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ARSENIC CONCENTRATIONS IN SEAGRASS AROUND THE MEDITERRANEAN COAST AND SEASONAL VARIATIONS

Abstract
Arsenic's occurrence in the environment could be due to human activities as well as to natural sources. In this study, Posidonia oceanica and Cymodocea nodosa are collected in 84 sites around the Mediterranean basin. In addition, both seagrass are collected monthly, in two sites (Calvi in Corsica and Salammbô in Tunisia). Arsenic concentrations in C. nodosa present seasonal variations in relation with spring phytoplankton blooms. For both species arsenic concentration is higher in the vicinity of geological sources (mining), lagoon outlets and industrial activities. Moreover, Mediterranean islands (Balearic, Sardinia, Corsica, Malta, Crete and Cyprus) and the Southern basin coastline exhibit lower concentrations in Arsenic than the rest of the Mediterranean basin. The wide spread distribution of these two species would encourage their use in a global monitoring network devoted to Arsenic contamination.

Key-words: Coastal contamination, *Posidonia oceanica*, *Cymodocea nodosa*, Arsenic, Human-induced pressure.

Introduction
Seagrasses are often used to monitor trace metals, and in the Mediterranean Sea most of these studies focused on *Posidonia oceanica* (L.) Delile meadows, due to their widespread distribution around the Mediterranean Sea and of their ability to accumulate the trace metals (see for instance: Augier *et al.*, 1978; Costantini *et al*., 1991; Pergent-Martini & Pergent, 2000; Lafabrie *et al.*, 2008; Luy *et al.*, 2012). Metals and metalloids are regarded as serious pollutants of the aquatic environment because of their toxicity, persistence, difficulties in biodegrading and their tendency to concentrate in aquatic organisms (Ikem & Egiebor, 2005 in Luy *et al.*, 2012). Among these metalloids, Arsenic (As) is widely distributed in the biosphere. Its occurrence in the environment could be due to human activities (mining, pesticides, burning of fossil fuel etc.) as well as to natural (geogenic) sources (Kim *et al*., 2002). As contamination of the environment from both anthropogenic and natural sources has occurred in many parts of the world and is recognized as a global problem (Storelli & Marcotrigiano, 2000).
Only few studies deal with the potential use of the main Mediterranean seagrasses as bio-integrator for As (Grauby *et al*., 1991; Luy *et al.*, 2012). However these two seagrasses constitute a continuous belt all around the Mediterranean coastlines. Thus, in order to increase the interest in using seagrass in Mediterranean monitoring programs, it would be interesting to confirm their capacities of bio-integrator for this element.

The aim of this study is (i) to assess the potential of *P. oceanica* and of *Cymodocea nodosa* (U.) Aschers. as As bio-integrators, (ii) to determine seasonal variations of As concentrations and (iii) to evaluate the level of As concentration along the Mediterranean coasts.

Material and methods

P. oceanica and *C. nodosa* leaves were collected monthly in two sites (Fig. 1) during the vegetative period; i.e. between March and October in Calvi and between January and December 2007 in Salammbô). Other 82 sites were sampled (between May 2007 and July 2008), 15 sites concerned *C. nodosa* and 68 *P. oceanica* (Tab. 1).

Tab. 1: Coordinates of sampling sites in decimal degrees (WGS 84). Site number is indicated for *Posidonia oceanica* and followed by c and in bold for *Cymodocea nodosa.*

N°	Station	North	East	N°	Station	North	East
				ALGERIA			
1	Annaba	36.893456	7.779814				
				CROATIA			
2	Brbinjscica	44.055117	14.991983	5	Seget Donji	43.511983	16.213789
3	Island Vlasnik	42.753353	16.796739	6	Zadar	44.103583	15.235861
4	Lavdara	43.948000	15.198533				
				CYPRUS			
7	Cape Greco	35.152778	34.173889	8	Larnaca	34.870117	33.655133
				EGYPT			
9	Alexandria	31.094064	29.692653				
				FRANCE			
10	Arinella	42.666667	9.450000	16	La Parata	41.883333	8.616667
11	Canari	42.783333	9.333333	17	Macinaggio	42.983333	9.483333
12	Cavalaire	43.178500	6.540380	18	Porto	42.250000	8.650000
13	Diane	42.116667	9.566667	19	Porto-Pollo	41.733333	8.766667
14	Ile Rousse	42.633333	8.933333	20	Sagone	42.083333	8.666667
15	La Chiappa	41.583333	9.366667	21	Toga	42.700000	9.450000
				GREECE			
22	Crete	35.399658	25.027119	24	Ligaria	35.401069	25.028414
23	Kalogria	40.129519	23.760458				
				ITALY			
25	Agropoli	40.375783	15.001250	34	Porto Ercole	42.436717	11.206133
26	Alghero	40.580044	8.309533	35	Porto Torres	40.838728	8.417222
1c	Bari	41.096733	16.964767	36	Santa Marinella	42.040267	11.894617
27	Bosa	40.284808	8.474883	37	Scarrupata	40.697056	13.916522
28	Cagliari 1	39.222444	9.239306	38	Talamone	42.559624	11.157310
29	Cagliari 2	39.207083	9.302083	39	Tavolara	40.849633	9.692300
2c	Civitavecchia	42.141716	11.743600	**4c**	Torre Canne	40.840417	17.469800
30	Gallipoli	40.057417	17.976183	**5c**	Torre Lapillo	40.282667	17.842183
31	Lacco Ameno	40.753817	13.891842	40	Torre Mozza	42.946567	10.694533
32	Olbia	41.000444	9.621306	**6c**	Torre Salinas	39.351722	9.593583
33	Oristano	39.833042	8.553292	41	Torre San Giovanni	39.897350	18.097500
3c	Porto Cesaro	40.257967	17.890850				
				LIBYA			
42	Garabouilli	32.814530	13.705220	43	Tajura	32.897528	13.355822
				MALTA			
44	Bahar ic-Caghaq	35.953650	14.448467	47	Qalet Marku Bay	35.949267	14.453017
45	Dahlet ix-Xmajjar	35.998983	14.366783	48	Rdum il-Kbir	36.065017	14.296233
46	Marsaxlokk Bay	35.828633	14.547200	49	St Paul’s Bay	35.954783	14.392733
				SLOVENIA			
50	Gulf of Trieste	45.548683	13.696233				
				SPAIN			
51	Alicante	38.251947	0.512400	54	Medes	42.048889	3.219167
52	Chafarinas	35.180006	0.569414	**11c**	Salines	40.599906	0.711792
7c	Depuradora	40.621092	0.636822	**12c**	Tancada	40.635772	0.737244
8c	Galatxo	40.587508	0.646989	**13c**	Torre Sant Joan	40.630797	0.740547
9c	Irta	40.625719	0.665625	55	Torredembarra	41.146983	1.426717
53	Mallorca	39.760083	3.406533	**14c**	Trabucador	40.618517	0.729028
10c	Mari	40.583769	0.608322				

Tab. 1 (continued): Coordinates of sampling sites in decimal degrees (WGS 84). Site number is indicated for *Posidonia oceanica* and followed by c and in bold for *Cymodocea nodosa*.

N°	Station	North	East	N°	Station	North	East
TUNISIA							
56	Kerkenah	34.681514	11.113286	61	Sidi Ali el Mekki	37.172792	10.262000
57	La Galite	37.536801	8.938876	62	Sidi Mechreg	37.164717	9.122424
58	La Goulette	36.813900	10.307603	63	Tabarka	36.954744	8.767150
59	Monastir	35.775128	10.843294	64	Zembra	37.136125	10.808897
60	Rades	36.767925	10.301869				
TURKEY							
65	Gokceada	40.224400	25.867894	67	Turgutlar bay	36.154100	33.445267
66	Mersin	36.790522	34.637447	68-**15c**	Urla	38.360847	26.794650

The *P. oceanica* and *C. nodosa* leaf blades were cleaned (epiphytes scraped off), rinsed (ultrapure water) and either lyophilised (Heto® FD4-85 freeze dryer, HetoHolten A/S) or dried at 30°C to constant weight, before they were manually reduced to powder. Analyses were run with quality assurance procedures at the Laboratory of Rouen / ETSA (Rouen, France). They were performed using the hydride generation and atomic absorption spectrometry method.

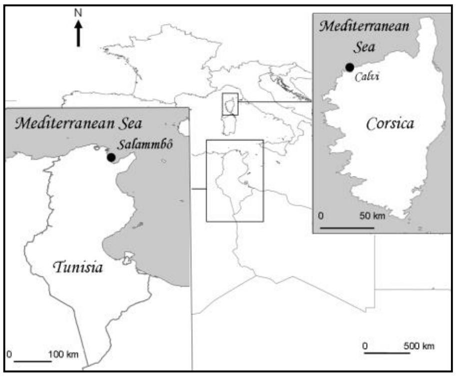

Fig. 1: Sampling sites of Calvi (Corsica) and Salammbô (Tunisia).

Results

Arsenic concentrations in *C. nodosa* were higher than in *P. oceanica* all year round (respectively a mean of 10.0 ± 1.3 and 0.9 ± 0.1 µg.g-1 DW in Calvi, and a mean of 0.6 ± 0.1 and 0.4 ± 0.0 µg.g-1 DW in Salammbô), with an important difference between the both species, observed in Calvi.

Seasonal variations were observed for *C. nodosa* in the two sites, with a maximum concentration in spring (27.8 ± 3.44 µg.g-1 DW) in Calvi, and at the end of winter (1.3 ± 0.1 µg.g-1 DW) in Salammbô. Two concentration peaks emerge during the sampling period: the first in April and the second in July in Calvi and the first in February and the second in September in Salammbô. A significant correlation (Pearson's correlation test, $p<0.05$) was observed between Calvi and Salammbô, with a lag of two months (probably due to seasonal gaps between the northern and southern Mediterranean coast).

For *P. oceanica*, As concentrations were similar all year round (repeated measures ANOVA, $p<0.05$ and Tukey HSD Test) even a maximum is observed in summer in Calvi (1.9 ± 0.1 µg.g-1 DW).

Highest As concentrations were reported in Trabuccador (Spain) for *C. nodosa* (Fig. 2A) and in Brbjinscica (Croatia) for *P. oceanica* (Fig. 2B). For both seagrass, the highest values are significantly different from the lowest values of arsenic concentrations (Nested design ANOVA, $p<0.05$).

Discussion

Very few studies concern As concentrations in seagrasses and their seasonal variations (Fourqurean *et al*., 2007, Malea & Krevekidis, 2013). Nutrients such as phosphorus (P) are required for growth of phytoplankton and seagrasses (Romero *et al*., 2006). The chemical similarities between As and P can explain substitutions of these elements. Thus, it has been suggested that indiscriminate uptake of As by marine biota can occur when P concentrations in the water column are depleted (Apte *et al*., 1986; Michel *et al*., 1998). In this study, peaks in As occur just after phytoplankton blooms, identified at the end of March and at the beginning of June by Goffart *et al*., (2002) in Corsica while arsenic concentration peaks occurred in April and July. The uptake of P by phytoplankton, which results in a depletion of this nutrient in the water column, could explain high As concentrations in seagrass during the following months (Howard *et al*., 1995). This phenomenon appears more visible in *C. nodosa*, perhaps due to the first's higher leaf growth rate (Cancemi *et al*., 2002) which induces a higher uptake of nutrients.

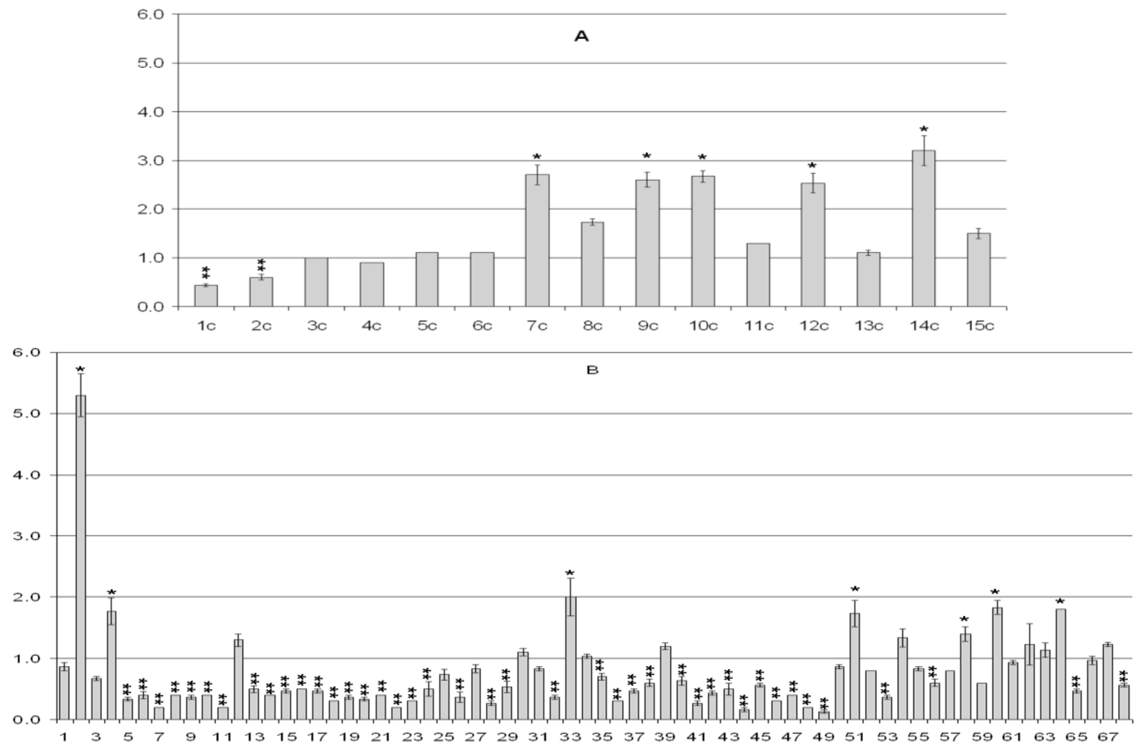

Fig. 2: Arsenic concentrations (µg.g^{-1} DW), in *C. nodosa* (A) and in *P. oceanica* (B) in Mediterranean sites. (Mean ± SE). Sites with * are significantly different from those with **.

The average concentrations in Calvi (more than 15 times higher than in Salammbô) could be explained by the presence, in proximity of Calvi, of a geological restricted zone rich in As and of a mine containing arsenopyrite (BRGM, 1994).

Concentrations of As, in *P. oceanica* around the western Mediterranean basin, exhibit a significant difference (Kruskal-wallis, $p < 0.05$) between Northern and Southern coasts. The significant higher concentrations observed on northern coast are probably linked to urbanization (UNEP, 2005). Indeed, metal contamination in coastal environments is often associated with impacts induced by human activities. Significant difference was also identified between continental sites and islands (Kruskal-wallis, $p < 0.05$), due to reduced human activities in the Mediterranean islands studied (Biggi, 2001). The highest value, recorded in Brbinjscica (Croatia), confirms high As contamination yet observed in Adriatic Sea (Fattorini *et al*., 2008).

The highest concentrations of As, in *C. nodosa* (> 2.0 µg.g^{-1} DW; Fig. 2A), were observed at the outlets of rivers and lagoons. The presence of As in estuaries has already been pointed out due to the accumulation of pollutants, released by upstream industries, and to specific mechanisms of desorption in estuaries (Michel *et al*., 1998). Conversely, the two sites with lowest values (less than 0.6 µg.g^{-1} DW) were located in open sea (Bari and Civitavecchia).

Conclusion

Concentrations of As, found in *P. oceanica* do not show important seasonal variations, conversely to *C. nodosa* where these variations seem linked to the availability of nutrients (P in water column); indeed similarities between As and P could induce an increased uptake of this metalloid after phytoplankton blooms.

For both seagrass species concentrations are higher in the vicinity of geological sources, lagoon outlets and in the vicinity of industrial activities. Moreover, Mediterranean islands (Balearic, Sardinia, Corsica, Malta, Crete and Cyprus), as well as the Southern basin coastline, exhibit lower concentrations in As.

The potential of seagrasses as contaminant bio-indicator is confirmed and the widely spread distribution of these two species in the Mediterranean basin would encourage their use in a global monitoring network devoted to As contamination, especially for *P. oceanica* where concentrations are few affected by seasonal variations.

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