POSIDONIA OCEANICA (L.) DELILE, A USEFUL TOOL FOR THE BIOMONITORING OF CHEMICAL CONTAMINATION ALONG THE MEDITERRANEAN COAST: A MULTIPLE TRACE ELEMENT STUDY

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Abstract

The concentrations of 19 trace elements (TE): Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi as well as Cr, Fe, Ni, Cu, Zn, Cd and Pb, were analyzed by DRC ICP-MS in Posidonia oceanica (L.) Delile leaves from the Mediterranean French coast. The first 12 TE have little been studied nowadays. Except for Al, Cr, Fe, Cu and Ag, TE were preferentially accumulated in photosynthetic part of leaves. Moreover, trace element concentrations of the third intermediate leaf are representative of the integral shoot, and could be used alone in biomonitoring. Environmental background concentrations of the 12 little studied TE were determined, and spatial variations were related to anthropic activities. Compared to previous publications, concentrations of the 7 other TE classically investigated present a diminution or a stabilization, reflecting the change of anthropogenic inflows. In conclusion, P. oceanica is a sensitive bioindicator for chemical contamination, even for the twelve little studied TE.

Keywords: Trace elements; Seagrass; *Posidonia oceanica*; Mediterranean; Pollution.

Introduction

Posidonia oceanica (L) Delile, the endemic seagrass of the Mediterranean, is sensitive to human disturbances, notably pollution by chemicals (Boudouresque, et al., 2006). Many metals, metalloids and non-metals are regarded as serious pollutants due to their toxicity, persistence and tendency to concentrate in organisms (Ikem & Egiebor, 2005). Concentrations of some trace elements (e.g. Pb, Hg, Cd, etc) were largely studied in *P. oceanica* tissues (Luy, *et al.*, submitted). For example, *P. oceanica* leaves give indications of trace element (TE) concentrations in seawater with accuracy (Pergent-Martini, et al., 2005), while sheaths and rhizomes memorize their temporal trends (Ancora, *et al.*, 2004). Romero et al. (2007) also suggested that the third intermediate leaf was representative of the integral shoot. However, human activities modify environmental TE concentrations (Zhou, *et al.*, 2008); quantification of other previously little studied potential pollutants (e.g. Sb, Bi, Mo, etc) is henceforth now relevant.

The objectives of this study were: (i) To measure concentrations of 12 TE little or never studied in *P. oceanica* (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi), to examine their tissue speciation and spatial variation, and to evaluate the potential use of *P. oceanica* as bioindicator. (ii) To investigate tissue speciation, spatial and temporal variations of 7 TE classically studied (Cd, Cu, Cr, Pb, Zn, Fe and Ni). (iii) To evaluate the ability to use *P. oceanica* third intermediate leaf instead of the integral shoot for the biomonitoring of these 19 TE.

Materials & Methods

In April 2007, 15 shoots of *P. oceanica* were collected at 15 ± 1 m depth in 18 sites located along the French Mediterranean coasts (Fig. 1).

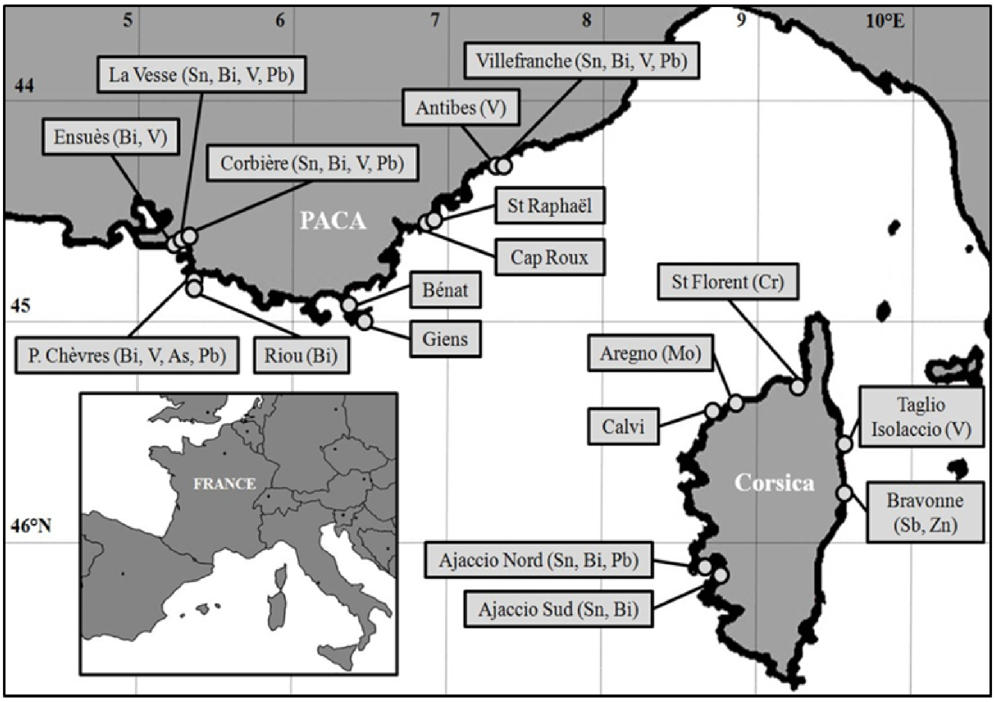

Fig. 1. Localization of the study area and repartition of sampling sites along the Provence-Alpes-Côte d'Azur (PACA) and Corsican coasts; for each site, trace elements presented in high amounts are listed.

Posidonia oceanica shoots were dissected according to the biometric method proposed by Giraud (1979). Epiphytes were scraped from leaves. Each shoot was sorted and noted 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, weighed, pooled, cryogenic ground and mineralized (closed microwave digestion labstation). Trace element concentrations were determined by DRC-ICP-MS. Analytical accuracy was checked by analyzing Certified Reference Materials: BCR 60, BCR 62, GBW 07603 and V463. Detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated for each TE.

Results & Discussion

1. Tissue speciation

Tissue speciation of V, Zn, Cr and Cu are illustrated below in 4 contrasted sites (Fig. 2). Profiles of the other TE were similar to 1 of these 4 characteristic graphs.

Most TE 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. Furthermore, Mn, Co, Ni, As, Mo, Sb, Pb, Bi and particularly V were present in higher concentrations in BAL (*e.g.* V in Fig. 2), contrary to Zn, Se and Cd. These TE presented similar concentrations in all 3 photosynthetic tissues (*e.g.* Zn in Fig. 2). In the particular case of Be and Sn, no clear tissue speciation could be found.

Al, Fe, Ag and Cr concentrations were higher in SAL only in sites which presented the highest concentrations (*e.g.* Fe in Fig. 2). Concerning Cu, concentrations in SAL were systematically higher than in other tissues, but differences were quite small (Fig. 2). This findings were in agreement with many previous studies: Campanella *et al.* (2001) for Cr, Zn, Cd and Pb, but not for Cu; Lafabrie *et al.* (2008) for Cr, Co, Ni, Cd and Pb; Conti *et al.* (2010) for Cr, Cu, Zn, Cd and Pb.

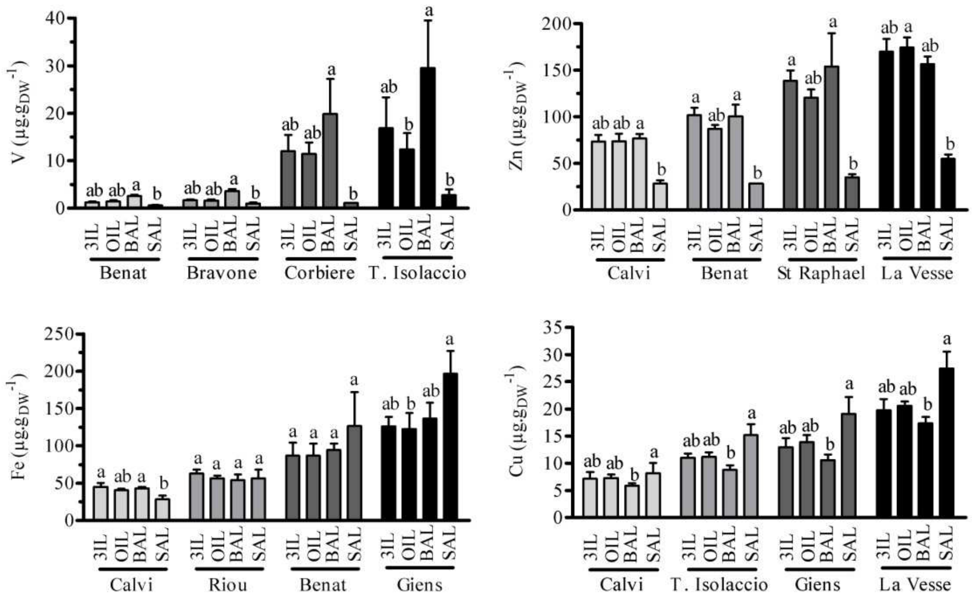

Fig. 2. Tissue speciation of V, Zn, Fe and Cu in 4 of the 18 sites; concentrations are expressed as mean ± standard deviation ($\mu g.g_{DW}^{-1}$); letters represent significant differences between tissues.

A preferential assimilation from the water column to photosynthetic tissues can be assumed for V, Mn, Co, Ni, Zn, Mo, Cd, Sb, Pb and Bi, which show higher concentrations, as suggested by Lafabrie *et al.* (2008) for Co, Ni, Cd, Hg and Pb. At least 2 hypotheses can be used to explain upper values in BAL: (i) A longer exposure to TE loaded in the ambient habitat (Warnau, *et al.*, 1996); (ii) A dilution effect due to the higher growth rate of intermediate leaves. These hypotheses can only play a major role for TE characterized by low kinetics of accumulation and little regulation. The different behaviour of TE preferentially accumulated in SAL suggests different uptake and distribution routes. For Cu, the systematic but quite limited upper level in SAL could be explained by an increase in metabolic activity during growth, when Cu is needed (Conti, *et al.*, 2010). Moreover, this work supports the hypothesis of Romero *et al.* (2007), as the TE concentrations of the 3IL are representative of the integral shoot.

2. Spatial (temporal) variation of TE concentrations

Based on average concentrations found in the shoots from the 18 sites (Tab. 1), TE levels decreased in the order: Zn, Fe, Al > Mn > Ni > Cu > V > Co, Cd, Pb, Mo, As > Ag > Cr, Se, Sb > Sn > Bi, Be. This confirms the sequence commonly observed in *P. oceanica* (e.g. Campanella, *et al.*, 2001, Conti, *et al.*, 2010). Some TE presented an important spatial variation of their concentrations in *P. oceanica* (higher than a factor 10 for Mo, Zn, V, Bi, Sn and As), others a moderate (from a factor 4 to 10 for Al, Cr, Fe, Pb and Sb) and many ones presented just a small (lower than a factor 4 for Cd, Cu, Ag, Be, Co, Ni, Mn and Se) spatial variation (Tab. 1).

Tab. 1. Minimum (Min.), maximum (Max.) and average (Av.) TE concentrations in *P. oceanica* shoots from the 18 sites (µg.g_{DW}^{-1}).

	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu
Min.	0.004	20	1.6	0.16	42	41	1.6	20	6.8
Max.	0.011	151	22.3	0.98	95	180	4.5	48	22.9
Av.	0.008	97	8.2	0.34	62	104	2.6	31	13.3

Zn	As	Se	Mo	Ag	Cd	Sn (BAL)	Sb	Pb	Bi
65	0.89	0.18	1.2	0.52	1.08	0.017	0.16	1.1	0.004
1273	9.44	0.31	27.1	1.63	4.21	0.115	0.70	4.8	0.049
163	2.24	0.24	3.7	0.92	2.46	0.048	0.22	2.3	0.013

Be, Se and Sn presented values close to L_D (0.007, 0.25 and 0.025 µg.g_{DW}^{-1} respectively); Bi levels were closed to L_Q (0.0048 µg.g_{DW}^{-1}). Be and Se concentrations, quite similar per site, reflected the background level of the northwestern (NW) Mediterranean Sea. A significant spatial variation was observed for Sn and Bi, the upper values being found in Marseille Bay (Ensuès, La Vesse and Corbière), Plateau des Chèvres, Riou, Villefranche and Ajaccio. Since they are principally used in advanced industry, it is not surprising to encounter them in industrialized areas and harbours.

V is a tracer of hydrocarbon pollutants (Amiard, *et al.*, 2003). The presence of 4 oil refineries in the department of Bouches-du-Rhône, an old oil-exporting harbour at Antibes and the petroleum depot of Lucciana can explain the high V concentrations in Marseille Bay, Antibes and Taglio Isolaccio. V concentrations varied from 1.6 µg.g_{DW}^{-1} at Riou and Aregno, to 22.3 µg.g_{DW}^{-1} at Antibes. The highest concentrations determined by Amiard *et al.* (2003) in 3 mollusc species (from 0.57 to 1.42 µg.g_{DW}^{-1}) along the south coast of Brittany after the sinking of the tanker *Erika* are in the lower range of the concentrations determined in this study. It confirms the use of *P. oceanica* as a good biological indicator of hydrocarbon pollution.

Mn presented just a small spatial variation (62 ± 15 µg.g_{DW}^{-1}). Furthermore, our SAL concentrations (14 ± 3 µg.g_{DW}^{-1}) were in the lower range of the values determined by Ancora *et al.* (2004) in *P. oceanica* scales in the Gulf of Naples (up to 200 µg.g_{DW}^{-1}). Mo is a micronutrient of significance for citrus nutrition (Srivastava & Shyam, 2007). The high Mo concentrations measured at Aregno (23.1 µg.g_{DW}^{-1}) might be due to this specific agricultural activity. However, the 17 other sites presented concentrations (1.2 – 5.2 µg.g_{DW}^{-1}) lower than those determined by Augier *et al.* (1991) in *P. oceanica* leaves (5.2 ± 0.6 µg.g_{DW}^{-1}) at the uncontaminated site of La Palu (Port Cros Island, France). So, average concentrations of these 2 TE reflect the background level of the NW Mediterranean Sea.

Ag contamination results from urban or industrial diffuse sources. RNO (2006), using wide mussel, determined the Ag contamination along the French Mediterranean coast as homogeneous and limited compared to the Atlantic coast. Moreover, lowest values of this study (0.52 µg.g_{DW}^{-1}) were found in Corsica. They corroborate the low values measured by Lopez y Royo *et al.* (2009) and reflect the Ag background level.

Sb, Zn and As had relatively small spatial variation, with the exception of Bravone (Sb and Zn) and Plateau des Chèvres (As). Average concentrations of Zn (163 µg.g_{DW}^{-1}) and As (2.24 µg.g_{DW}^{-1}) corresponded to unpolluted sites (Fourqurean, *et al.*, 2007). The high Sb concentrations only detected at Bravone could be attributed to the As/Sb deposit at Matra. However, if the high Sb concentrations were associated with high Zn concentrations, it is not the case with As, as previously shown in mussels by Andral *et al.* (2004). The high As concentrations detected at Plateau des Chèvres could be attributed to the industrial history of the south of Marseille (Andral, *et al.*, 2004), which suffers from a chronic contamination of As and Pb (Lassalle, 2007).

Al and Fe presented similar profiles. This is consistent with Barabasz *et al.* (2002) observation: one effect of Al on plants is the stimulation of Fe absorption by roots. Our Fe average concentration (104 $\mu g.g_{DW}^{-1}$) corroborates the low values determined by Fourqurean *et al.* (2007) on the Illes Balears. Fe and Al spatial variations would result from a natural heterogeneity rather than an anthropogenic disturbance.

The high Cr levels determined at Saint Florent (0.98 $\mu g.g_{DW}^{-1}$) corroborates similar high values measured at west of Cape Corse (1.07 $\mu g.g_{DW}^{-1}$) by Lafabrie *et al.* (2008). These values have been linked to waste from the disused Canari asbestos mine. Contrary to Lafabrie *et al.* (2008), the high Cr levels at Saint Florent were not associated with high levels of Co or Ni. Furthermore, Co, Ni and Cd spatial distributions were similar, and their measured concentration ranges were low and should reflect a diffuse contamination. Moreover, when compared to Lafabrie *et al.* (2008), a slight decrease in Cd level in Corsica was likely a result of the application of European rules (CE, 2009).

Our Cu average concentration (13,3 $\mu g.g_{DW}^{-1}$) is similar to the NW Mediterranean background level determined by Campanella *et al.* (2001). All sites sampled can thus be considered as having low levels of Cu contamination. Since Pb interdiction of use as an anti-knock additive, contamination levels are in decline, as determined by Ancora (2004) between 1989 and 1999 in the Gulf of Naples. However, its significant environmental persistence in the sediment explains the relatively higher end values determined in the highest industrialized sites (Marseille Bay, Plateau des Chèvres, Villefranche and Ajaccio).

Conclusion

Tissue speciation allows us to construct hypotheses concerning the uptake and distribution routes of these 19 TE. Such hypotheses have to be confirmed by the analysis of different parts of the same leaf and both above and below-ground tissues, and by experimental contaminations. Furthermore, future analyses of most TE in *P. oceanica* could be realized only on the single 3IL. The NW Mediterranean natural levels of most of the 12 little studied TE measured in *P. oceanica* could be determined, and their spatial variation and their contamination sources (punctual, diffuse and/or chronic) could be explained. *P. oceanica* might be used *de facto* as a sentinel species for their monitoring. The temporal variations of the other 7 widely studied TE present a diminution, or at least a stabilization of their concentrations, probably due to their reduced anthropogenic inflows. These observations suggest that *P. oceanica* is a sensitive bioindicator for the monitoring of the 19 TE studied.

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