± 0,18	c*	0,57 ± 0,16	a*	1,11 ± 0,35
Ni	ad*	1,70 ± 0,64	b*	5,91 ± 2,51	c*	0,89 ± 0,45	ac*	1,09 ± 0,42	d*	2,26 ± 0,86
Cu	a*	4,27 ± 0,63	b*	9,89 ± 2,45	ac*	4,04 ± 0,96	c*	3,19 ± 0,57	a*	5,07 ± 0,95
Zn	a	133 ± 59	ab*	112 ± 47	b*	98 ± 34	b	84 ± 30	ab	93 ± 38
Se	a	4,23 ± 0,64	b	3,56 ± 0,70	bc	3,08 ± 0,50	c	2,71 ± 0,38	b	3,19 ± 0,45
Ag	ac*	0,0212 ± 0,0063	b*	0,0509 ± 0,0210	c*	0,0172 ± 0,0073	c*	0,0165 ± 0,0049	a*	0,0252 ± 0,0079
Cd	a	1,08 ± 0,18	a	1,03 ± 0,29	b*	0,66 ± 0,23	b	0,60 ± 0,12	b	0,75 ± 0,15
Sn	ad*	0,0503 ± 0,0208	b*	0,2346 ± 0,1015	c*	0,0197 ± 0,0041	d*	0,0373 ± 0,0153	ab*	0,0763 ± 0,0222
Sb	ad*	0,0200 ± 0,0065	b	0,0659 ± 0,0207	c*	0,0092 ± 0,0024	cd	0,0112 ± 0,0021	ab	0,0258 ± 0,0069
As	a*	18,4 ± 3,2	b*	27,2 ± 2,7	ac*	16,1 ± 1,5	c*	13,1 ± 1,7	a*	17,7 ± 1,4
Mo	a*	93,8 ± 29,2	b*	43,0 ± 14,1	c*	29,5 ± 8,7	c*	21,5 ± 7,2	b*	38,6 ± 12,4
Be	a	0,0155 ± 0,0054	b*	0,0835 ± 0,0311	a*	0,0075 ± 0,0043	a*	0,0150 ± 0,0053	b*	0,0295 ± 0,0088
Pb	ad*	0,96 ± 0,24	b	3,01 ± 0,95	c*	0,52 ± 0,17	ac*	0,76 ± 0,18	bd	1,27 ± 0,34
Bi	ad	0,0207 ± 0,0049	b*	0,1107 ± 0,0310	c*	0,0128 ± 0,0048	ac*	0,0157 ± 0,0037	bd*	0,0381 ± 0,0115

4. Conclusion

M. galloprovincialis efficiently accumulates the 10 little studied TEs (Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb, Bi) in addition to the 9 TEs classically investigated (Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V). However, the important variability of TE levels sometimes recorded at regional or even local scale required a coordination of monitoring programs between neighbouring countries, in order to define sensible reference conditions to properly monitor the chemical pollution of the coastal environment. Low levels measured in the Diane pond may serve as baseline levels for comparison with ulterior surveys, at least for the northwestern Mediterranean.

Relationships between TE concentrations and the body dry weight or between TE contents and the mussel shell length, all individuals together, are better modelled with power functions or linear regressions, respectively; *a contrario*, relationships between TE contents and the mussel shell length are properly modelled with both linear regressions and power functions when only considering individuals longer than 55mm. Small size *M. galloprovincialis* have an antagonist effect: they drive to elect the linear function to model relationships between the mussel size and TE contents, but diminish the significance of the power function modelling relationships between the mussel weight and some TE concentrations. Small class-size mussels furthermore concentrate more chemicals, and show an important variability. If a large range of sizes can be used for monitoring purposes, one will take care not to use very small or large individuals.

Many differences were observed between sexes, prior to spawn. Although the influence of gametogenesis in determining female body higher TE concentrations is not clear yet and need further studies, the role played by metallothioneins could be fundamental. The hepatopancreas preferentially accumulates TEs, except for Zn, Se, Cd and Mo which are more concentrated in gills. Body distribution is not influenced by the reproductive status, but gametogenesis dilutes TE due to the important tissue production prior to spawn. Based on these observations, it appears judicious to monitor the 19 studied TEs during mussel sexual dormancy. However, temporal variations of environmental TE concentrations mostly hide this gametogenic dilution effect.

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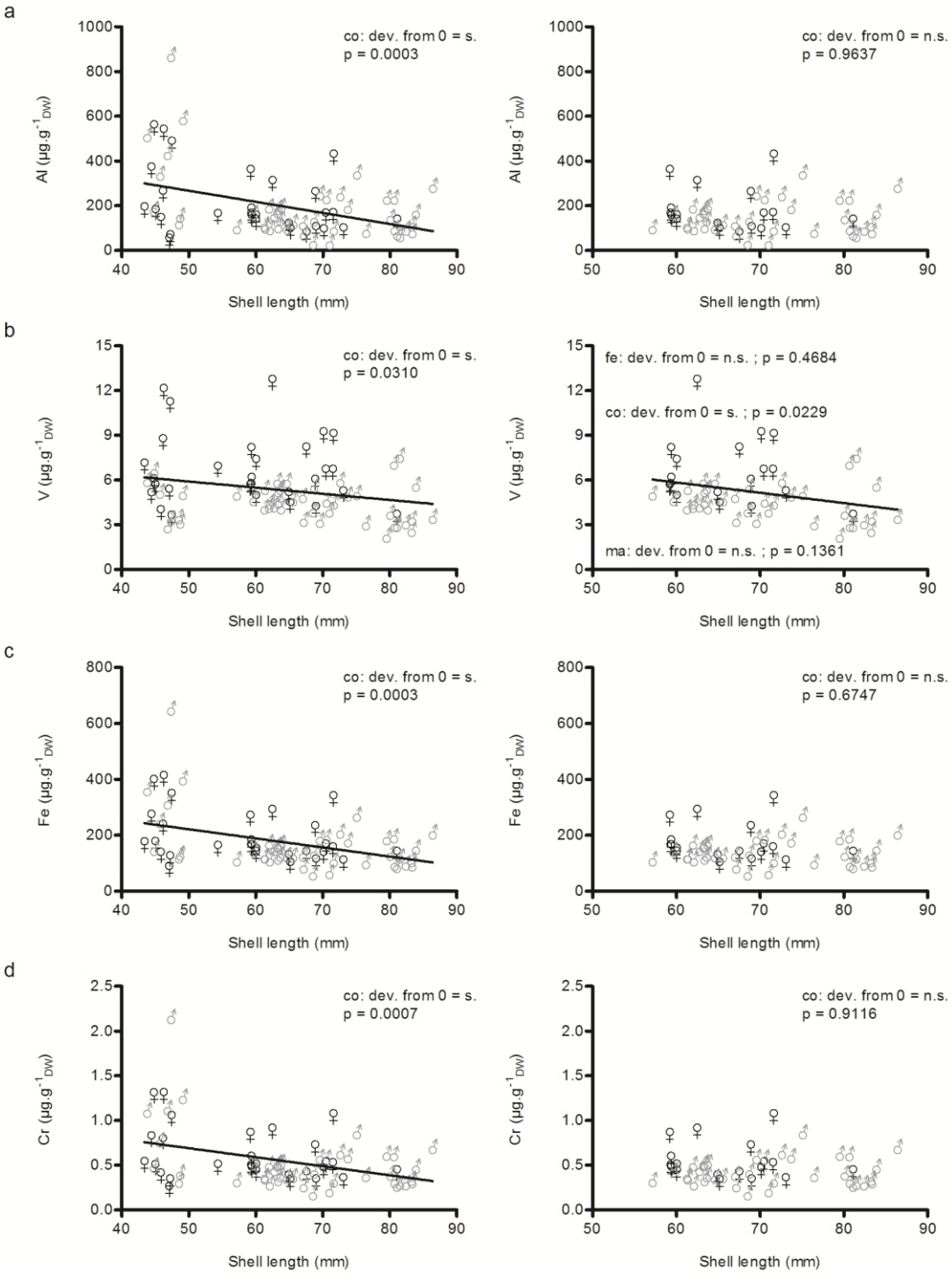
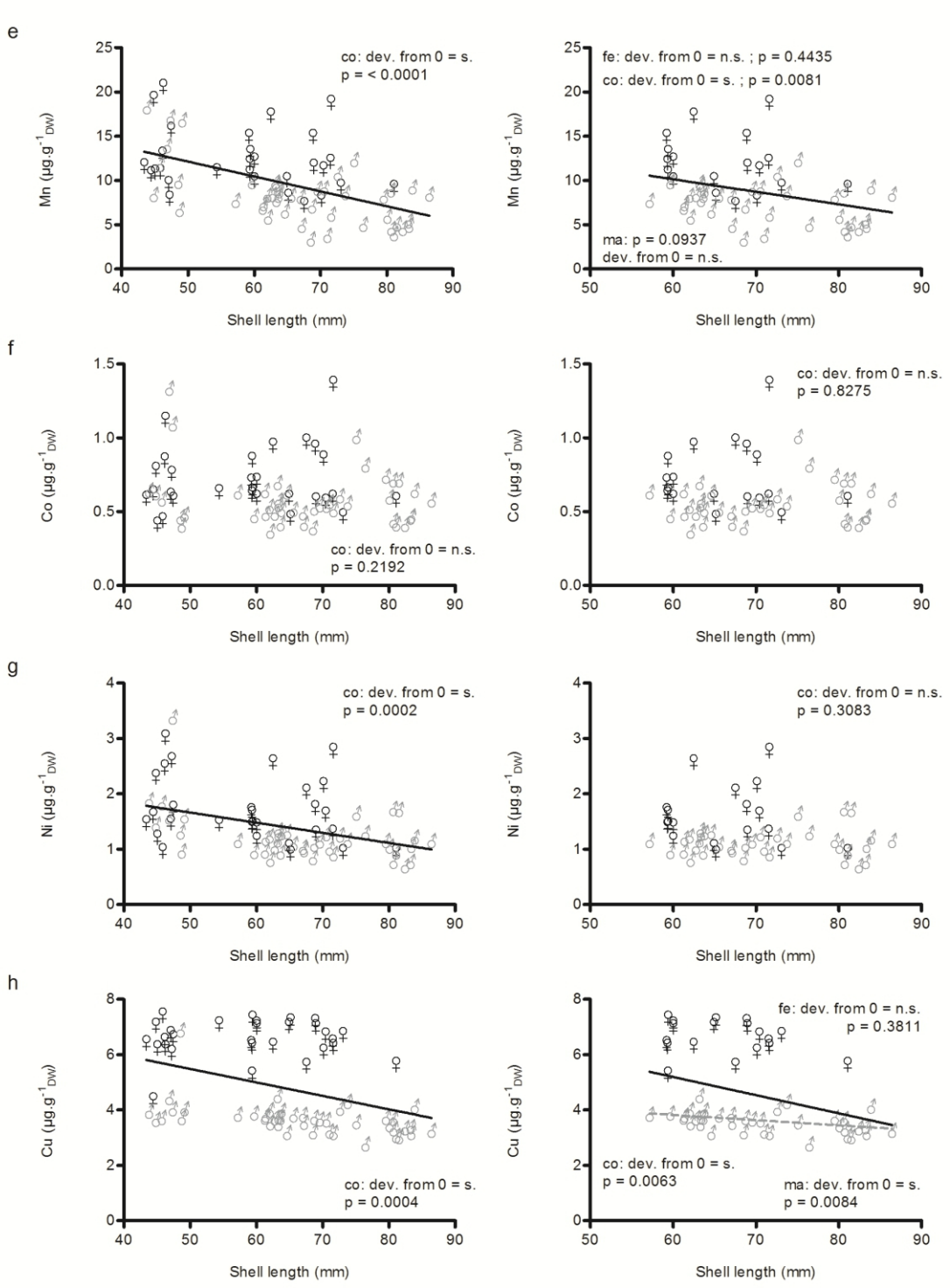
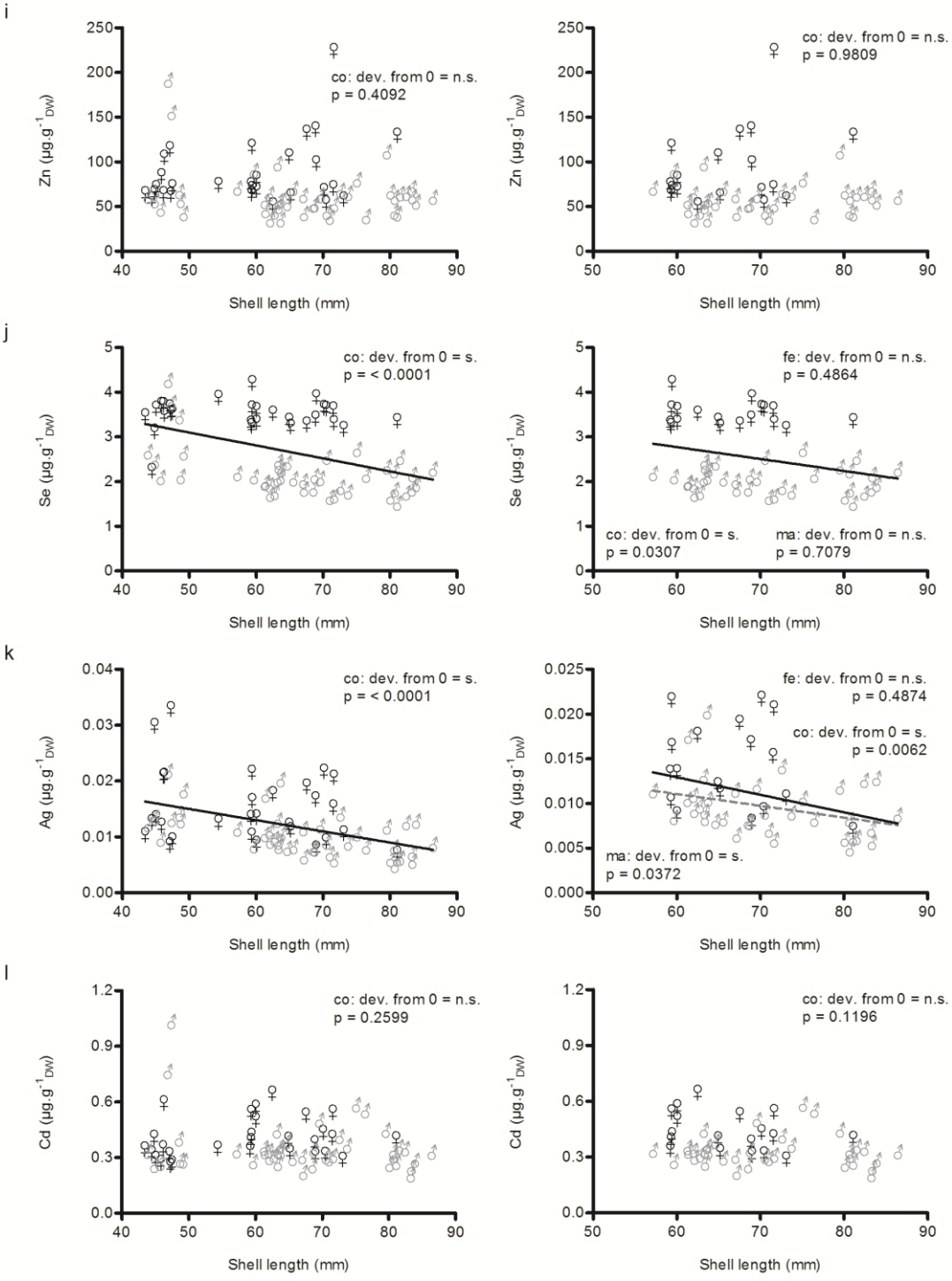


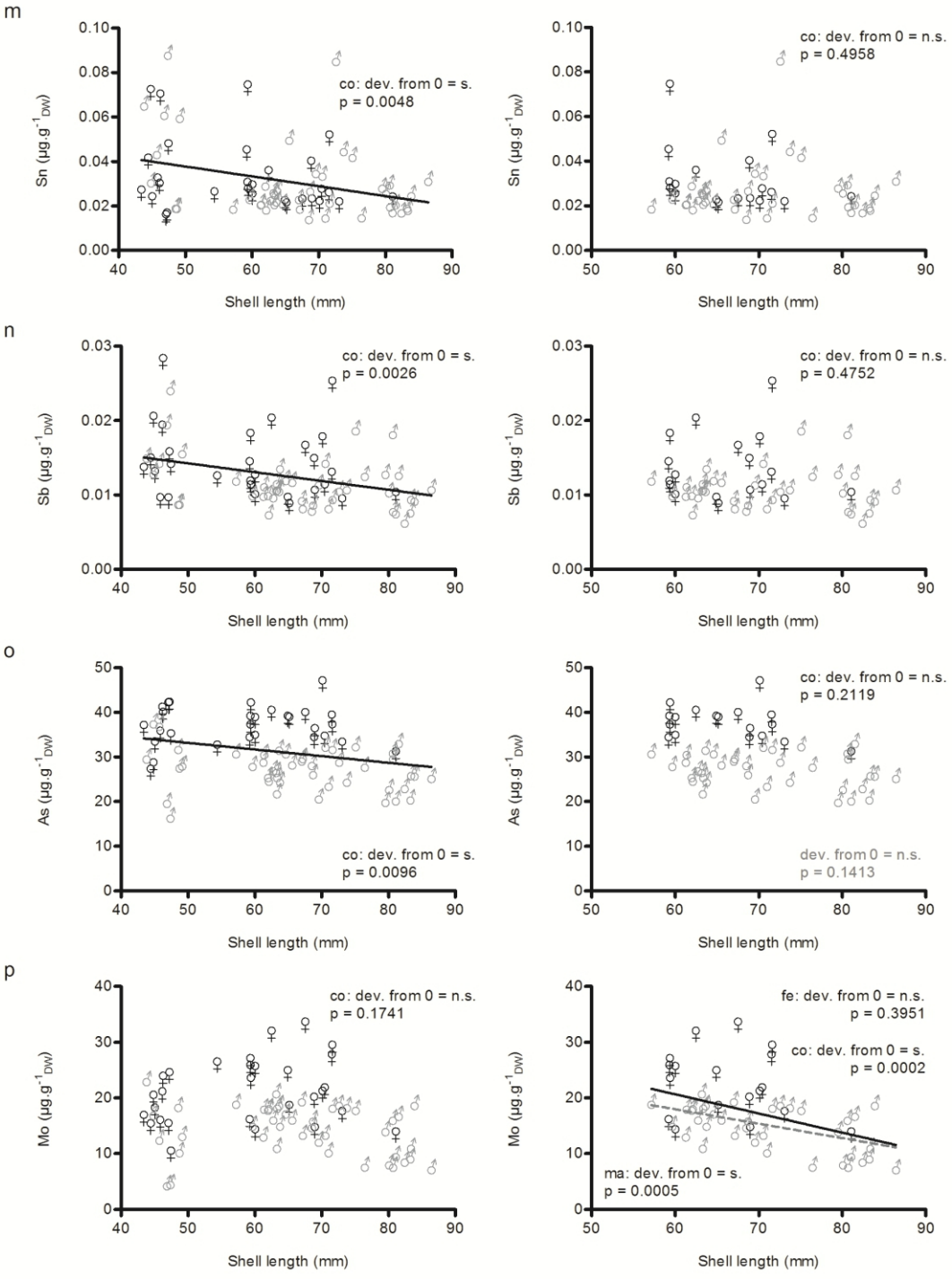
Annex A. Linear regressions modelling relationships between the mussel shell length and a) Al, b) V, c) Fe and d) Cr concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Al, V, Fe and Cr when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for V, significant ($p < 0.05$) when considering only mussels longer than 55 mm (right graphs), underlines the difference of V accumulation linked to sex prior to spawn, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$).



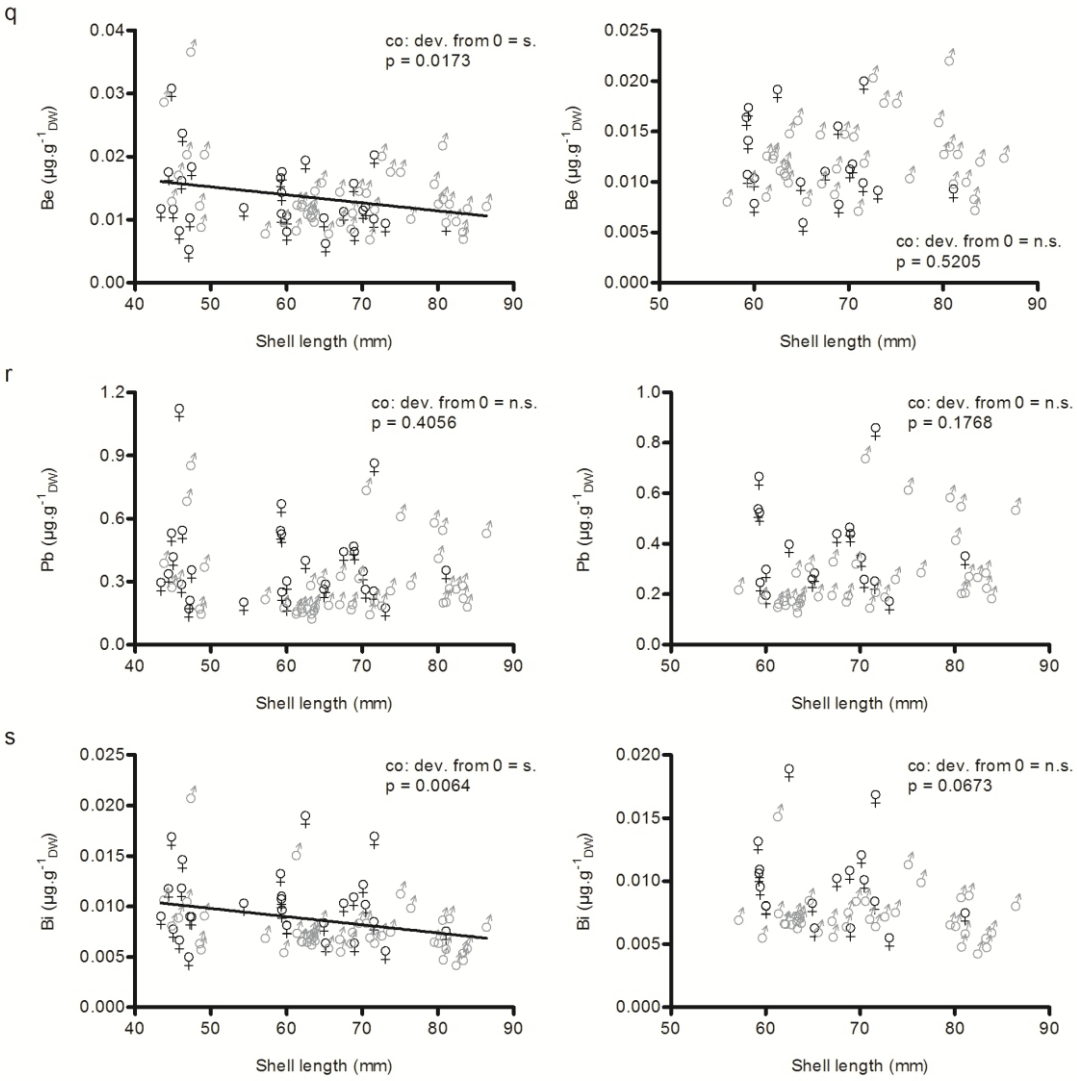
Annex A (*Continued*). Linear regressions modelling relationships between the mussel shell length and e) Mn, f) Co, g) Ni and h) Cu concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Mn, Ni and Cu when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for Mn and Cu, significant ($p < 0.05$) when considering only mussels longer than 55 mm (right graphs), underlines the difference of Mn and Cu accumulation linked to sex prior to spawn, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$), except for Cu accumulation in males.



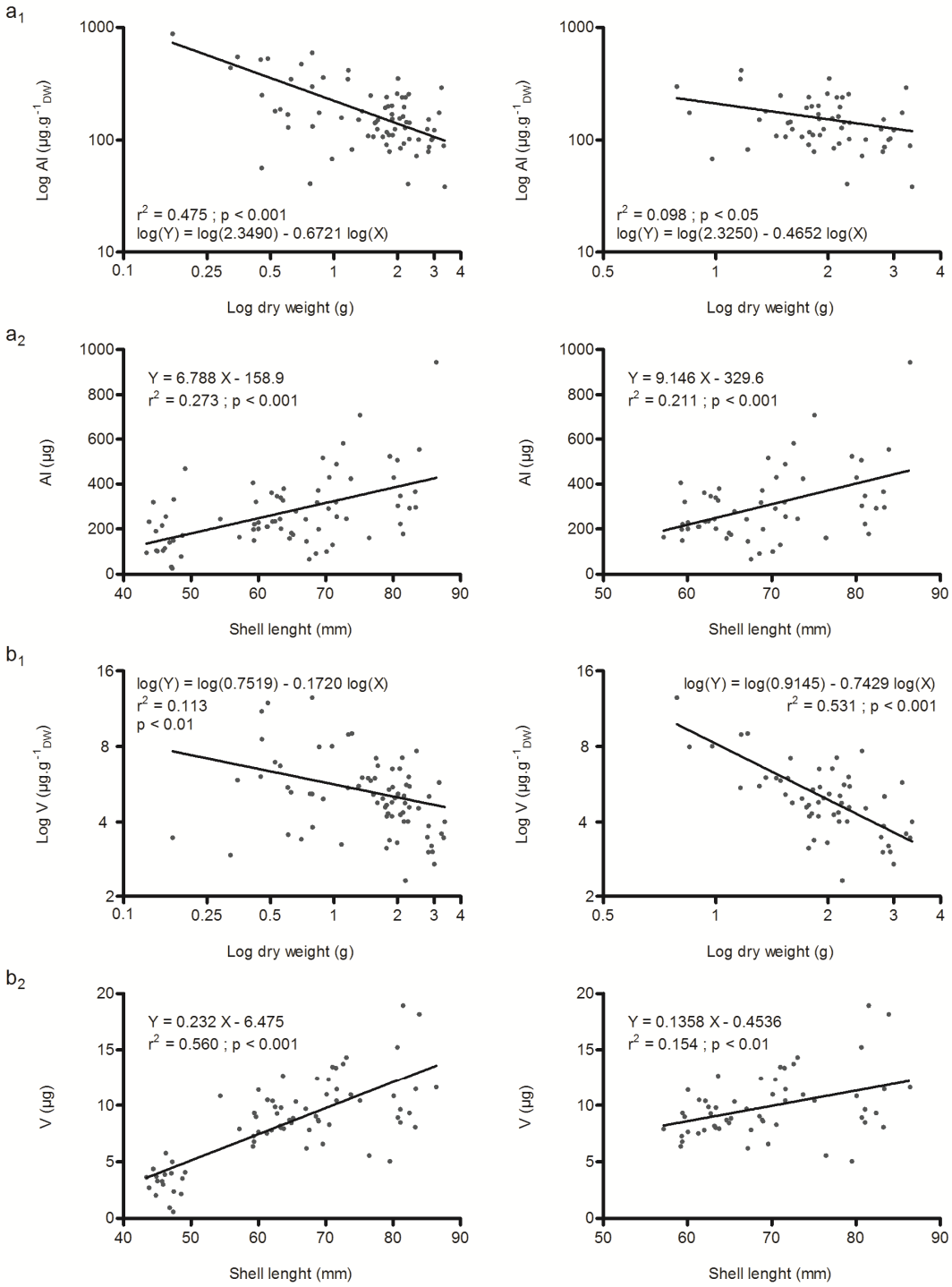
Annex A (*Continued*). Linear regressions modelling relationships between the mussel shell length and i) Zn, j) Se, k) Ag and l) Cd concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Se and Ag when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for Se and Ag, significant ($p < 0.05$) when considering only mussels longer than 55 mm (right graphs), underlines the difference of Se and Ag accumulation linked to sex prior to spawn, superimposed on the size effect; regressions specific to each sex (fe., ma.) are moreover not significant ($p > 0.05$), except for Ag accumulation in males.



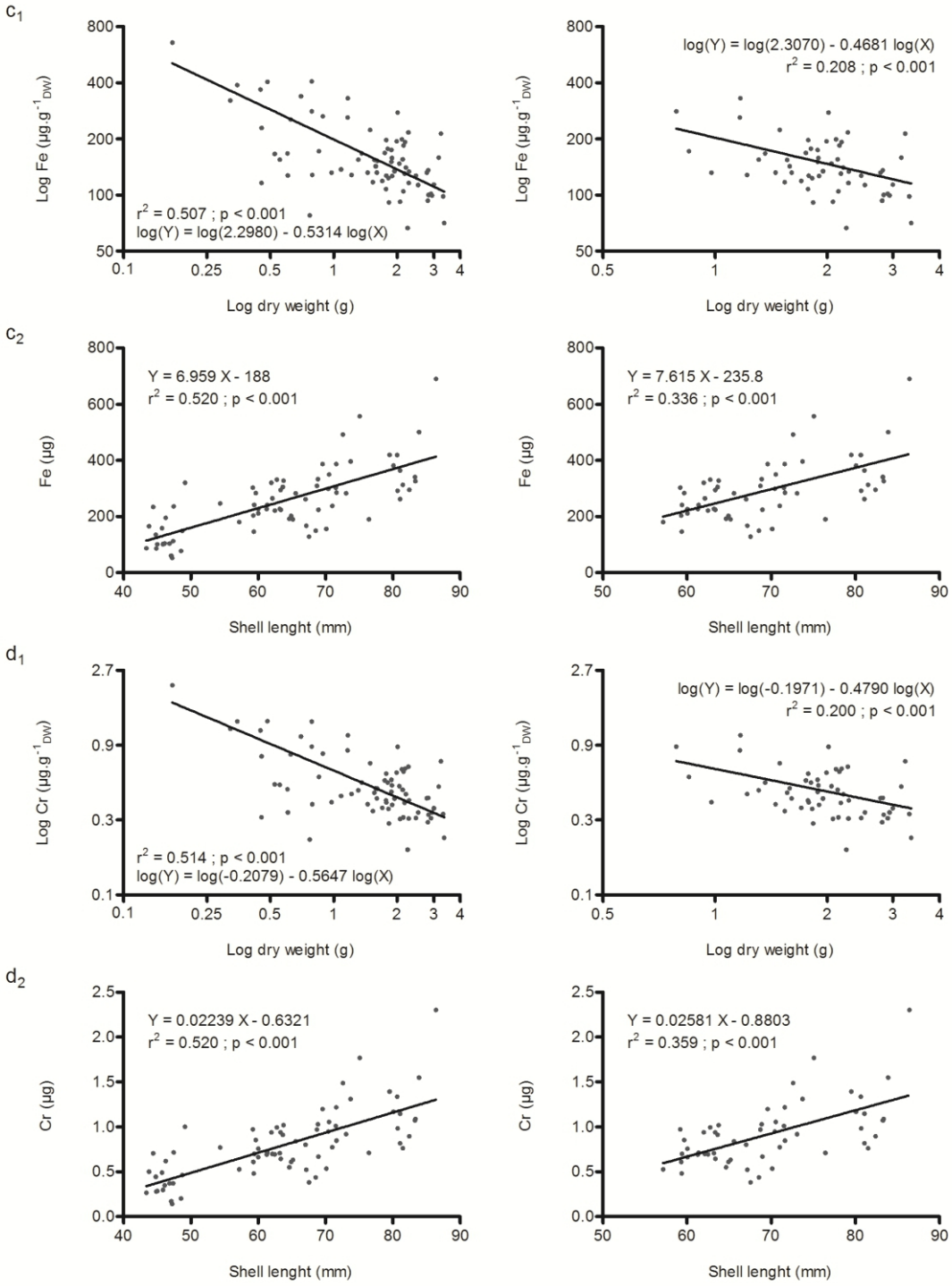
Annex A (*Continued*). Linear regressions modelling relationships between the mussel shell length and m) Sn, n) Sb, o) As and p) Mo concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Sn, Sb and As when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. The regression common (co.) to females and males for Mo, significant ($p < 0.05$) when considering only mussels longer than 55 mm (right graphs), does not depend on a difference in Mo accumulation related to the sex prior to spawn, but rather on a diminutiotn in Mo concentrations in large size males. The relationship between the size of males longer than 55 mm and their Mo content is moreover very highly significant ($p < 0.001$).



Annex A (*Continued*). Linear regressions modelling relationships between the mussel shell length and q) Be, r) Pb and s) Bi concentrations ($\mu g.g^{-1}_{DW}$). Females and males are represented by their gender symbol. Probabilities (p-levels) of linear slopes to deviate (dev.) from 0 are given (s. = significant, n.s. = non-significant). The regression common (co.) to females and males, significant ($p < 0.05$) for Be and Bi when considering all individuals (left graphs), underlines the determining effect played by small-size mussels. There is no significant ($p > 0.05$) regression common (co.) to females and males when considering only mussels longer than 55 mm (right graphs).



Annex B. Double log transformed power functions modelling relationships between a_1-b_1) the mussel soft tissue dry weight (g) and Al or V concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between a_2-b_2) the mussel shell length (mm) and Al or V total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



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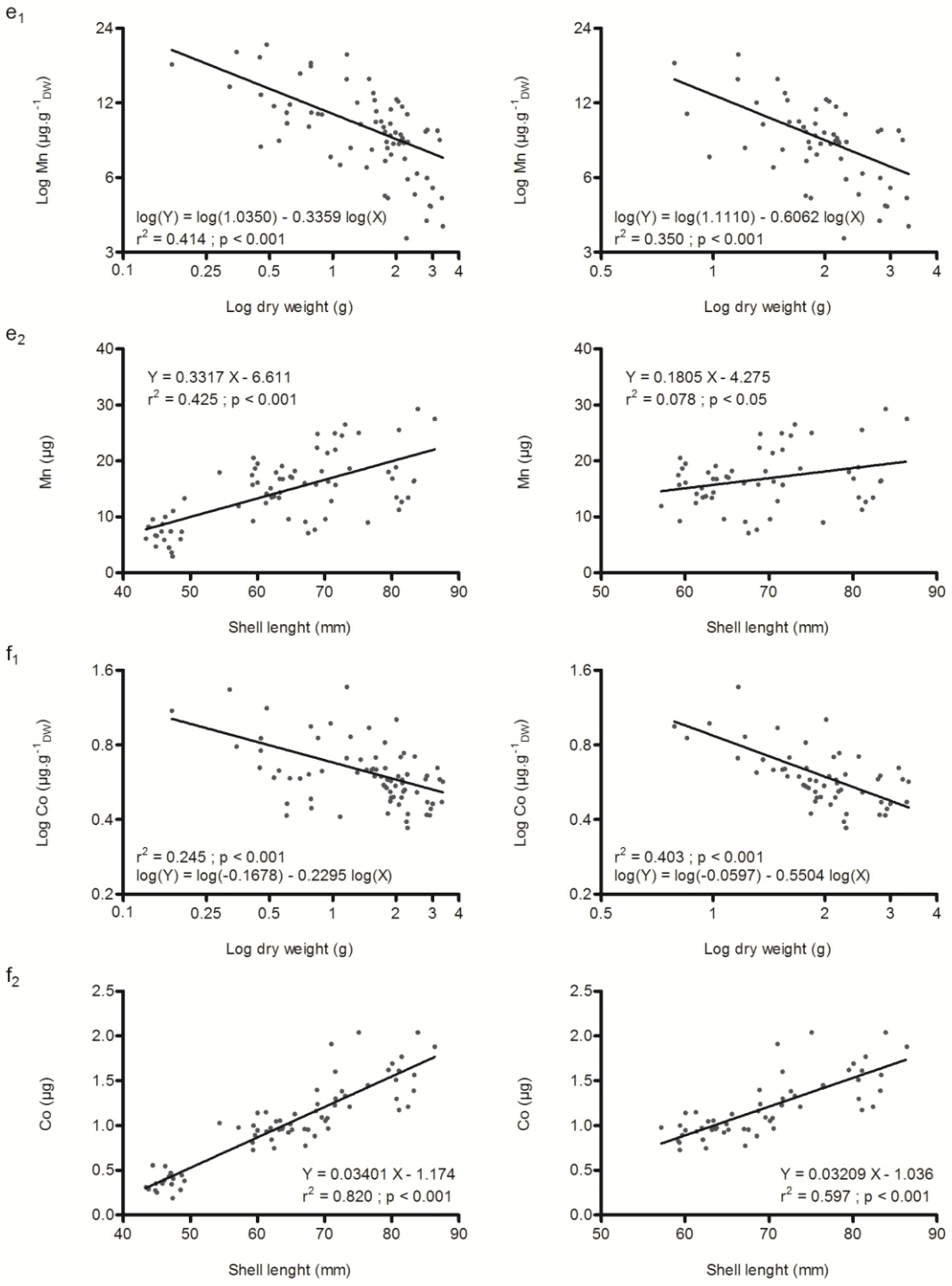
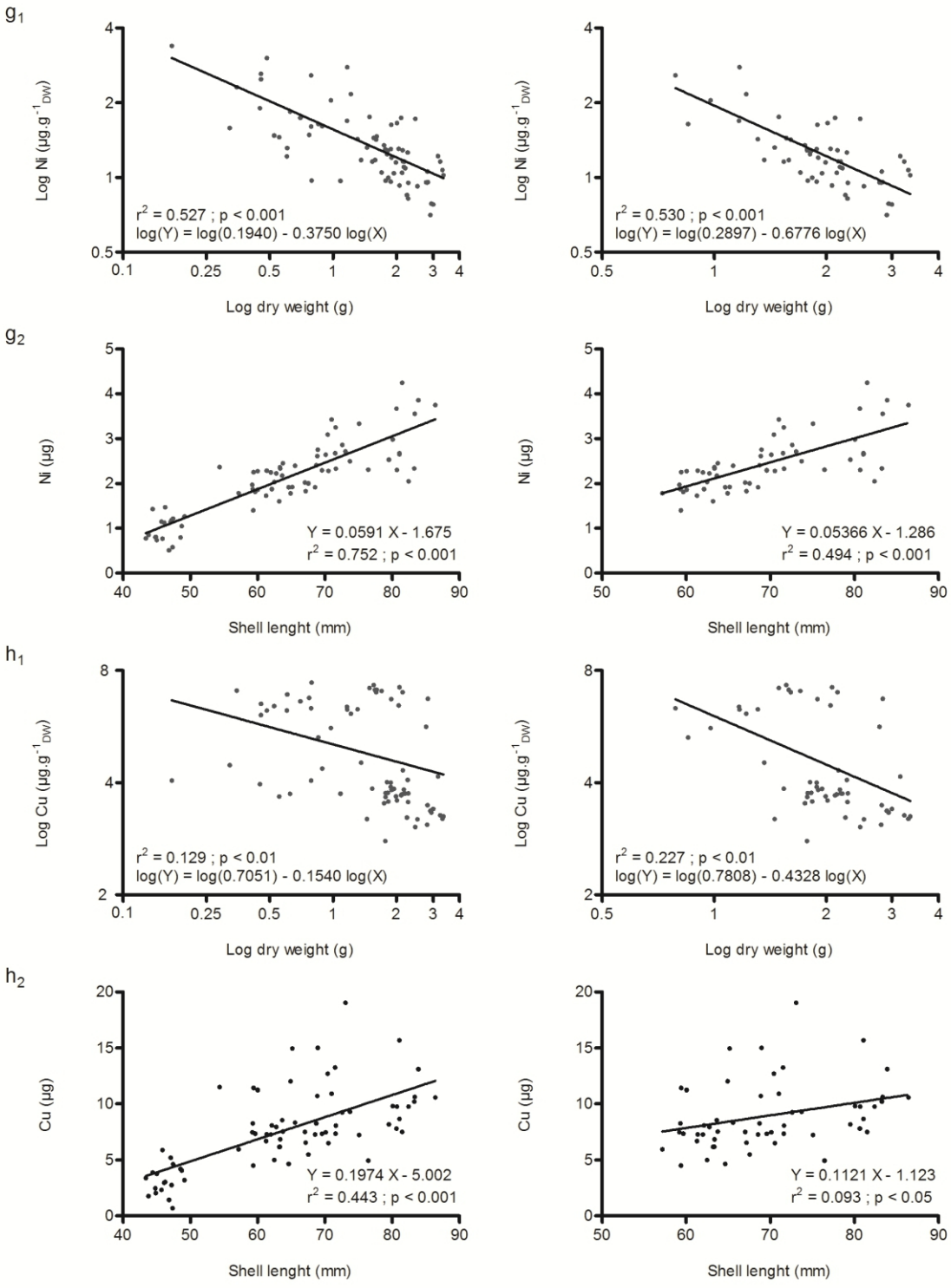
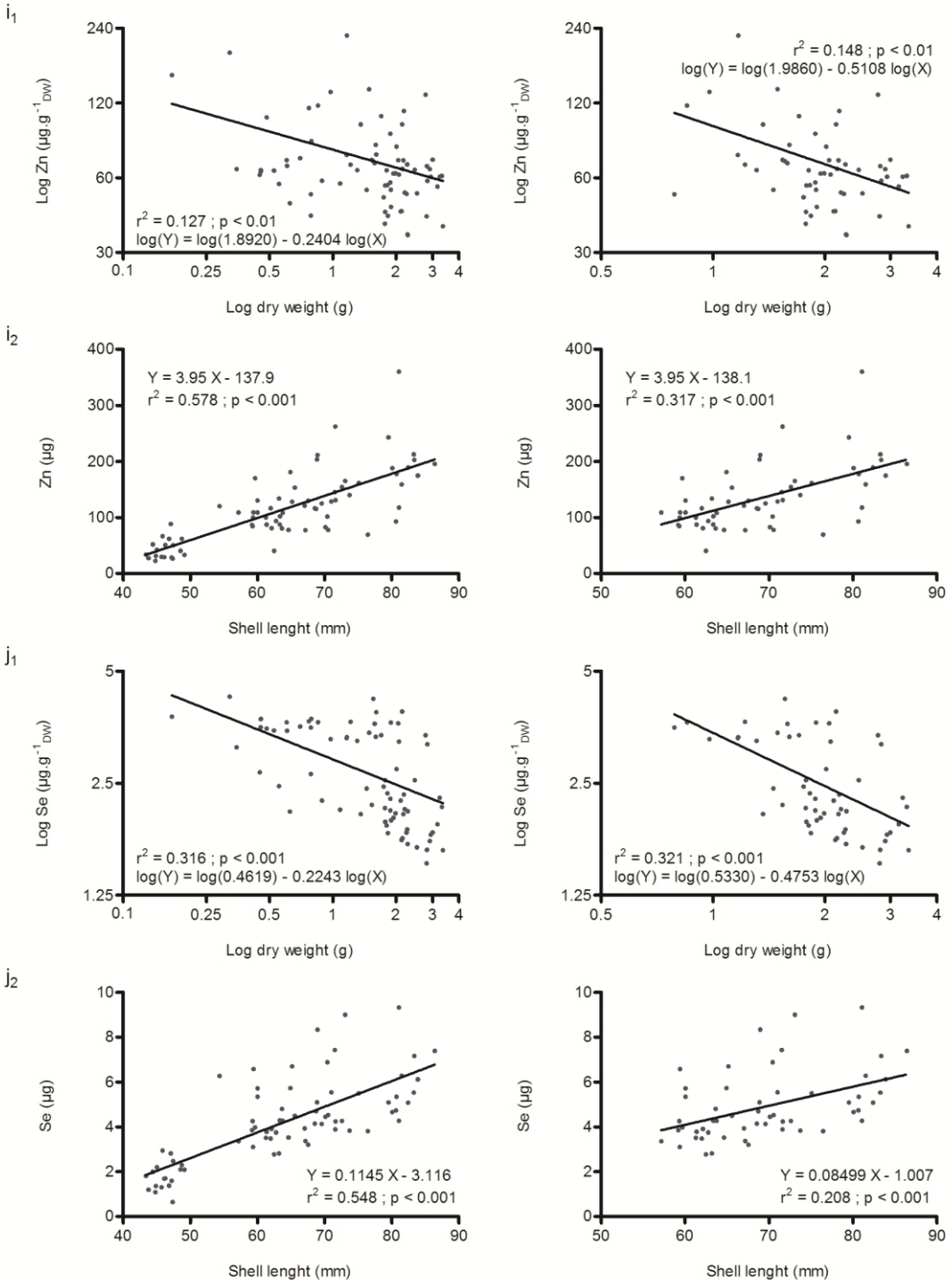


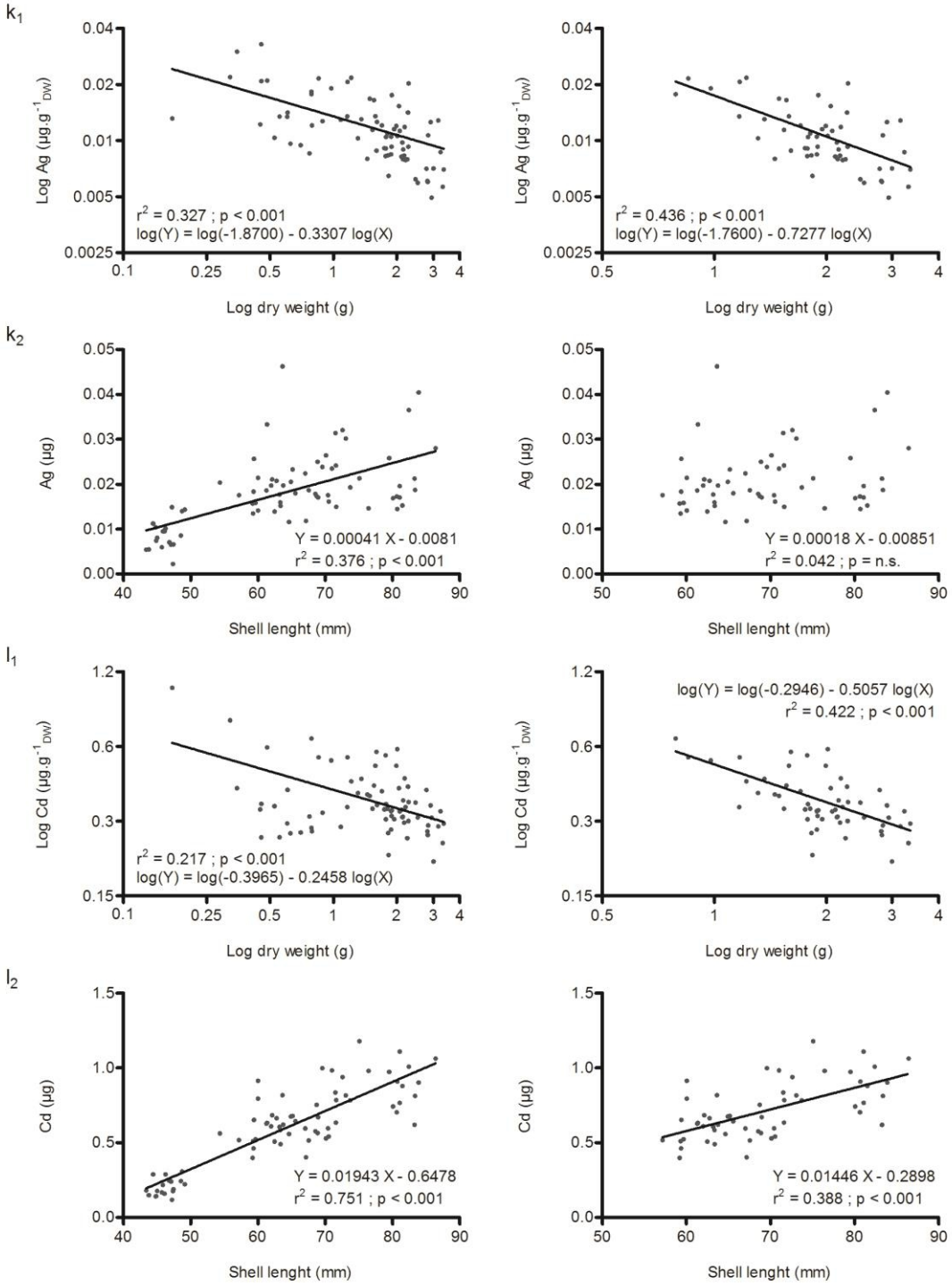
Annex B (*Continued*). Double log transformed power functions modelling relationships between e_1-f_1) the mussel soft tissue dry weight (g) and Mn or Co concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between e_2-f_2) the mussel shell length (mm) and Mn or Co total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



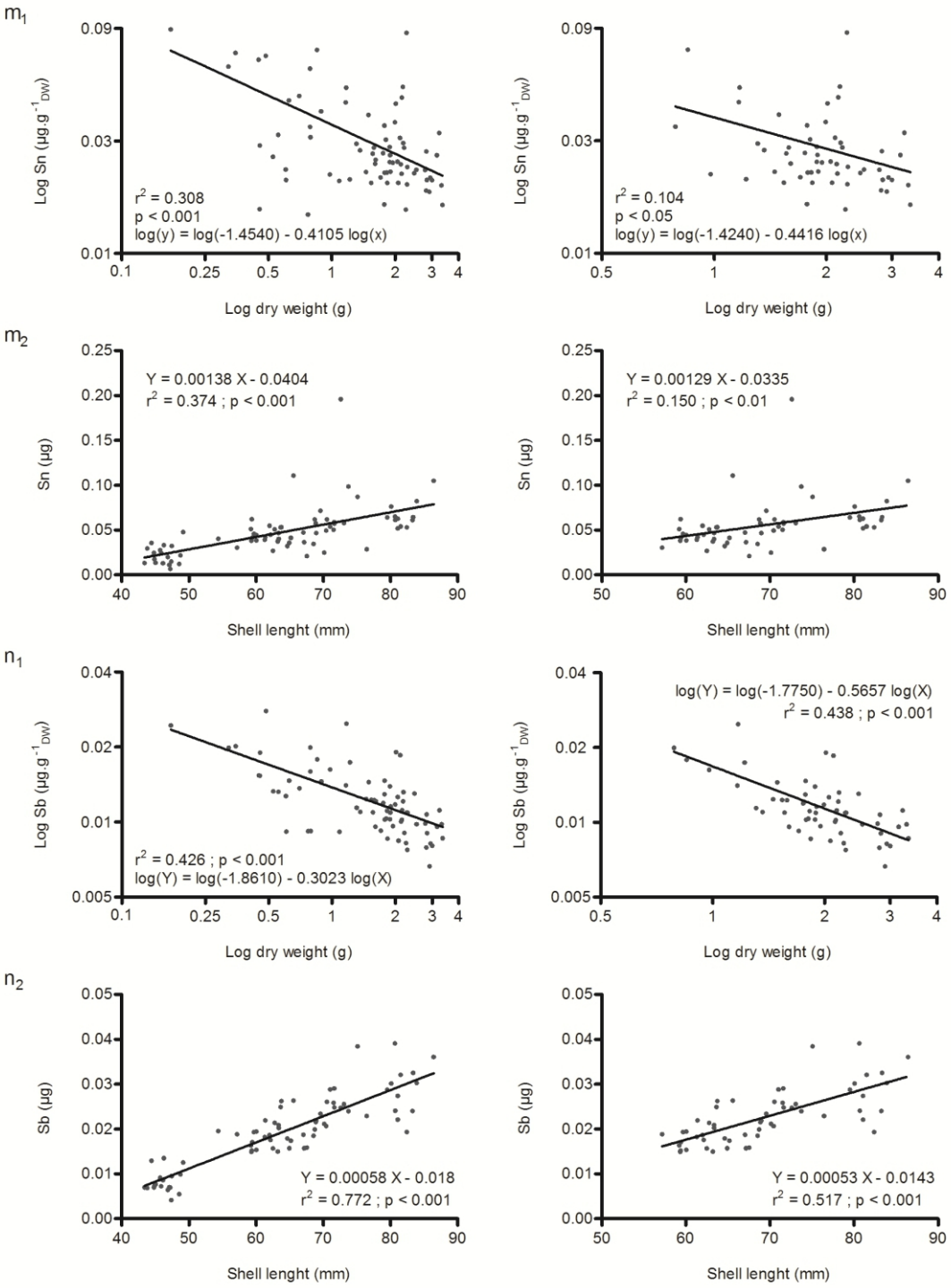
Annex B (*Continued*). Double log transformed power functions modelling relationships between g_1-h_1) the mussel soft tissue dry weight (g) and Ni or Cu concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between g_2-h_2) the mussel shell length (mm) and Ni or Cu total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



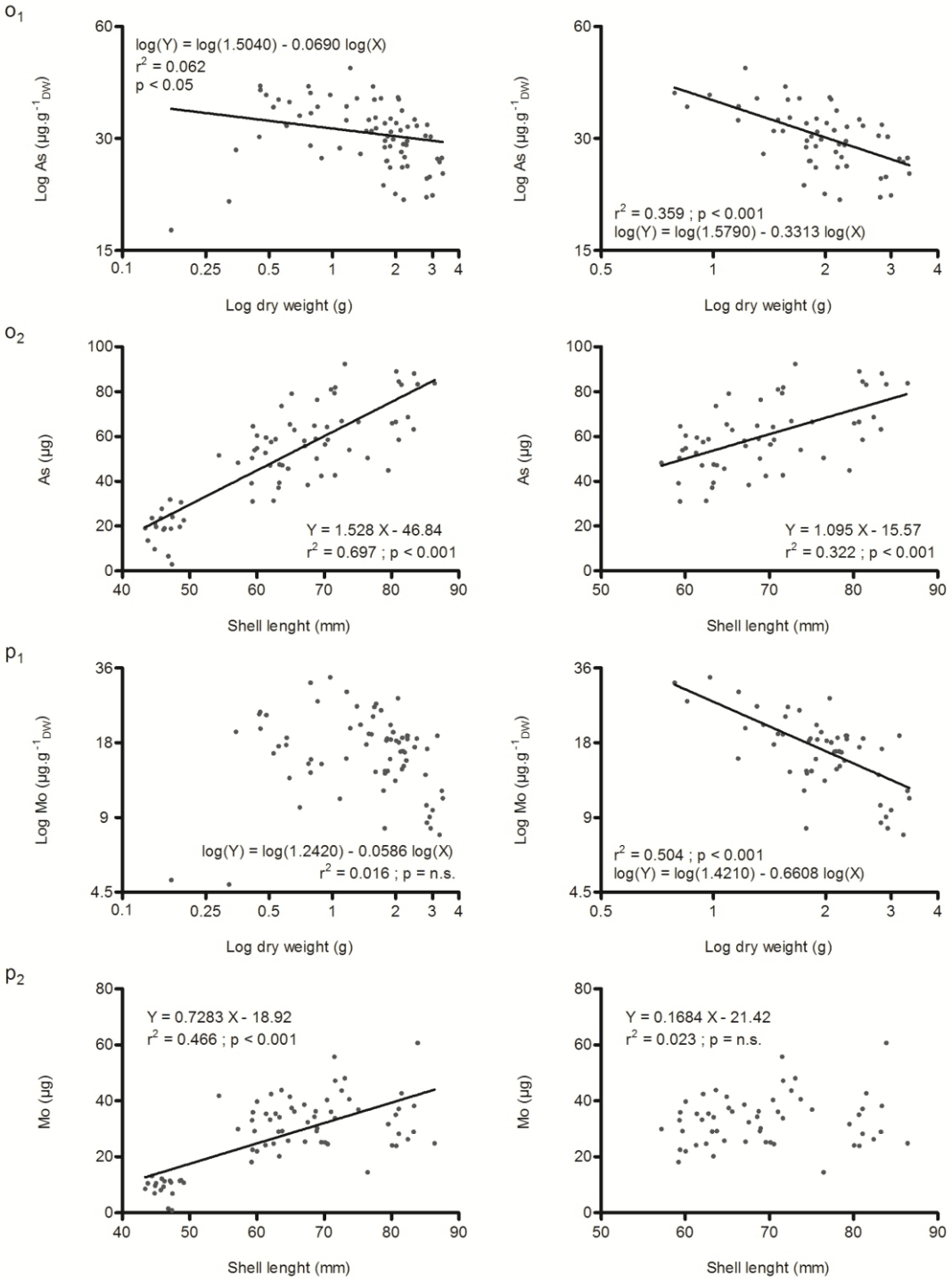
Annex B (*Continued*). Double log transformed power functions modelling relationships between i_1-j_1) the mussel soft tissue dry weight (g) and Zn or Se concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between i_2-j_2) the mussel shell length (mm) and Zn or Se total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



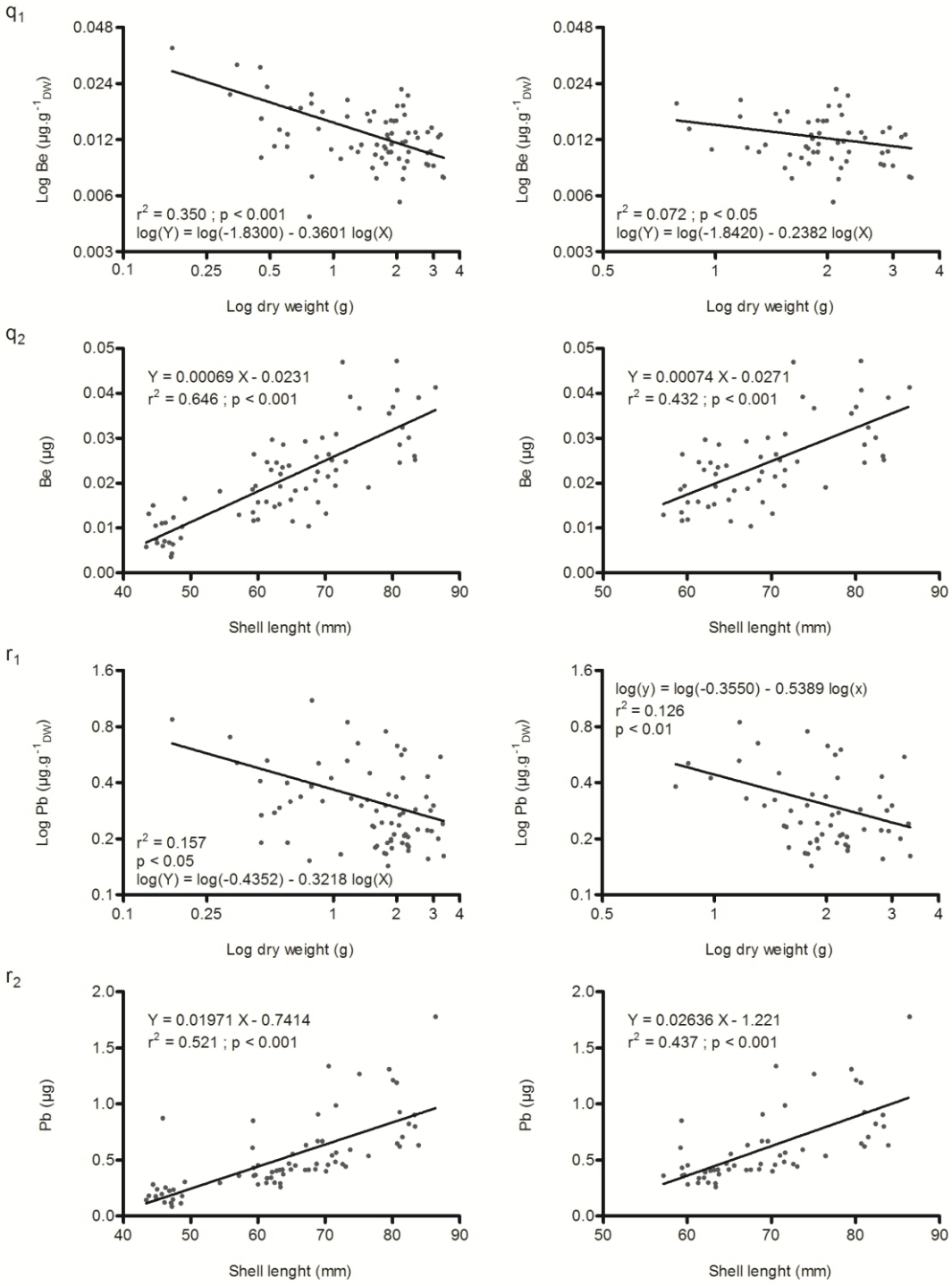
Annex B (*Continued*). Double log transformed power functions modelling relationships between k_1-l_1) the mussel soft tissue dry weight (g) and Ag or Cd concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between k_2-l_2) the mussel shell length (mm) and Ag or Cd total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



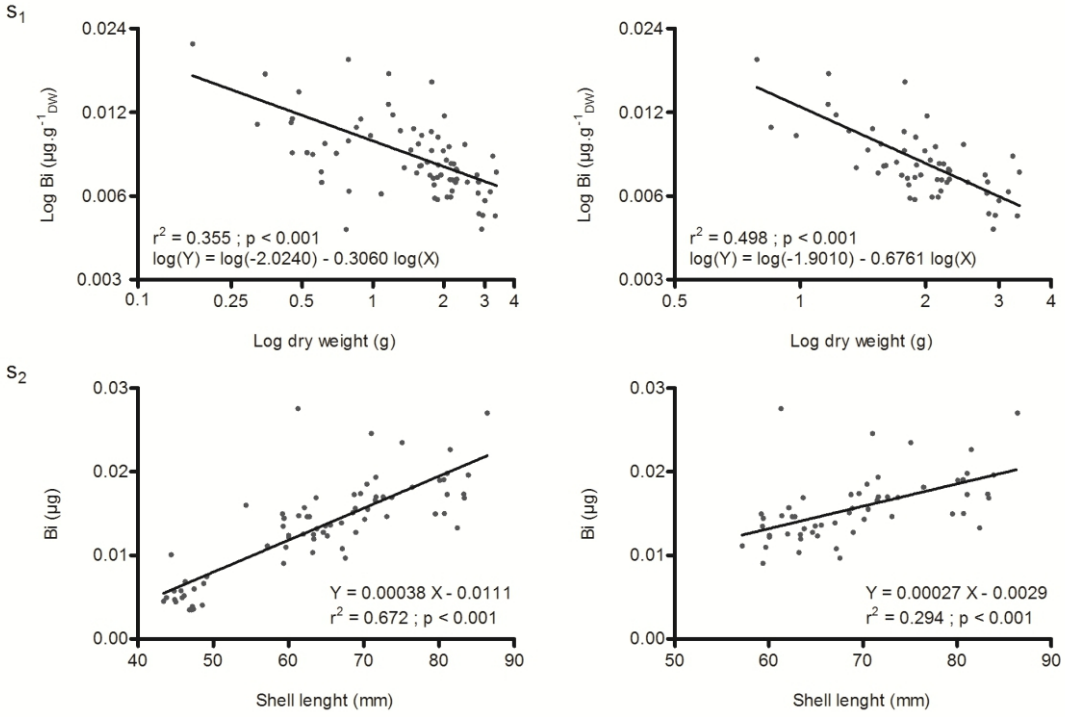
Annex B (*Continued*). Double log transformed power functions modelling relationships between m_1-n_1) the mussel soft tissue dry weight (g) and Sn or Sb concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between m_2-n_2) the mussel shell length (mm) and Sn or Sb total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



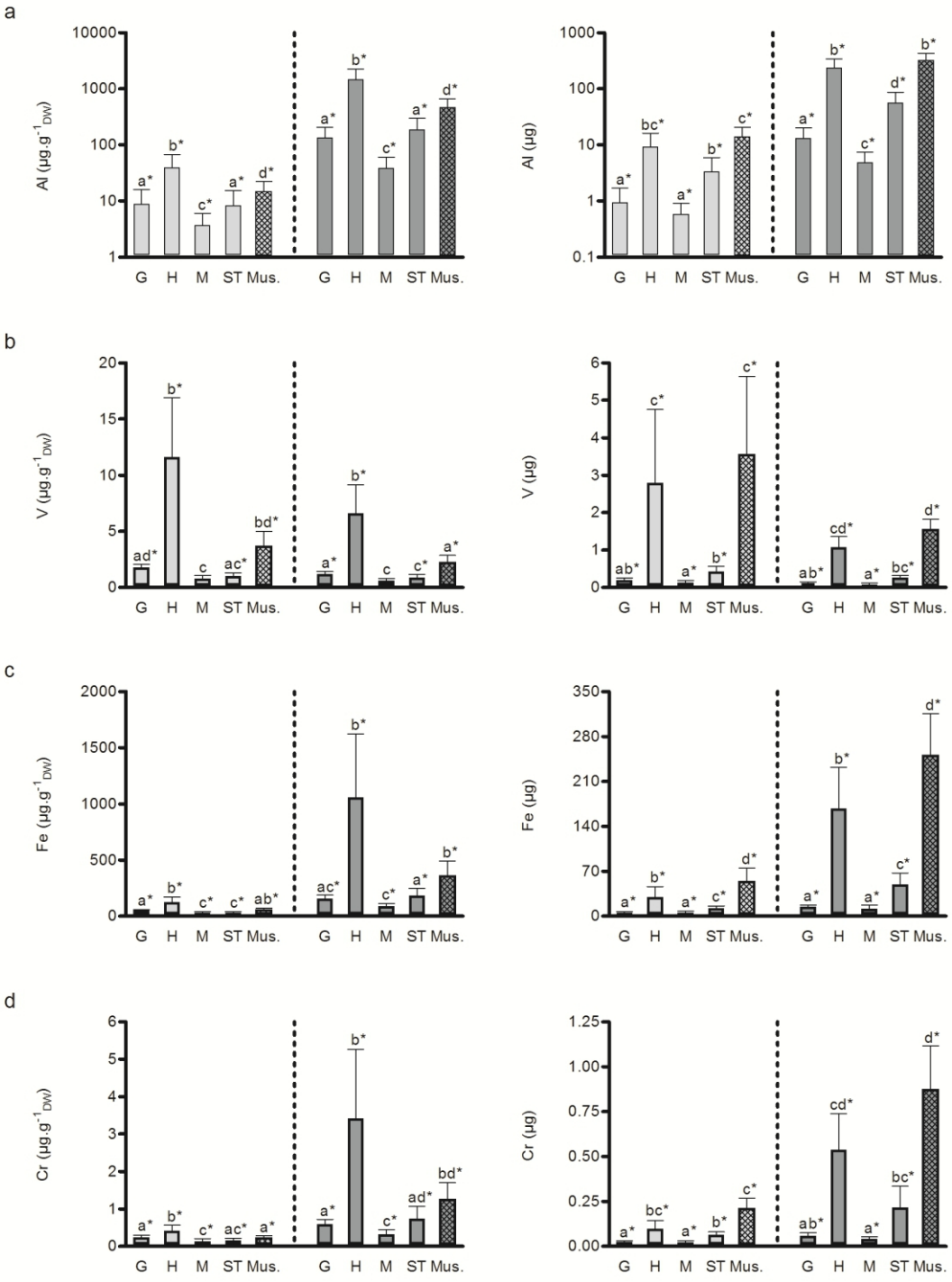
Annex B (*Continued*). Double log transformed power functions modelling relationships between o_1-p_1) the mussel soft tissue dry weight (g) and As or Mo concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between o_2-p_2) the mussel shell length (mm) and As or Mo total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



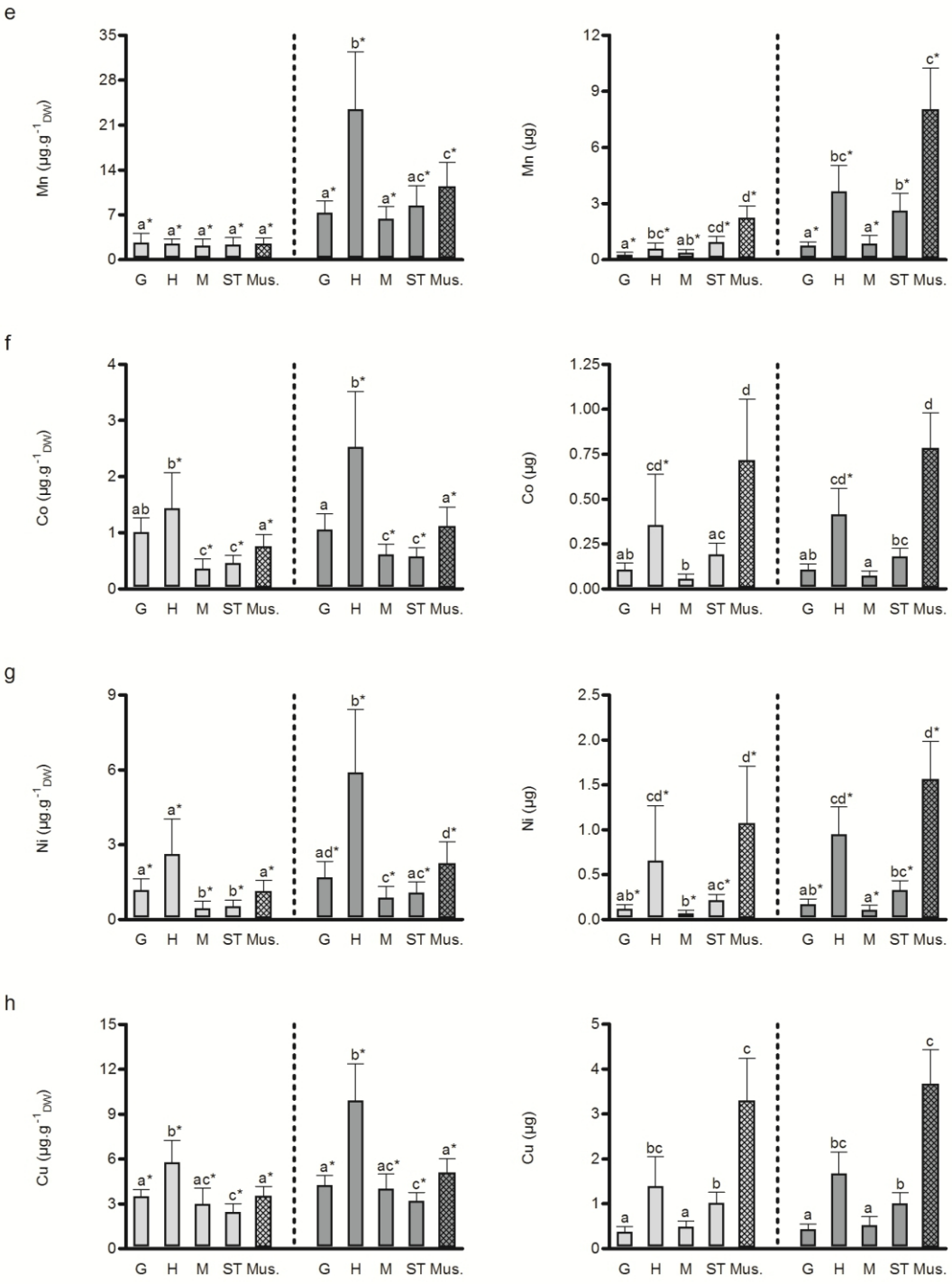
Annex B (*Continued*). Double log transformed power functions modelling relationships between q_1-r_1) the mussel soft tissue dry weight (g) and Be or Pb concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between q_2-r_2) the mussel shell length (mm) and Be or Pb total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



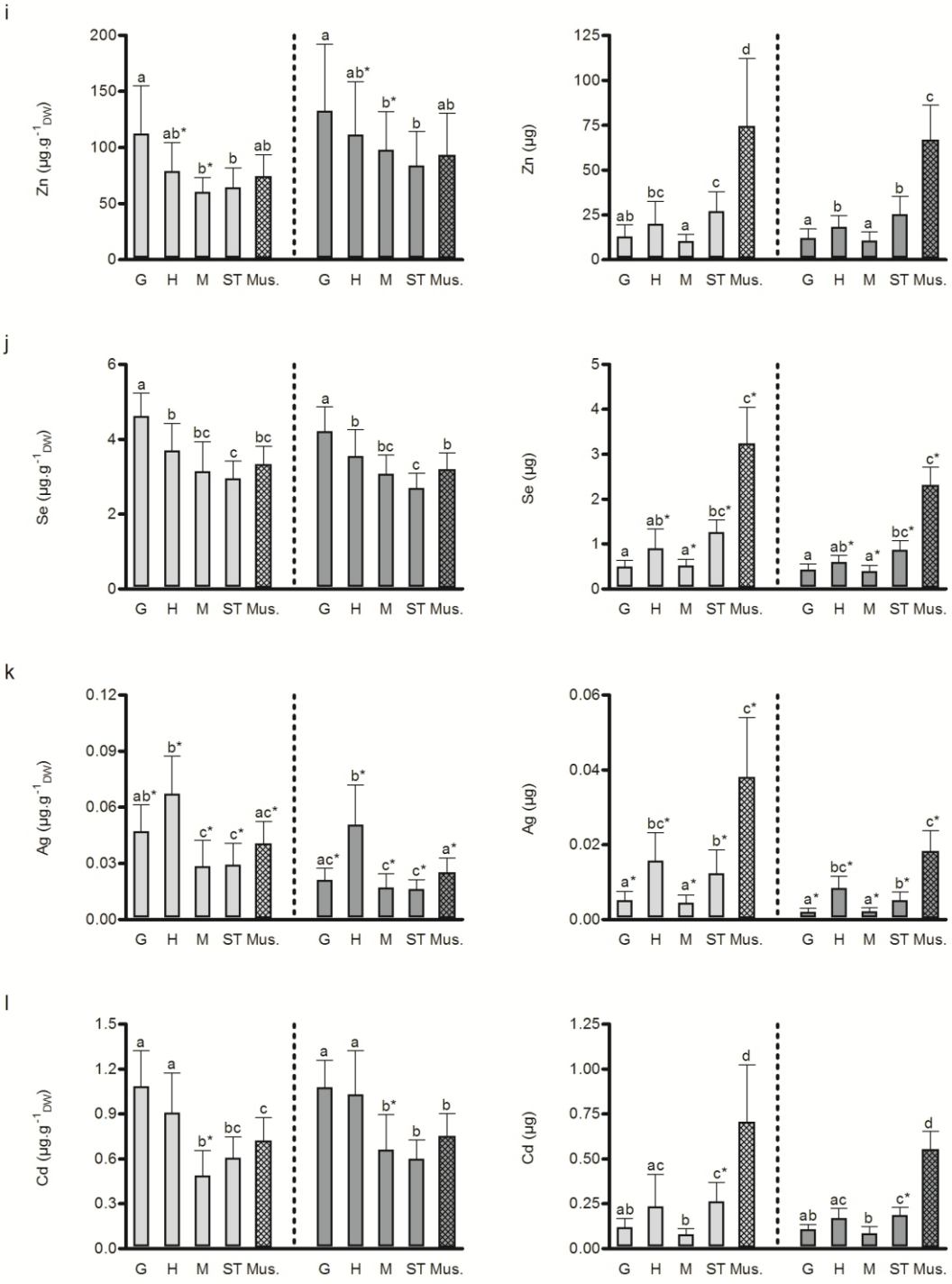
Annex B (*Continued*). Double log transformed power functions modelling relationships between s_1) the mussel soft tissue dry weight (g) and Bi concentrations ($\mu g.g^{-1}_{DW}$) and linear regressions modelling relationships between s_2) the mussel shell length (mm) and Bi total contents (µg), all individuals together (4 left graphs) or limited to mussels longer than 55 mm (4 right graphs). Linear equations and their corresponding fitting parameters (r^2 and p-levels) are reported on graphs.



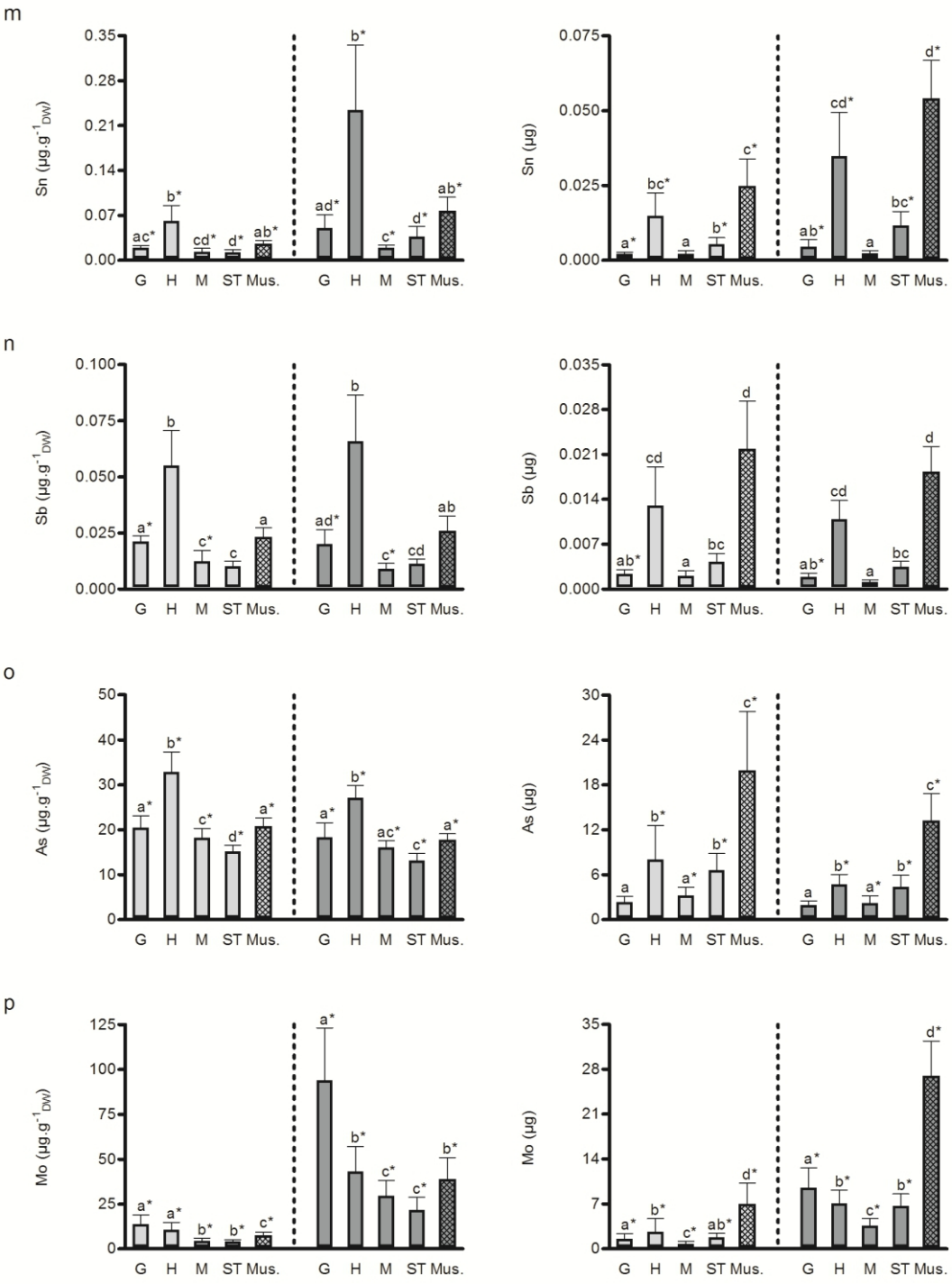
Annex C. a) Al, b) V, c) Fe and d) Cr levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status; (i.e. multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.



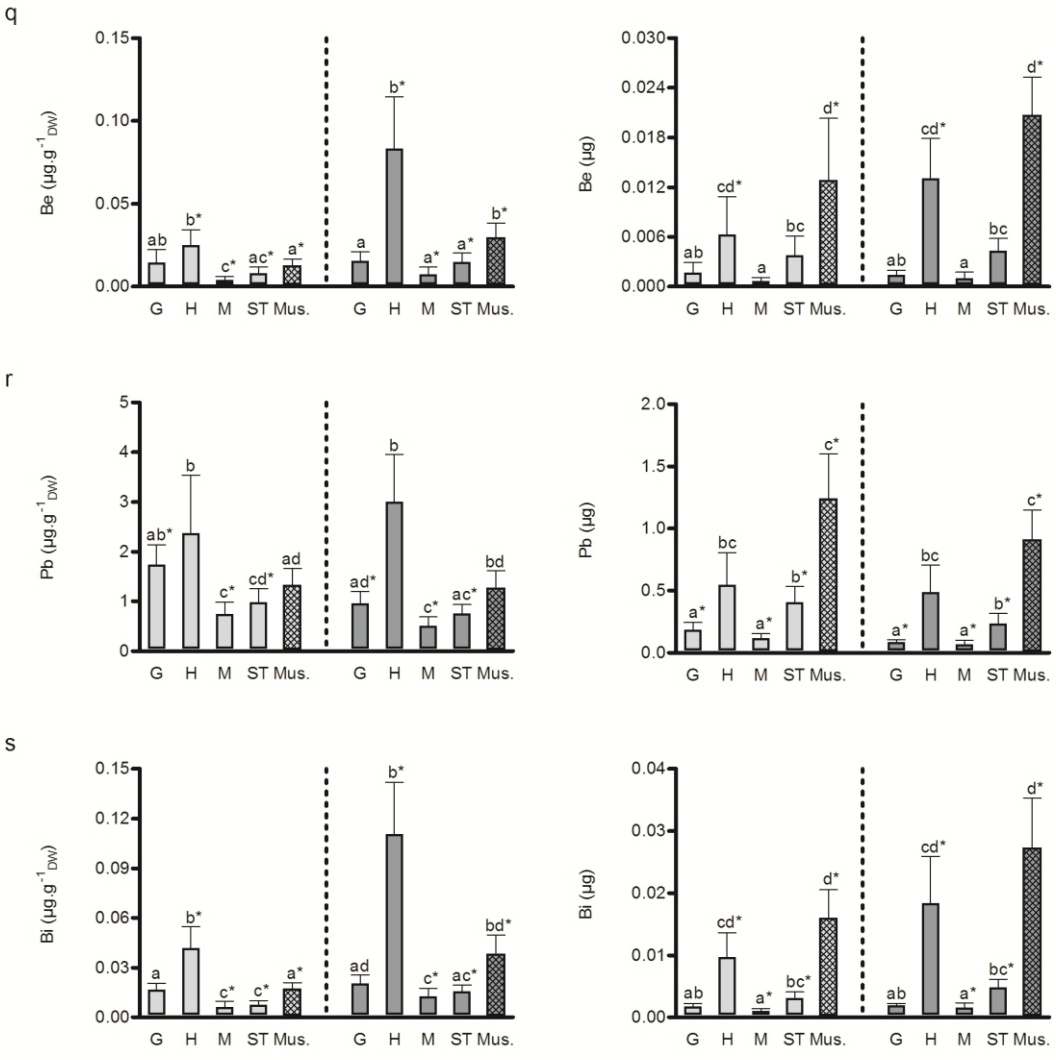
Annex C (*Continued*). e) Mn, f) Co, g) Ni and h) Cu levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status; (i.e. multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.



Annex C (*Continued*). i) Zn, j) Se, k) Ag and l) Cd levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status; (i.e. multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.



Annex C (*Continued*). m) Sn, n) Sb, o) As and p) Mo levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status; (i.e. multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.



Annex C (*Continued*). q) Be, r) Pb and s) Bi levels in gills (G), hepatopancreas (H), mantle (M), remaining soft tissues (ST) and whole individuals (Mus.) of mussels sampled before (light gray) or after (dark grey) spawning. Levels are expressed in concentrations ($\mu g.g^{-1}_{DW}$ - 4 left graphs) or total contents (µg - 4 right graphs). Letters represent significant differences ($p < 0.05$) between the 4 body compartments and whole individuals of a same gametogenic status; (i.e. multiple comparison tests of means); * represent significant differences ($p < 0.05$) for a same body compartment or between mussels before or after spawning (i.e. pairwise comparison tests of means), respectively.

Chapter 5

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General discussion

1. Context summary

The pollution by trace elements is still today a topical subject: (i) 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); (ii) on the other hand because. the global production and use of trace elements, after having suffered a slight slowdown at the end of the 1990s, experienced a new growth (US Geological Survey website, http://www.usgs.gov/), result of the emergence of all a series of nations (e.g. the People's Republic of China, India etc.; Sievers et al. 2010, Tiess 2010).

Water is the primary vector of a series of pollutants, including trace elements, and the public health problems resulting from the exposure to contaminated waters are very many (Table 2, chapter 1). In addition, access to quality water is one of the main drivers of development (WHO 2011). If in industrialized countries, connectivity to municipal wastewater treatment plants is in the range of 50 % to 95 %, more than 80 % of the municipal wastewater in low-income countries is *a contrario* discharged without any treatment, polluting rivers, lakes, and finally coastal areas (Schwarzenbach et al. 2010). Furthermore, 60 % of the world's population lives within 100 km of the coasts, and this demographic trend will continue to increase in the future (Tanaka 2006). Chemical pollutions, including the one by trace elements, must therefore be continuously monitored, from their emission sources down to their ultimate repository, *i.e.* the oceans.

We have widely developed in the introductory chapter the advantages related to the use of bioindicator species – *i.e.* key species of ecosystems accumulating pollutants at levels representing the rate of contamination of their environment (Blandin 1986) – in place of direct chemical measurements in environmental compartments (water and sediments; Zhou et al. 2008). The marine magnoliophyte *Posidonia oceanica* and the Mediterranean mussel *Mytilus galloprovincialis* are part of these key species integrated for a long time in monitoring programs in the Mediterranean (e.g. Montefalcone 2009, Andral et al. 2011).

These two bioindicators respond appreciably and quantitatively to the coastal pollution, including the one by trace elements, and complement each other: the two species accumulate pollutants dissolved in the water column; *P. oceanica*, deeply rooted in sediments, also reflects the contamination of this compartment; *M. galloprovincialis*, as filter feeder, accumulate pollutants from their particulate phase. Together, they give an estimate of the

overall pollution (water, sediments, suspended matter) of the Mediterranean coastal environment. We have extended their use as bioindicators to the monitoring of various trace elements of environmental emerging concern (e.g. Bi, Sb, Mo etc.); we have further measured levels of trace elements classically monitored with these species (e.g. Pb, Cd, Zn etc.) as a time-integrated efficient monitoring of pollutants requires the continuous survey of their environmental levels.

The main objectives of this study were:

• to monitor the present status of chemical pollution by trace elements along the French Mediterranean coasts;

• to investigate the potential use of *P. oceanica* and *M. galloprovincialis* to biomonitor trace elements of environmental emerging concern;

• to study the underlying physiological mechanisms determining trace element accumulation in both species, under reference conditions or when exposed to environmental changes of pollutant loads.

Our results presented in chapters 2 to 4 are globally discussed below. In order to look further into certain aspects of this general discussion, results of complementary studies (master thesis[†] co-directed with Dr. Sylvie Gobert, in collaboration with STARESO (STARE-CAPMED contracts), IFREMER and the French Water Agency) related to the present work will be presented each time that is sensible.

[†] Master thesis related to the present work, listed by academic year:
2008-09: Nicolas Luy - Trace element concentrations in *Posidonia oceanica* (L.) Delile along the French Mediterranean littoral: relationship with anthropisation (Oceanography, ULg).
2009-10: Pierre Serpe - Measurement of the concentration of 18 trace element in *P. oceanica* and *P. lividus* from the Revellata Bay: seasonal and pluriannual variations (Oceanography, ULg).
2010-11: Pierre-Karl Louis - Non-destructive technique for the definition of the biological quality of water masses in the Mediterranean: elaboration, validation and measurements on the physiology of *P. oceanica* (Agronomy, Campus of Isia); Alexandre Deraikem - The monitoring of pollution by 19 trace elements in the Calvi Bay (Corsica): use of caged transplanted Mediterranean mussels (Biology of Organisms and Ecology, ULg); Marjorie Fassin - Trace element content dynamics in *Mytilus galloprovincialis* from the Diane pond (Corsica) (Biology of Organisms and Ecology, ULg); Muriel Roland - Health status of the *Posidonia oceanica* (L.) Delile meadow in the Ajaccio Bay (Corsica): human activity effects on trace element contents (Oceanography, ULg). Lucie Lefèbvre - Spatiotemporal variations of trace elements in *Posidonia oceanica* (L.) Delile meadows (Oceanography, ULg).

2. Contamination of the French Mediterranean littoral

The French Mediterranean coasts are submitted to diverse contaminations by trace elements which can be local, diffuse and/or chronic. We detected point and non-points sources of pollution, and we highlighted the present and past human activities related to these pollutions: they were of agricultural origins (Mo), they resulted from old mining (Cr, Sb, Zn) or industrial (As) activities, they arised from the transportation, the storage, and the refinement of petroleum products (V, Pb), or they were related to the existence of important ports and urban centres (Sn, Bi, Ag).

With the exception of Be and Se, we showed that *P. oceanica* was a good accumulator of the trace elements. Its usefulness as a bioindicator in the monitoring of the coastal pollution by trace elements, already recognized for a large number of elements classically studied with that species (*i.e.* Cr, Fe, Ni, Cu, Zn, Cd and Pb), can therefore be extended to elements of emerging concern (Al, Mn, Co, Mo, Sn, Sb and Bi). We also showed that *P. oceanica* could be used to survey trace elements broadly monitored with *M. galloprovincialis* (Ag, As, V) but little with that species.

TE broadly monitored with *P. oceanica*

TE	$\sum(x_{max}/x_i)/17$	x_{max}/x_{min}	index	Site x_{max}
Cr	3.7 ± 1.2	6.0	1.6	St Florent
Fe	2.1 ± 0.9	4.4	2.1	Bravone
Ni	1.7 ± 0.3	2.4	1.4	St Raphaël
Cu	2.0 ± 0.6	3.4	1.7	Villefranche
Zn	14.0 ± 3.3	19.6	1.4	Bravone
Cd	1.9 ± 0.6	3.9	2.0	St Raphaël
Pb	2.8 ± 1.2	4.4	1.6	Ajaccio N.

TE little monitored with *P. oceanica*

TE	$\sum(x_{max}/x_i)/17$	x_{max}/x_{min}	index	Site x_{max}
Al	2.3 ± 1.8	7.5	3.3	Ajaccio N.
V	6.2 ± 5.0	14.5	2.4	Antibes
Mn	1.6 ± 0.3	2.2	1.4	St Raphaël
Co	1.9 ± 0.4	2.9	1.5	St Raphaël
As	6.2 ± 2.5	10.6	1.7	P. Chèvres
Mo	14.3 ± 5.6	22.8	1.6	Aregno
Ag	2.0 ± 0.5	3.1	1.6	La Vesse
Sn	3.6 ± 1.8	6.9	1.9	Corbière
Sb	3.7 ± 0.4	4.4	1.2	Bravone
Bi	6.4 ± 3.4	13.6	2.1	P. Chèvres

Table 1. The 2 distinct parts of Table 1 synthetize, all sites together, give for each trace element either broadly (left) or little (right) monitored with *P. oceanica*: • the weighted sum of the ratios of the maximum concentration (x_{max}) over each of the 17 remaining concentrations (x_i): $\sum(x_{max}/x_i)/17$; • the ratio between the maximum (x_{max}) and the minimum (x_{min}) concentrations recorded among the 18 sites: x_{max}/x_{min} ; • the index equals to the ratio of the 2 previous measures, that gives a good estimate of the overall spatial variability of concentrations measured in *P. oceanica* collected at the 18 sites along the French Mediterranean coasts: the more that index is close to one, the less trace element concentrations vary spatially. The site with the highest mean concentration is also reported for each trace element.

We studied the spatial variability of trace element levels recorded in *P. oceanica* sampled at the 18 sites along the French Mediterranean coasts (Fig.1, chapter 2). The spatial range of trace element levels can be expressed as the ratio between their maximum and minimum concentrations (Table 1). Then, this ratio calculated for trace elements little monitored with *P. oceanica* went from 2.2 for Mn to 14.5 for V, and up to 22.8 for Mo. This variability was more important that the one recorded for trace elements classically monitored with that species (from 2.4 for Ni to 19.4 for Zn).

We have also calculated a weighted index that expresses the overall variability of trace element levels between the 18 sampled sites (Table 1): the more that index is close to one, the less trace element concentrations vary spatially, as illustrated in Fig. 1.

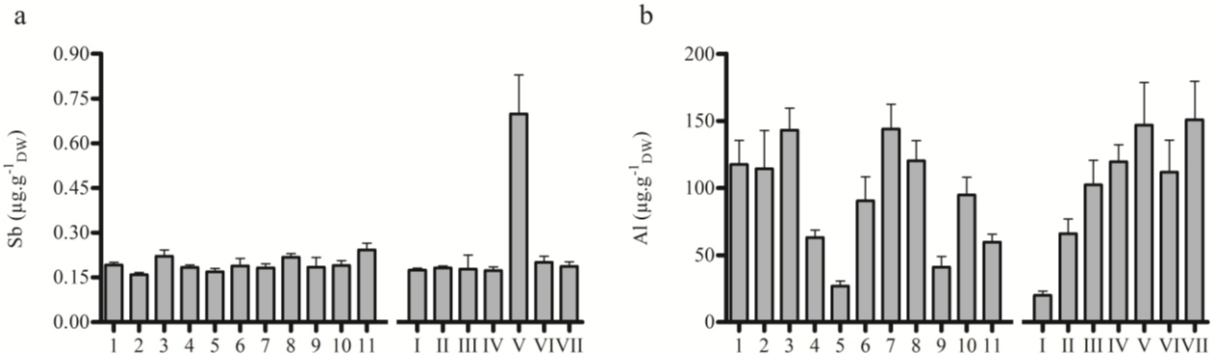

Fig. 1. Spatial variability of a) Sb and b) Al mean concentrations ($\mu g.g^{-1}_{DW}$ ± SD) in *P. oceanica* shoots sampled at the 18 sites along the French Mediterranean coasts: Ensuès (1), La Vesse (2), Corbière (3), Plateau des Chèvres (4), Riou (5), Bénat (6), Giens (7), Cap Roux (8), St Raphaël (9), Antibes (10), Villefranche (11), Calvi (I), Aregno (II), St Florent (III), Taglio Isolaccio (IV), Bravone (V), Ajaccio Sud (VI) and Ajaccio Nord (VII). X-axis labels indicate the sites along the coasts (1–11: PACA; I–VII: Corsica).

The 2 examples given for Sb and Al properly show that this index, close to 1 for Sb, underlines the little spatial variability of concentrations of this element between the 18 sites: with the exception of the site of Bravone, all the remaining concentrations are very similar (Fig. 1a). *A contrario*, the spatial heterogeneity of Al concentrations is reflected by its high index value (3.3, the highest value among the studied trace elements; Fig. 1b). This useful index therefore gives complementary and summarized information to the graphical representation of trace element concentrations.

Multielement analysers such that the ICP-MS used during the present study allow, from a same sample, precise measurements of numerous trace elements at the same time

(Ammann 2007). Because we have showed that the spatial variability of concentrations of trace elements of emerging concern was as important as that of elements classically investigated with *P. oceanica*, and because we could associate the higher environmental concentrations to anthropogenic sources of pollution, we strongly suggest to broaden the list of trace elements monitored along the Mediterranean coasts to all the chemicals studied in this work.

3. The Calvi Bay as a reference site

In order to qualitatively compare the results of different *ad hoc* biomonitoring studies (in time, but also often in space), there is a need to define reference conditions. We have shown that trace element concentrations measured in the healthy *P. oceanica* bed (shoots, water, sediments; chapters 2 and 4) in front of the the Oceanographic Station STARESO, Calvi Bay (Fig. 1 in chapter 4), were low to very low. We therefore suggest considering this site as a reference site in the study of the pollution of the Northwestern Mediterranean by trace elements. This seagrass meadow presents furthermore an overall good ecological status, with a low anthropization index of its water body (Gobert et al. 2009), and also fulfilled the criteria of "a good reference monitoring site", as defined in the SeagrassNet Monitoring Manual (representative of the location, homogeneous, accessible, and removed from any large obvious impact; Short et al. 2002).

Salivas-Decaux et al (2010) proposed a "quality scales" based on quintile (from very low to very high contamination levels) to evaluate the level of trace element (As, Ag, Cd, Cu, Hg, Ni, Pb) contamination in the whole Mediterranean, biomonitored with the two external (adult) leaves of *P. oceanica*. These authors showed that there existed a gradient of contamination for some metals between the Eastern Basin and the Western Basin: (i) the Tyrrhenian Sea and the Algero-Provencal basin were more contaminated with Ni than the Ionian Sea and were more contaminated with Ag than the Levant Sea and the Aegean Sea; (ii) sites situated in the North of the Western Mediterranean Basin presented higher concentrations in Ag, As, Cd, Hg, Ni and Pb than sites located in the South of this basin. However, we consider that such a quality scale common to the whole Mediterranean doesn't take into account regional specificities (natural or human-induced). Thus, if we compare our results with their quality scales, the *P. oceanica* meadow of the Calvi Bay then appears to be

moderately to highly contaminated with Ag, As, Cd, Cu and Ni, which goes against our conclusions.

Recently, Benedicto et al. (2011) studied the variation of trace metal contamination (Hg, Cd, Pb and Ni) by means of caged mussels in the Western Mediterranean, considered either in its entirety or cut into its 4 natural sub-basins (the Alborán, Northwestern, Southwestern and Tyrrhenian sub-basins). Their complementary approach at two spatial scales provided an overview of the problematic of the pollution of the Western Mediterranean, while allowing defining realistic and attainable reference conditions by sub-basins. We consider that such an approach by sub-basins has to be privileged. As we showed that the Calvi Bay was little contaminated compared to many sites monitored in the Northwestern Mediterranean, we again suggest electing this site as a regional reference site for the Northwestern basin of the Mediterranean.

4. *P. oceanica* and *M. galloprovincialis* trace element bioaccumulation behaviours

From the average trace element concentrations measured in *P. oceanica* from the Calvi Bay (chapters 2 and 3) and in *M. galloprovincialis* from the salty pond of Diane (chapter 4), we built for both species graphs ordering trace elements by decreasing order of concentrations (Fig. 2).

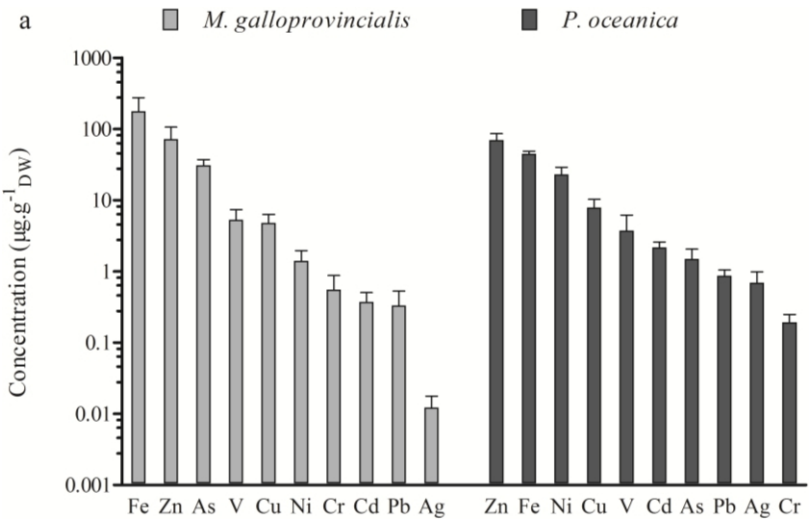

Fig. 2. Range of concentrations (mean ± SD, in $\mu g.g^{-1}_{DW}$; logarithmic scale) of trace element a) classically monitored in *M. galloprovincialis* collected from the salty pond of Diane in February 2011 and in *P. oceanica* shoots collected seasonally between years 2008 and 2010 in the Calvi Bay.

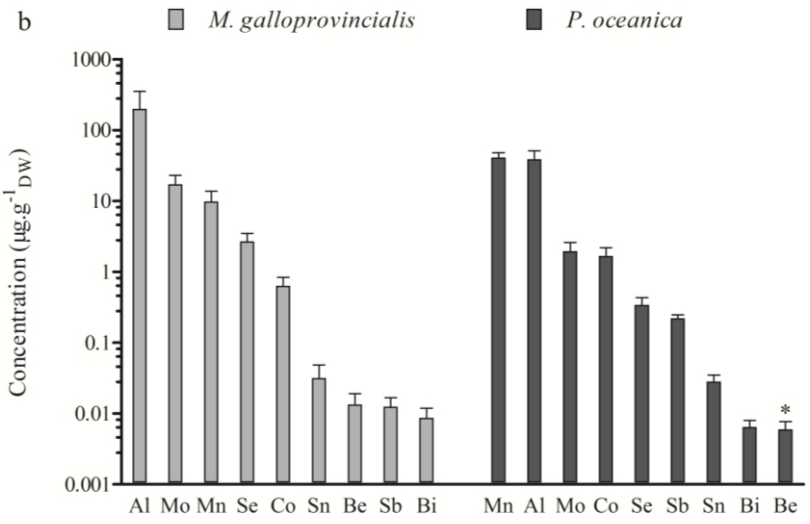

Fig. 2 (*Continued*). Range of concentrations (mean ± SD, in $\mu g.g^{-1}_{DW}$; logarithmic scale) of trace element a) of emerging concern monitored in *M. galloprovincialis* collected from the salty pond of Diane in February 2011 and in *P. oceanica* shoots collected seasonally between years 2008 and 2010 in the Calvi Bay. Number of replicates: n = 74 for *M. galloprovincialis* (all trace elements); n = 166 (all trace element, except 4), 135 (Se, Be, Sb) or 15 (Sn) for *P. oceanica*. * represents concentrations < L_D.

We showed that these 2 sites were little contaminated by trace elements; Fig. 2 thenceforth presents the natural aptitude of the two bioindicators to accumulate trace elements classically monitored (Fig. 2a) or of emerging concern (Fig. 2b) in relatively clean environmental conditions. As this aptitude can differ between species, the order of trace elements on graphs can differ.

Trace elements classically monitored with the two bioindicators species globally show similar graphic profiles. Essential trace elements such as Fe, Cu, Ni and Zn are accumulated in a more important way, while non-essential toxic trace elements such as Cd, Pb, Ag, As and V concentrations remain lower. Nevertheless, there exist some differences between the 2 species. The somewhat higher V levels measured in *M. galloprovincialis* remain limited to our sampling, as V data for the Diane pond continuously monitored by the Ifremer (http://www.ifrmer.fr/envlit/) are in a range of lower values, closer to the ones recorded for *P. oceanica* in STARESO. With regard to As is naturally more accumulated by *M. galloprovincialis* than by *P. oceanica* (Table 1 in chapter 2, Table 2 in chapter 4). Finally, the preferential accumulation of Cr by mussels can be attributed to the essential aspect of this trace element to animals, compared to plants (Kapustka et al. 2004).

We have moreover calculated the bioaccumulation factors of trace elements from seawater (Table 1 in chapter 3; Sohrin and Bruland 2011) to the 2 bioindicator species (Table 2).

TEs broadly monitored			TEs of emerging concern		
TE	*M. galloprovincialis*	*P. oceanica*	TE	*M. galloprovincialis*	*P. oceanica*
Cr	2.9E+03	1.0E+03	Al	2.5E+05	4.9E+04
Fe	1.6E+05	4.1E+04	Mn	2.3E+04	9.7E+04
Ni	3.8E+03	6.2E+04	Co	8.2E+03	2.2E+04
Cu	1.5E+04	2.5E+04	Se	1.9E+04	2.5E+03
Zn	5.7E+04	5.5E+04	Mo	1.5E+03	1.8E+02
Cd	7.8E+03	4.5E+04	Sn	2.7E+04	2.5E+04
Pb	2.6E+03	6.6E+03	Sb	6.6E+01	1.2E+03
Ag	1.2E+02	6.9E+03	Bi	7.2E+04	5.4E+04
As	2.0E+04	9.6E+02	Be	6.7E+04	3.0E+04
V	3.0E+03	2.1E+03			
mean	2.7E+04	2.5E+04	mean	5.2E+04	3.1E+04
SD	± 4.9E+04	± 2.4E+04	SD	± 7.9E+04	± 3.2E+04
min.	1.2E+02	9.6E+02	min.	6.6E+01	1.8E+02
max.	1.6E+05	6.2E+04	max.	2.5E+05	9.7E+04

Table 2. Bioaccumulation factors of trace elements broadly monitored (left) or of environmental emerging concern (right) to *M. galloprovincialis* and *P. oceanica*. Mean ± SD, minimum and maximum bioaccumulation factors are given for each category of elements.

Bioaccumulation factors ranged from 1.2 10^2 (Ag) to 1.6 10^5 (Fe) for *M. galloprovincialis* and 9.6 10^2 (As) to 6.2 10^4 (Ni) for *P. oceanica*, for a mean factor of 10^4 for the two species. Only Cu, Ag (*P. oceanica*) and As (*M. galloprovincialis*) differed by more than one order of magnitude between the two species. Our study once more demonstrated that the 2 bioindicator species, since they efficiently bioaccumulate trace elements broadly surveyed, could be used to monitor their environmental levels.

The range of concentrations of trace elements of emerging concern accumulated in *P. oceanica* and *M. galloprovincialis* is similar to that of elements classically monitored with these species (Fig. 2b). Our results show that environmentally abundant and/or essential trace elements such Al, Mo and Mn are accumulated in a more important way, while non-essential and potentially toxic Sn, Sb, Bi and Be concentrations remain low to very low. By comparing our results with available data from the literature, we concluded that levels of these trace elements of emerging concern could be considered as low to very low both in the Diane pond (chapter 3) and the Calvi Bay (chapters 2 and 4). They can therefore be regarded as reference baselines values for subsequent monitoring surveys in the North-Western Mediterranean.

But we also showed that there further existed some differences between the accumulation behaviours in the 2 species with respect to some elements. Thus, Be and to a certain extent Se were accumulated at quantifiable levels by *M. galloprovincialis* only;

mussels should therefore be preferred to seagrasses for their monitoring. As it was the case for Cr, the higher level of Se accumulation by *M. galloprovincialis* could be attributed to the essential aspect of this trace element to animals, compared to plants (Kapustka et al. 2004).

Bioaccumulation factors from seawater (Table 1 in chapter 3; Sohrin and Bruland 2011) ranged from 6.6 10^1 (Sb) to 2.5 10^5 (Al) for *M. galloprovincialis* and from 1.8 10^2 (Mo) to 9.7 10^4 (Mn) for *P. oceanica*, for a mean factor of 10^4 for the two species. Only Sb (*P. oceanica*) bioaccumulated concentrations differ by more than one order of magnitude between the two species. Our study demonstrates that the 2 bioindicator species, since they efficiently bioaccumulate trace elements of emerging concern, can be used to monitor their environmental levels. Moreover, we showed that many of these elements actually threatened the French Mediterranean littoral, and had therefore to be monitored.

We compared so far *P. oceanica* and *M. galloprovincialis* sampled from 2 distinct reference sites. However, we can wonder how these species will respond when they are both sampled from the same stations, and how their specific lifestyle will modulate this response. To answer this question, we immersed caged mussels in four different locations in the Calvi Bay (Fig. 3): in front of the STARESO; besides an aquaculture farm; in the vicinity of the pipe discharging the treated domestic wastewaters of the Calvi city; at the Punta Bianca, just outside of the Calvi Bay influence; sites were separated by a distance of 1 to 3 km.

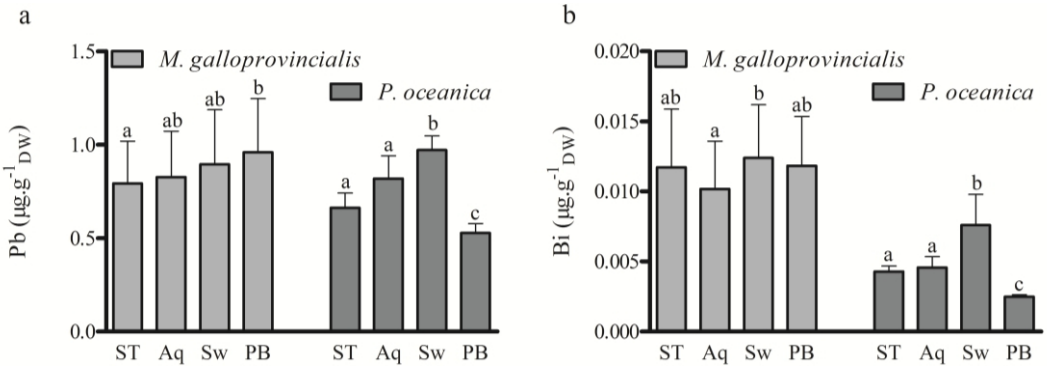

Fig. 3. Spatial resolution of *M. galloprovincilias* (n = 48-49) and *P. oceanica* (n = 15) used to biomonitor the a) Pb and Bi) contamination in the Calvi Bay. Stations STARESO (ST), aquaculture farm (Aq), Calvi city sewer (Sw) and Punta Bianca (PB) are separated by a distance of 1 to 3 km. Concentrations are given in $\mu g.g^{-1}_{DW}$ ± SD. Letters represent significant differences between stations.

Cages stayed immerged for 3 months, from March to June 2010, and when they were retrieved from water, *P. oceanica* shoots were concomitantly collected. We showed that the homogenous response given by caged *M. galloprovincialis* reflected the clean homogenous

status of the water column of the Calvi Bay. *P. oceanica*, as a rooted organism, was influenced by the accumulation of pollutants in sediments, and made it possible to highlight weak point sources of long-term contaminations at the scale of the Bay. Thus, only *P. oceanica* highlighted the local impact of the Calvi city on Pb (Fig. 3a) and Bi (Fig. 3b) environmental levels at the station sewer just as the general weak containment effect played by the Bay (concentrations at station Punta Bianca – outside of the Bay – are lower).

We further studied the fine monitoring sensitivity of *P. oceanica* at even smaller spatial scale. We therefore analysed *P. oceanica* shoots collected from the Ajaccio Bay, West coast of Corsica (Fig. 1 in chapter 2). Shoots were sampled in 9 stations aligned along the same radial following the coastline, each spaced by approximately 300 m (Fig. 4a). The radial was located at the back of the Ajaccio Bay, between sites Ajaccio Sud (site VI, Fig. 1 in chapter 2) and Ajaccio Nord (site VII, Fig. 1 in chapter 2).

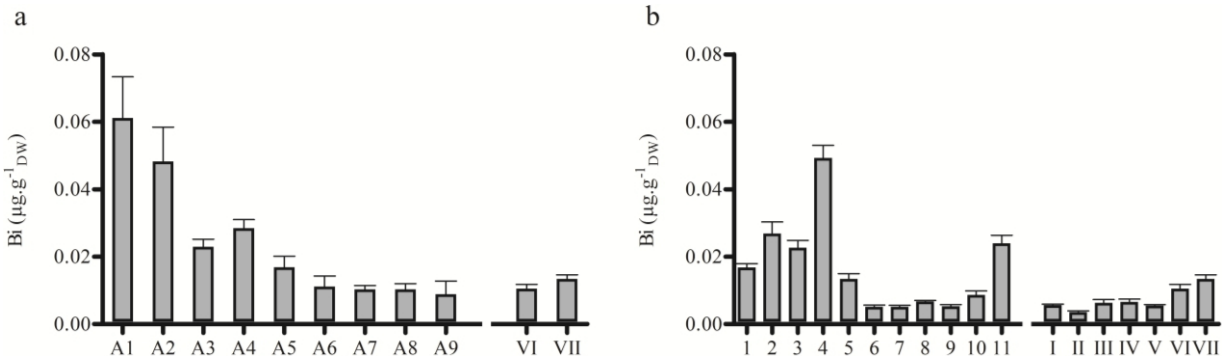

Fig. 4. Spatial variations of Bi concentrations ($\mu g.g^{-1}_{DW}$ ± SD) in *P. oceanica* a) sampled in 9 stations (A1-A9), each one separated by 300 m along a radial in the back of the Ajaccio Bay and in two supplementary sites – Ajaccio Sud (VI) and Nord (VII) – distant of approximately 4 km from the radial. The variability of Bi concentrations along the radial is as important as b) the large spatial scale variability of Bi levels measured in *P. oceanica* (among them sites VI and VII) sampled in 18 sites along the French Mediterranean coasts (1–11: PACA; I–VII: Corsica)

Our results showed that the more the stations were remote from the back of the Ajaccio Bay – *i.e.* the more stations were remote from the urban centre of Ajaccio – the more the concentrations of trace elements decreased. For example, Bi levels exponentially decrease along the radial to finally achieve the values reported for Ajaccio Sud (VI) and Nord (VII) sites (Fig. 4a).

Based on the spatial variability of Bi concentrations recorded at the scale of the of the Mediterranean French coasts (Fig. 4b), we categorized the 2 sites Ajaccio Sud (VI) and Nord

(VII) of moderately impacted by Bi pollution compared to the Plateau des Chèvres, a site located in the vicinity of the pipe discharging domestic wastewaters of the Marseille city. But present results show that stations located very close to the urban centre of Ajaccio recorded pollution by Bi as high as the one recorded near Marseille.

In conclusion, our results showed how difficult it can be to designate representative sampling stations, all the more if the selected bioindicators are associated to sediments. Since *M. galloprovincialis* can be easily used to characterize the overall state of a water body without having to multiply the number of monitored stations within each site (*i.e.* one mussel cage within a bay is sufficient), we recommend to elect this bioindicator for large scale monitoring studies (e.g. Andral et al. 2011, Benedicto et al. 2011).

However, *P. oceanica* offers the added benefit of allowing the fine-spatial scale mapping of coastal pollution. Sediments offer a degree of time integration, contrary to the water column (Rainbow 1995, Amiard 2011). We showed that weak human impacts, not discernible with caged mussels, could be highlighted by using an indicator associated to sediments, as *P. oceanica* (Fig. 3). However, when using *P. oceanica* as unique bioindicator in large-scale programs (e.g. chapter 2), we recommend to attentively select the stations to be sampled, *i.e.* not too close to punctual sources of contaminants, in order not to overestimate the contamination status of the whole area under study (Fig. 4).

5. Trace element kinetics

Contrary to *M. galloprovincialis*, available data on the uptake and loss kinetics of trace elements by *P. oceanica* are scarce, except in Ledent (1992) for Cd and Warnau et al. (1996) for Zn, Ag, Cd, Cs, and Am. Contrary to caged mussels, *P. oceanica* shoots cannot be transplanted from clean stations to contaminated ones and *vice versa* (e.g. Lepoint et al. 2004); However, we have demonstrated that the *in situ* experimental contamination of seagrass bed portions exposed to cocktail of chemicals at realistic environmental levels gave relevant results.

We showed that *P. oceanica* shoots contaminated *in situ* accumulated quickly trace elements (Fig. 3 and Annex B in chapter 3); this accumulation depended of the nature of the trace element, of its concentration, of the interactions between the different pollutants (multielement solutions of 15 trace elements) and of the length of the exposure. Once the

uncontaminated conditions were restored, trace element concentrations in shoots quickly evolved toward a return to their initial levels.

We measured a significant increase of several pollutants in below-ground rhizomes during the entire experiment, decontamination phase included (Fig. 4 and Annex B in chapter 3), whereas superficial sediments had remained uncontaminated. We therefore conclude that the acropetal translocation of trace elements from shoots to rhizomes, recently evaluated by Sanz-Lazarro (2012) in the cycling of trace elements within *P. oceanica* meadows, had to be fast and efficient even at low levels of contaminants. If shoots depurate quickly after an exposure to contaminants, this exposure can therefore be recorded in below-ground tissues. We therefore suggest to study these tissues to assess the past pollution by trace elements of emerging concern, as it has often been done for elements classically monitored (e.g. Pergent-Martini and Pergent 1994, Copat et al. 2012). However, the destructive uprooting of *P. oceanica* raises again the question of its status of protected species (Boudouresque et al. 2006, Montefalcone et al. 2007) and should be limited as much as possible.

Complementary results showed that the accumulation of trace elements of emerging concern by *P. oceanica* shoots, just as the accumulation of elements classically monitored, was influenced by the seasonal biological cycle of the plant, i.e. the growth, the aging and the leaf fall.

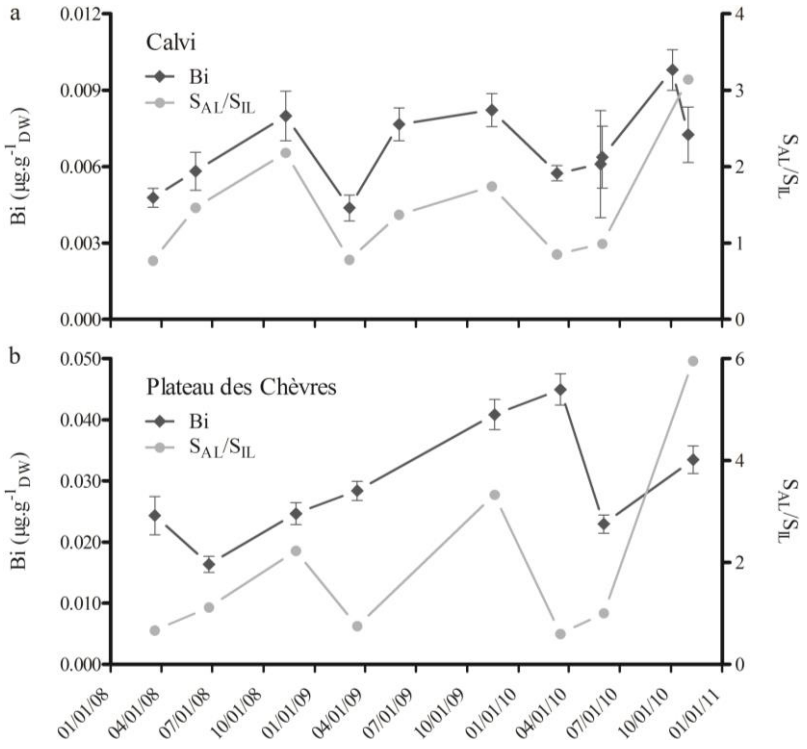

Fig. 5. Bi ($\mu g.g^{-1}_{DW}$ ± SD kinetics and seasonal cycle of shoot aging in *P. oceanica* collected seasonally from March 2008 till November 2011 in a reference site, a) the Calvi Bay and in a contaminated site, b) the Plateau des Chèvres. The indicator of the seasonal aging of shoots is given as the ratio between the Surface of Adult Leaves (S_{AL}) and the Surface of Intermediate Leaves (S_{IL}).

We showed that Bi concentrations followed a graphic profile similar to the one of the ratio between the surface of *P. oceanica* adult leaves and the surface of intermediate leaves (*i.e.* an indicator of the aging of shoots): the more shoots aged, *i.e.* the longer shoots were exposed to Bi, the more its concentration increased (Fig. 5a). The late winter, early spring drop in concentrations corresponds to the renewal of leaves. However, in an environment submitted to anthropogenic disturbances, the natural seasonal trend can be completely perturbed.

We showed that the Plateau des Chèvres, located in the vicinity of the pipe discharging domestic wastewaters of the Marseille city, was the site more contaminated out of Bi of the 18 sites studied in chapter 2 (Table 1 and Fig. 3b in chapter 2). *A contrario,* Bi concentrations in *P. oceanica* shoots from the Calvi Bay were among the lowest. In the Plateau des Chèvres (Fig 5b), Bi levels increased continuously during 2 years rather than following a seasonal trend characterized by maximum concentrations in autumn and minimum concentrations in late winter, early spring as in the Calvi Bay. From this example, we conclude that the exposure of *P. oceanica* to ambient trace element levels is a major factor determining concentrations measured in the plant; in addition, trace element kinetics are prone to smaller temporal variations linked to the evolution of their environmental bioavailability and to the physiology of the organism.

Available data on trace element kinetics in *M. galloprovincialis* are many. Trace element (Hg, Cd, Pb, Cu et Zn) uptake and loss kinetics have moreover recently been deeply investigated in natural conditions with caged mussels placed in reference and contaminated sites, during a period of time covering the different physiological status of that species (Casas 2005, Casas and Bacher 2006, Casas et al. 2008).

When caged mussels are transfer from a clean to a contaminated environment, they are exposed to a significant increase of trace element concentrations in their surrounding waters and rapidly take up trace elements into their soft tissues; when caged mussels are transplanted from the contaminated site back to a clean one, trace element flow goes then from the organism into the water. These kinetics obtained in field conditions by Casas et al. (2005, 2006, 2008) are consistent with those of previous laboratory studies, and match in a broader way with the observations obtained in a transplantation experiment we carried out in 2011 in the Calvi Bay and the Diane pond (Fig. 6).

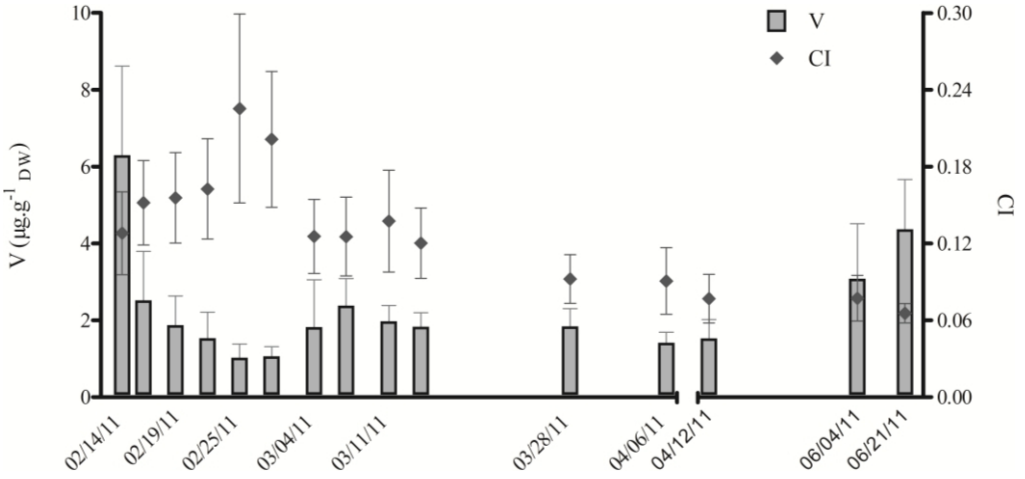

Fig. 6. Variations of V concentrations (µg.g^{-1}_{DW} ± SD) and Condition Index (CI ± SD; ratio of flesh dry weight over shell dry weight) in *M. galloprovincialis* (purchased from the Diane pond) during a transplantation experiment in STARESO from February to June 2011. Caged mussels rapidly equilibrated within 2 weeks with the new environmental conditions; V concentrations increased in early March, corresponding to the main spawning event indicated by the abrupt decrease of the CI; a second increase in June corresponded to a V contamination of the water column, likely related to the resumption of the touristic period.

Our results showed that during the adaptation of caged *M. galloprovincialis* to their new environmental conditions, their biological cycle acted on trace element concentration as shown for V in Fig. 6. Thus, the important loss of body weight (up to 40 %; Cossa 1989) of spawning mussels caused an increase in V concentration in soft tissues.

But our results further confirmed that the closer *M. galloprovincialis* were to spawning, the more the differences in traces element concentrations between individuals of opposite sexes were important (Table 3 in chapter 4). In order to get rid of this important physiological variable, we suggest using this bioindicator during his phase of sexual dormancy, as previously recommended for trace elements classically monitored.

Furthermore, we showed that the seasonal (Fig. 6) and interannual (Fig. 4 and Annex C in chapter 4) variations of the bioavailability of trace elements (pollution level, quality and quantity of food etc.) could modulate in an important way their accumulation rates, even in reference sites. The exposure of *M. galloprovincialis* to ambient trace element levels is therefore a major factor determining its bioaccumulation behaviour.

We also pointed out that there was no consensus on the question of the influence of the size of farm-grown mussels in the monitoring of traces elements. Our results showed that a

large range of sizes of mussels grown on ropes could be used for monitoring purposes, and that only small size individuals derogated to this rule. Since small size mussels only represent a small percentage of the total of individuals fixed on a rope, this practical consideration can consequently be minimized.

We can conclude that the uptake and loss kinetics of trace elements are under the influence of various parameters that interact such as the environmental conditions, the nature of the pollutants and its characteristics, the physiology of the species etc. Ideally, all these interacting parameters should be controlled, which is mostly the case for trace elements classically monitored with *M. galloprovincialis*. Moreover, for this species, consensual methods allow to intercompare results between sites and studies: correction for the trophic heterogeneity of the immersion sites, caging experiment during the period of sexual dormancy etc.

A contrario, for *P. oceanica*, no general rule prevails, and each one collects shoots at any time of the year, at any place, without considering the seasonality of the bioaccumulation behaviour of this indicator. However, Pergent- Martini et al. (2000) had pointed out already years ago the importance of this seasonality and our results confirmed their statement. Malea et al. (in prep.), who recently monitored the seagrass *Cymodocea nodosa* during one year, concluded the same. We consequently strongly recommend developing consensual monitoring protocols in order to improve the use of *P. oceanica* as bioindicators of pollution. We are however conscious of the difficulty of such a task, as recently recalled during the 3rd Mediterranean Seagrass Workshop held in 2012 in Morocco.

6. Trace elements compartmentalization

We have largely discussed different aspect of the biomotoring of trace elements based on the use of entire *P. oceanica* and *M. galloprovincialis*. However, many studies select specific tissues instead of working on entire individuals (e.g. Adami et al. 2002, Romero et al. 2007a, Salivas-Decaux et al. 2010), mostly to facilitate sample treatment and subsequent analyses, or to find the tissue that accumulate the most the studied pollutants. *P. oceanica* and *M. galloprovincialis* main compartments were therefore also considered independently in order to give some insights about the distribution and tissue kinetics of trace elements within both organisms.

In *P. oceanica* sampled from the reference site of STARESO, we showed that trace elements 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; Table 4 in chapter 3).

Furthermore, we showed that trace elements were compartmentalized differently between leaves according to their age, and that element concentrations varied even along a same leaf, as shown for adult leaves dissected in their limb and their base (Table 1 in chapter 2). In addition, we highlighted that this compartmentalization could vary according to the state of contamination of the studied sites (Fig. 2 and Annexes B and C in chapter 2), and could moreover varied between reference sites themselves (chapter 3).

Posidonia oceanica adult leaves usually accumulated, under given environmental conditions, more chemicals than intermediate leaves owing to the fact that they remained exposed longer to the pollutants. However, we showed that when trace elements were experimentally injected in mesocosm, the physiologically more active intermediate leaves uptoke most chemicals more rapidly than adult leaves. We also showed that both leaf types decontaminated rapidly when initial conditions were restored (Fig. 3 and Annex B in chapter 3). Accumulated trace elements also underwent redistribution processes between *P. oceanica* compartments, particularly in the case of essential micronutrients such as Fe, Zn or Cu (Table 1 and Fig. 2 in chapter 1, Fig. 4 in chapter 4).

We showed that the 3rd intermediate leaves were globally representative of entire shoots sampled in April in 18 sites along the French Mediterranean coasts (Table 1, Fig. 2 and Annex C in chapter 2), whatever their levels of pollution. This leaf is however not systematically present on shoots all year round, as in summer when spring young intermediate leaves have aged to give adult leaves.

Romero et al. (2007a, 2007b) measured *P. oceanica* physiological metrics (of which trace elements) of their POMI index on the 3rd youngest leaf (juvenile leaves excluded). Their index was defined to assess the ecological status of coastal waters. In a preliminary study, these authors had selected the 2nd youngest leaf (Martínez-Crego 2005, Martínez-Crego et al. 2008). However, this 2nd leaf was thereafter regarded as not having had sufficient time to accumulate enough trace elements (Fe, Zn, Ni, Mn, Cr, Pb, As, Cu; Martínez-Crego pers. com.). We showed that it was also the case for Sn in our study: Sn concentration remained below detection limit, except for some more polluted sites (Annexe B in chapter 2).

For analytical or anatomical reasons, we can thenceforth conclude that no compartment of *P. oceanica* is fully satisfactory for the monitoring of trace element pollution. Moreover, additional results showed that each compartment of *P. oceanica* shoots underwent a seasonal cycle of its trace element content that could differ from that of the entire shoot.

As regards *P. oceanica*, the definition of water quality index based on different metrics of the plant must evolve toward non-destructive methods (Montefalcone 2009). The non-destructive index recently proposed by Gobert et al. (2012) requires the measurement of the epiphytic load of the plant, of its foliar surface etc., of entire shoots cut close to their base to ensure their regrowth (hence the designation of non-destructive method). We have demonstrated that when special care was given to the sampling, the storage and the processing of samples, it was then possible to retrieve these shoot leaves, cleaned of their epiphytes, for the analyse of the 19 trace elements investigated in the present study. Such an approach allows optimizing the preparatory work carried out as both a quality index of the water body and a complete description of the chemical contamination of the studied site can be performed on the same sample. It also reduces the amount of collected material.

On the basis of all these observations, we conclude that the analysis of trace elements in entire shoots of leaves (cut with scissor and not uprooted) is the most appropriate approach for the monitoring of the pollution of the Mediterranean by trace elements.

About *M. galloprovincialis*, we showed a well-marked compartmentalization of their trace element contents, most of them accumulating preferentially in the hepatopancreas. This specific organ could therefore be specifically analysed in monitoring surveys. We also showed that the distribution of trace elements was rather similar between individuals sampled before and after the release of their gametes, at one year of interval (Fig. 4 and Annex C in chapter 4). This conservative character of the compartmentalization of trace elements implies their internal regulation and their quantitative redistribution between tissues (Gabbott 1975, Lobel and Wright 1982) after the significant loss in weight (up to 40 %; Cossa 1989) associated with the release of gametes.

We measured that trace elements concentrations were usually the lowest in the mantle where the gonad follicles are dispersed. The complementary caging experiment we conducted in the Calvi Bay showed that when mussels spawned, most trace elements underwent a short-time increase in their concentration to recover thereafter a pseudoequilibrum level with the environment (Fig. 5). Trace elements are consequently less concentrated in spawn materials

than in the rest of the organism. Only some essential trace elements (Zn, Mn, Cu and Se) and As departed from this common rule. Because of these important regulatory processes within the mantle, we suggest not using this tissue in biomonitoring surveys, and we recall the importance of working with individuals in sexual dormancy.

Mussels are important shellfish products consumed worldwide which thenceforth raises certain health issues of food security (Fig. 16 in chapter 1). 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, mussels analysed can come from sites designated for the production of shellfish products (e.g. the Diane pond). 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. Then, in the case of mussels sampled from the Diane pond, we showed that Cd and Pb levels were well below the phytosanitary standards defined by the European Union (EC 2001). Because of this phytosanitary aspect, we suggest using preferentially entire mussels in biomonitoring studies. In addition, existing trace element kinetic models (e.g. Casas 2005, Casas and Bacher 2006, Casas et al. 2008) apply to entire *M. galloprovincialis*, not to individual organs. This supplementary consideration also suggest to biomonitor trace elements with entire mussels.

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Chapter 6

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Conclusion

Results of the present study brought answers to the main questions asked in the beginning of this research, namely:

- What is the current status of chemical pollution by trace elements along the French Mediterranean coasts?
- Are *Posidonia oceanica* and *Mytilus galloprovincialis* suitable bioindicator species to monitor coastal pollution by trace elements of emerging concern?
- How does their physiology modulate their bioaccumulation behaviours and how do they respond to changes of pollutant load in their habitat?

We firstly concluded that trace elements of environmental emerging concern were of concern. The spatial variability of their environmental levels recorded along the French Mediterranean coasts could be attributed to specific anthropogenic sources. Levels of pollution by trace elements of emerging concern were further similar, or even higher, to those of elements classically monitored with *P. oceanica*. We consequently suggest enlarging the list of trace elements conventionally monitored in the Mediterranean to the 19 chemicals investigated in this study.

In addition, we validated the clean chemical status of the *P. oceanica* bed of the Calvi Bay, as suggested in past studies. This site can therefore be considered as a reference site in the Northwestern Mediterranean for the monitoring of the pollution by trace elements of emerging concern just as for the monitoring of element classically surveyed.

We showed that *P. oceanica* and *M. galloprovincialis* efficiently accumulated trace elements from their environment. The decreasing classification of their body concentrations was similar: both species preferentially accumulated essential or naturally abundant elements, while maintaining low body concentrations of non-essential and potentially toxic elements. The mean bioaccumulation factor from seawater was about 10^4 for both species. They further gave complementary information about the health status of the coastal environment. *M. galloprovincialis* appeared to be a good indicator of the overall quality of a water body. *P. oceanica* further provided a fine-spatial scale mapping of coastal pollution as this deeply rooted species reflected the long-term integration of weak pollution sources in sediments. We conclude that both species are relevant bioindicators species and that they should be used concomitantly in the monitoring of the pollution by trace elements of emerging concern just as in the monitoring of the pollution by elements classically monitored.

We also showed that these two bioindicators rapidly equilibrated with the trace element load of their ambient environment, as shown with *P. oceanica* experimentally *in situ* contaminated and with *M. galloprovincialis* transplanted from the Diane pond to the Calvi Bay. Both species therefore properly reflected the contamination state of their surrounding water body within days to weeks, depending on the kinetic of each element. But because of this fast balancing, some punctual pollutions of importance could be missed. We experimentally showed that the acropetal translocation of chemicals from contaminated *P. oceanica* shoots to rhizomes could record these punctual events; rhizomes could therefore be a convenient candidate for the monitoring of past pollutions by trace elements of emerging concern, just like it is the case for elements classically monitored. However, as a protected species, the destructive uprooting of shoots and rhizomes should be limited to specific case studies.

We showed that both species were further influenced by their biological cycle. In *M. galloprovincialis*, the gametogenic cycle played an important role by diluting the levels of pollutants in the organism. Females and males bioaccumulation behaviours also differed during the breeding period, females accumulating more chemicals than males. For *P. oceanica*, the seasonal aging of their deciduous leaves modulate trace element 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*. We therefore strongly recommend developing consensual monitoring protocols in order to improve the use of *P. oceanica* as bioindicator of pollution.

We also showed that each body compartment of both species accumulated more or less pollutants according to its age, its function, its exposure etc. It is therefore interesting to study the compartmentalization of trace elements between organs and tissues to better understand their dynamics within organisms. However, none of these organs completely represented the accumulation behaviour of entire organisms (e.g. 3rd intermediate leaf in *P. oceanica*). Furthermore, detailed kinetic models incorporating environmental variables which further modulate the accumulation processes of trace elements by organisms are designed for entire individuals, as is the case for *M. galloprovincialis*. For these two main reasons we recommend to biomonitor the chemical contamination of coastal environments with entire seagrasses and mussels.