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Rym Zakhama-Sraieb[1,2], Yassine Ramzi Sghaier[1,3], Ahmed Ben Hmida[1], Giovanna Cappai[4], Alessandra Carucci[4] & Faouzia Charfi-Cheikhrouha[1]

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1. Université Tunis El Manar, Faculté des Sciences de Tunis, Département de Biologie Animale, S11UR11 Bio-Ecologie et Systématique Evolutive 2092 Manar II, Tunisia.

2. Higher Institute of Biotechnology of Sidi Thabet, Manouba University, Tunisia.

3. Regional Activity Centre for Specially Protected Areas (RAC/SPA), Boulevard du Leader Yasser Arafat, BP 337, 1080 Tunis Cedex, Tunisia.

4. Department of Geoengineering and Environmental Technologies (DIGITA), University of Cagliari, Piazza d'Armi 1, 09100 Cagliari, Italy

Corresponding author:

Rym Zakhama-Sraieb

E-mail : zakhamarym@yahoo.fr

Telephone: +216 22 581 681

Abstract

Accumulation of five Metallic Trace Elements (MTEs) cadmium, copper, lead, nickel and zinc was measured in *Posidonia oceanica* leaves. Seasonal sampling was carried out in Port El Kantaoui; a total of 180 shoots were collected by SCUBA divers within the 8 - 10 m depth range. Levels of the five MTEs were analysed using inductively coupled plasma atomic emission spectrometry (ICP-AES) in three components of *P. oceanica* shoots: blades and petioles of adult leaves, and intermediates leaves. Results showed a preferential accumulation of Cd, Pb, Ni and Zn in adult leaf blades and, irrespective of season, the recorded mean level of MTEs decreased in the following order: Zn > Ni > Cu > Pb > Cd. One-way ANOVA indicated a significant difference in levels of the five MTEs in adult leaf blades between seasons. Levels of Cd and Cu showed a seasonal pattern; Cd

decreased from spring to winter while Cu shown the opposite trend. A highly significant correlation was found between Cd-Cu and Cd-Pb. A significant correlation was also noted between Cd-Ni. A relationship was recorded between leaf adult area and Zn accumulation.

Keywords: Metal Trace Elements, *Posidonia oceanica*, blade, seasonal variation, Port El Kantaoui, Tunisia.

1. Introduction

Seagrass meadows play a crucial role as a habitat for a multitude species, for which they provide food and shelter, and protect the coast against erosion. Additionally, they improve environmental quality by increasing transparency and oxygenation of the water, and contribute to carbon storage (Green and Short 2003). Organic carbon produced by seagrass is added to the carbon sink in sediments, which are hotspots for carbon sequestration in the biosphere (Duarte et al. 2005). The highest carbon levels recorded in sediments are from the Mediterranean where seagrass meadows store carbon several meters deep down in the sediment. A decline and/or loss of seagrass will lead to release of this carbon to the atmosphere, thereby accelerating global warming. Among seagrasses, the endemic *Posidonia oceanica* (L.) Delile is widely distributed in the Mediterranean Sea, is the most important contributor to coastal primary production (Pergent et al. 1994) and plays a major role in contributing to coastal stability of sediments and in protecting beaches from erosion (Boudouresque et al. 2006). However, meadows of this species are very susceptible to pollution and disturbance by human activities, including industrial and harbour activities, coastal development including for tourism, maritime activities, destructive fishing practices amongst others, have a large adverse impact leading to regression and even loss of the habitat.

Recently, according to Richir and Gobert (2014), *P. oceanica* meadows are a very good indicator of water quality of the marine environment. This plant has been widely used to provide important information on the vitality and dynamics of seagrass systems, and to assess human impacts on the environment (Boudouresque et al. 2006; Romero et al. 2007). Furthermore, *P. oceanica* has been used as a bioindicator for metals for the last three decades (Panayotidis et al. 1990; Costantini et al. 1991; Grauby et al. 1991; Taramelli et al. 1991; Paterno et al. 1991; Carlotti et al. 1992; Pergent-Martini et al. 1993; Augier et al. 1994; Catsiki et al. 1994; Malea et al. 1994; Bougerol et al. 1995; Duarte et al. 1995; Ledent et al. 1995; Romeo et al. 1995; Warnau et al. 1996; Pergent and Pergent-Martini 1999; Sanchiz et al.2001; Campanella et al. 2001; Baroli et al. 2001; Ancora et al. 2004 ; Kljakovic-Gaspic et al. 2004; Tranchina et al. 2005; Maserti et al. 2005; Gosselin et al. 2006; Fourqurean et al.

2007; Lafabrie et al. 2009; Lopez y Royo et al. 2009; Cozza et al. 2013; Conti et al. 2014; Richir and Gobert 2014) given its capability to accumulate metals such as copper, lead, cadmium, nickel, zinc.

In Tunisia, studies on seagrass meadows are few and recent, and most of these have focussed on distribution (Ben Mustapha and Hattour 1992), vitality and biometric parameters (Sghaier et al. 2013), and associated epiphytes (Ben Brahim et al. 2010; 2013; Mabrouk et al. 2012; 2014), mainly in order to assess the "health status" of seagrass and consequently the habitat. At El Kantaoui, *P. oceanica* shoot density and temporal patterns in leaf and rhizome production and other flowering parameters using lepidochronology have been reported and compared with those of nine other meadows distributed along the Tunisian coast (Sghaier et al. 2013).

The development of port activities in Tunisia has clearly led to economic benefits but they have also resulted in environmental problems. A major concern is the presence of toxic pollutants generated by port activities and their effects on marine ecosystems and human health. In the present study, five MTEs were selected and their levels measured in three components of the *P. oceanica* shoot. *P. oceanica* was chosen since it is a powerful bioindicator; it is sensitive to different types of disturbances, it has a wide distribution along Mediterranean coasts and high longevity, and it is a strong accumulator of MTEs and is resistant to these contaminants. The present study focuses on metallic contamination of this plant, and aims: 1) to provide an overview of levels of MTEs in seagrass regarding two toxic elements Pb and Cd and three essential elements Cu, Ni and Zn; 2) to assess levels of these metals in the environment (sediment and seawater), and in the plant; and 3) to compare levels of these metals in *P. oceanica* present in Port El Kantaoui with those in the same seagrass present in other areas.

2. Material and Methods

2.1. Study area and sampling technique

Sampling was carried out in Port of El Kantaoui (35° 35' N - 10° 36' E - Eastern coast of Tunisia). Four sampling sessions of seagrass were carried out according season (Spring = 24/03/2012, Summer = 05/06/2012, Autumn =20/09/2012 and Winter =17/12/2012), at three stations located inside the marina and four stations located outside the marina (Fig. 1 and Table 1).

Sampling of seawater and superficial sediment were also conducted at the seven stations during spring. Three replicates (5 l) of water samples were collected from the seawater surface at each station using plastic carboys. A homogenized aliquot of 100 ml from each seawater sampling station was transferred in a High Density

Polyethylene (HDPE) container, which was completely filled in order to minimize the interaction of the sample with air, and then acidified with 1 ml of 65% nitric acid. Samples were stored at 4°C until analysis.

The sediment samples were collected using a box corer (13.5 x 13.5 x 16 cm) operated by hand. A homogenized sediment aliquot of 500 ml from each sediment sampling station was transferred to a HDPE container, completely filled in order to minimize the interaction of the sample with air and stored at 4°C until analysis. Samples were dried in the laboratory and only sediment fractions having a mean grain size less than 2 mm were analysed.

Sampling of *P. oceanica* was carried out only at the four stations outside the marina of Port El Kantaoui, with three replicates taken per station. No sampling of seagrass was possible inside the marina, as the plant had disappeared from there. At each station, 45 orthotropic shoots (15 shoots per replicate sample) were collected by SCUBA divers from 8 m to 10 m depth range. The total 180 shoots were then transported in plastic bags and preserved at -20°C until analysis.

2.2. Plant samples treatment

For each sample of 15 *P. oceanica* shoots, biometric analysis was performed according to Giraud (1977). Leaves were classified as: 1) adult in which a distinction can be made between blade (photosynthetic upper part) and petiole (basal part); 2) intermediates without petiole and 3) juveniles having a length less than 5cm. The mean adult leaf area (LA), which is the mean surface area of adult leaves, was estimated per station and per season, and expressed in cm^2. For the chemical analysis, intermediate and the adult leaves were used, which were separated into petioles and blades. Epiphytes were carefully removed from leaves using a glass slide. Samples were then dried at 60°C for 48 h and afterwards ground in a ceramic mortar to obtain a homogeneous powder.

2.3. Chemical analysis

Seawater: Following filtration through a 0.45-μm Whatman filter, analysis of Cd, Cu, Ni, Pb and Zn were performed using Inductively Coupled Plasma spectrometry (ICP-OES, Perkin Elmer Optima DV 7000).

Sediment: Approximately 50 g wet weight of sediment was taken from each sample and placed in a porcelain crucible and oven dried at 100°C until constant weight was achieved (2 days). Analysis of Cd, Cu, Ni, Pb and Zn, was performed in duplicate, and out on dry sediment following metal acid solubilisation and subsequent analysis by Inductively Coupled Plasma spectrometry. A standardized microwave assisted acid digestion method was applied (EPA method number 3052). Representative samples of 0.7 g were placed in a teflon microwave vessel and a mixture of analytical grade concentrated acids was added: 9 ml of 65% nitric acid, 3 ml of 37% hydrochloric acid and 5 ml of 40% hydrofluoric acid. Vessels were sealed and heated in the microwave system to 180 ± 5°C for 12 minutes, and for a further 15 minutes at the same temperature. After cooling, the vessel contents were evaporated on hot plate at 80°C to almost dry and then re-dissolved in nitric acid on hot plate at 80°C. The extract was finally filtered through 0.45µm Whatman filters into 50 ml volumetric flasks containing 1% of nitric acid. For quality control, the standard reference material sediment GSD-12 was analysed (Geostandars newsletter 1994).

Plant: In order to remove organic material, 500 mg of dried samples of the three *P. oceanica* components were calcinated at 450°C for 2 hours. To each sample, 5 ml of nitric acid (65%) and 3 ml of hydrogen peroxide (35%) were added in a closed teflon cup. Digestion was performed using a microwave oven. Calibration was performed using aqueous standards for the five tested trace elements. These standards were prepared by diluting the concentrated solutions with water at concentrations of between 0.05 and 5 ng mL-1. The certified reference samples were analysed in the same manner. The analytic procedure was verified using certified reference material NIST-1515 apple leaves. Mineralized samples were analysed and trace element rates measured using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, OPTIMA 2100 DV and PERKIN ELMER 8000).

2.4. Statistical analysis

Statistical analysis was performed using R Statistical Software. Significant differences between seasons were tested using a one-way analysis of variance (ANOVA) after testing the homogeneity of variances using Cochran's C-test. ANOVA was followed with the Student-Newman–Keuls (SNK) comparison test (with a significant level of 0.05; 0.01 and 0.001).

The assumption of normality was tested using the Shapiro-Wilk test, which indicated non-normality of the data. Spearman’s test was used to explore correlation between levels of metals in he tested matrix. In the statistical analyses, values below the detection limit were considered as half the detection limit value.

3. Results

3.1. Seawater

Table 2 shows mean levels of MTEs in seawater recorded from the sampling stations. For comparison purposes, the environmental quality standards (EQS) proposed by the European Union (European community directive 2008/105/EC) for ‘good chemical status’ of surface waters, is also reported. Levels of metals in seawater were very low at all stations. Some metals, namely Cd, Ni and Pb, were recorded in levels that were always below the analytical method detection limit. Levels of Zn and Cu were relatively higher at stations located inside the port compared to ones recorded from outside the port.

3.2. Sediment

Mean levels of metals in sediments collected from sampling stations in are reported in Table 2. Mean levels of MTEs in sediment decreased in the following order: Cu > Zn > Ni, Pb > Cd in S1, S2 and S3; and it's the different for the stations outside the port Zn > Pb > Ni > Cu > Cd. All measured MTE were detected in almost all sampled stations with an increasing gradient from outside the port to inside the port. S2, station located in front of the fuel pump of the port show the highest Pb and Zn concentrations.

3.3. Levels of MTEs in *Posidonia oceanica* compartments

Levels of MTEs in the different leaf components of *P. oceanica* are summarized in Table 3. Results show that *P. oceanica* accumulated the five metals (Cd, Cu, Pb, Ni and Zn) in the three leaves components. A variable distribution of MTEs according to the compartment of the plant was observed. A preferential accumulation of Cd, Pb, Ni and Zn was recorded in blades of adult leaves, which exhibited significantly higher levels than intermediate leaves; the lowest value was reported in the basal part (petiole). Regarding Cu, the highest level was recorded in intermediate leaves, followed by the petiole. Given that the majority of the MTEs were concentrated in blades of adult leaves compared to intermediate leaves and petioles, focus is made on this component hereafter.

3.4. Accumulation of MTEs in blades of *Posidonia oceanica*

Mean levels of MTEs in the blades of *P. oceanica* decreased in the following order: Zn > Ni > Cu > Pb > Cd (Figure 2).

3.5. Seasonal levels of MTEs in *P. oceanica* blades

Seasonal levels of MTEs are shown in Fig. 2. Levels of Cd and Cu in blades of *P. oceanica* showed a seasonal pattern; levels of Cd decreased from spring to winter as opposite of levels of Cu. One-way ANOVA indicated a significant difference in levels of the five MTEs in blades between different seasons. The results of SNK indicated the following:

Cadmium: a significant difference in levels of Cd between spring-autumn, spring-winter, summer-autumn, summer-spring and summer-winter. However, no significant difference in levels of Cd was indicated between winter-autumn.

Copper: a significant difference in levels of Cu was observed between winter-spring, winter-summer, winter-autumn and autumn-spring. No significant difference in levels of this metal was detected between summer-spring and autumn-summer.

Lead: a significant difference in levels of Pb was observed between spring-autumn, winter-autumn, spring-summer, summer-autumn and spring-winter. No significant difference was detected in levels of this metal between winter-summer.

Nickel: a significant difference in levels of Ni between winter-autumn, winter-spring, summer-autumn, spring-summer and summer-winter. Non-significant difference was detected between autumn-spring.

Zinc: a significant difference in levels of Zn between summer-autumn, summer-spring and summer-winter. No significant difference was detected between winter-autumn, winter-spring and spring-autumn.

3.6. Correlation between MTEs in *P. oceanica* blades

Given that the results for levels of MTEs suggested some relationship between trace metals, Spearman's correlation test was applied (Table 4). Significant negative and positive correlation was reported between Cd and Cu and between Cd and Pb, respectively. Cd and Ni were also positively correlated.

3.7. Correlation between MTEs and adult leaf area LA

Values of LA exhibited a seasonal pattern with a minimum in winter and a maximum in summer (Fig. 3). Levels of MTEs recorded from the four stations (S4, S5, S6 and S7) and four seasons, as well as mean values of LA were tested using Spearman test. Of the five MTEs tested, a significant correlation was reported only between LA and Zn (Table 4).

4. Discussion and conclusion

P. oceanica leaves had high levels of the five MTEs, compared to sediments and seawater, indicating the strong ability of this species to accumulate the metals. Furthermore, the overall of MTEs level in *P. oceanica* are relatively similar to those in the environment; this is the case for Zn, Cu and Cd. Overall, this is considered to reflect uptake in proportion to levels of metals available in the environment (seawater). Furthermore, the present results indicate that seagrass meadows play an important role as "reservoir" through the assimilation and the storage of MTEs from the surroundings.

The metals analysed in this study (Cd, Cu, Pb, Ni and Zn) were chosen as they represent the most common trace and toxic metals that can affect coastal communities (Roberts et al. 2008). All were found in detectable and variable concentrations in the *P. oceanica* components, at all sampled stations and in all seasons. Several factors, such as the metal tested for, availability of MTEs in the environment, the sampling period, the tissue analysed, and the metabolic processes linked to plant physiology, may influence the distribution of MTEs in the different components of *P. oceanica* leaves, as indicated in the literature (Pergent-Martini and Pergent 2000, Luy et al. 2012).

Marine Magnoliophytes assimilate MTEs available in the water column using their leaves or in the interstitial water using their roots. Our results indicate that levels of MTEs in *P. oceanica* tissues depend on leaf class and component. Indeed, Zn, Cd, Pb and Ni were preferentially accumulated in photosynthetic tissue rather than in non-photosynthetic tissue. Luy et al. (2012) and Lafabri et al. (2008) suggest that photosynthetic tissue preferentially assimilates several MTEs from the water column, particularly Co, Hg, Zn, Cd, Pb and Ni. Furthermore, these workers noted that the metals were present in higher levels in adult leaf blades compared to the other shoot components. Warnau et al. (1996) explain the observed differences of accumulation of MTEs between photosynthetic tissues (adult and intermediate leaves) by the fact that adult leaves are exposed longer to MTEs than intermediate leaves. Campanella et al. (2001) suggest that the tip of the leaf compared to its younger basal part presents a dilution effect due to the higher growth rate of intermediate leaves. Regarding Cu, which is an essential micronutrient for *P. oceanica* (Conti et al. 2010), the present levels recorded in the intermediate leaves and in the petiole of adult leaves were systematically higher than in the adult leaf blades. These findings are in agreement with previous studies (Catsiki and Panayotidis 1993; Luy et al. 2012) and can be explained as suggested by Luy et al. (2012) by an increase in metabolic activity during leaves growth.

Besides the observed variation of levels of MTEs between different shoot components, the present results reveal seasonal variation in accumulation of MTEs in adult leaves of *P. oceanica*, probably due to metabolic factors. Previous studies mention seasonal variation of levels of MTEs in seagrass, which is not always significant and differs depending on the metal and plant compartment examined (Malea et al. 1994). In much the same way, Ledent et al. (1993) revealed that the sampling season significantly influenced the observed levels of metals (Cd, Cr, Fe, Pb, Ti and Zn) in photosynthetically active leaves. The differences recorded by different authors appear to be due to physicochemical and/or biological parameters that are capable of modifying availability of metallic elements. Indeed, Malea et al. (1994) pointed out the importance of epiphyte biomass, which is highly variable from one season to the next, and will affect the accumulation levels of some elements (e.g. Ca). In addition, several authors attributed the low levels of some MTEs recorded in spring and summer to an irreversible fixation of the metal in the plant and to dilution of these contaminants during the growth period (Malea and Haritonidis 1995).

Our results are in agreement with a survey carried out in other Mediterranean Sea areas using *P. oceanica* (Catsiki and Bei 1992; Luy et al. 2012) as bioindicator, and which indicated the highest mean levels for Zn and the lowest ones for Cd.

The pattern of seasonal variation of levels of Zn probably results from the growth dynamics of *P. oceanica*. The leaf area of this magnoliophyte reaches a minimum value in winter and increases towards autumn and spring, attaining its maximum value in summer. An increase in levels of Zn in seagrass leaf blades within the active growth period has been also previously reported by Malea et al. (2013). Zinc is a micronutrient that is involved in several physiological processes in plants (Hafeez et al. 2013). It plays an important role in plant metabolism such as activation of several enzymes involved in carbohydrate metabolism; it maintains the integrity of cellular membranes, protein synthesis, regulation, and stability of the gene expression required for the tolerance of environmental stresses.

The interaction of MTEs may also influence the seasonal variation of levels of these metals in *P. oceanica*, Cadmium and Cu exhibit an antagonistic activity, hence the negative correlation obtained in our results. For some species of macroalgae, Foster (1976) and Bryan (1983) point out that Cu inhibits Cd uptake.

The levels of Pb recorded from the sediments of Port El Kantaoui are lower than those reported from the main ports in Tunisia (Ports of Sidi Mansour and Gabès) by Chouba and Mzoughi-Aguir. (2006), and fromother Mediterranean sites such as the Antikrya Gulf in Greece (Malea et al. 1994) and Livorno in Italy (Lafabrie et al. 2007). However, Pb levels recorded from the present study are similar to those reported from sites that are free from direct pollution sources e.g. Canari, France and Porto-Torres, Italy (Lafabri et al. 2007). Pollution by MTEs is essentially due to phenomena occurring at the air-sea interface, and this particularly true for lead deposition in water and sediment (Schneider et al. 2000).

A comparison of levels of metals in *P. oceanica* leaf blades recorded from Port El Kantaoui with those reported from populations of the seagrass in other parts of the Mediterranean Sea (Table 5) suggest the following: *P. oceanica* in El Kantaoui exhibits low levels of Cd, Cu and Ni compared with Italian (Conti et al. 2007; Lafabrie et al. 2007), French (Gosselin et al. 2006; Lafabrie et al. 2007; Luy et al. 2012) and Greek populations (Catsiki and Panayotidis 1993; Malea et al. 1994, Sanz-Lazaro et al. 2012) of the same seagrass. However, levels of Pb recorded from the present study are similar to those reported by Conti et al. (2007; 2008) and Lafabrie et al. (2007), and lower than those recorded by Malea et al. 1994 and Sanz-Lazaro et al. 2012 in Green coasts, Gosselin et al. (2006) for the Sicilian coast and Luy et al. 2012 for French coast. Levels of Zn from the present study were higher than those reported by Malea et al. 1994 from the Gulf of Antikyra (Greece), similar to those reported by Conti et al. 2008 from Sicily (Italy), and lower than one indicated in other studies. According to the classification by Pergent (2007), one can consider the *P. oceanica* present at El Kantaoui as having low levels of contamination by Cd (<1.92) and Ni (<18.10), and a moderate level for Pb (1.83 - 2.42). In general, when comparing the present results to ones in the literature, levels of MTEs in *P. oceanica* at El Kantaoui indicated "unpolluted" status for the area.

The presence of MTEs in the port area of El Kantaoui can be explain by the presence of boats in the marina that have antifouling paints and ship rustproof enamel (a source of Cu and Pb), a fishing sinker, a fuel pump (Pb can be an additive in gasoline), and emissions to the atmosphere which may lead to fallout into the marine environment.

The present work underlines once more the importance of using *P. oceanica* to assess the quality of the marine environment. *P. oceanica* can used as a good bioindicator of levels of MTEs over a short time (4 seasons) and for assessing levels of Cd, Pb and ZN in the environment. Furthermore, the present study provides the first data on levels of MTEs in El Kantaoui. Assays of other MTEs and further assessments of seagrass data using lepidochronological method are on-going; these will enable the compilation of a historical log of pollution in this area. We suggest that similar future studies can consider sampling only the *P. oceanica* adult leaves blade, since this avoids uprooting the shoots and permits the subsequent re-growing of leaves, hence reducing the need for destructive sampling.

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Figure

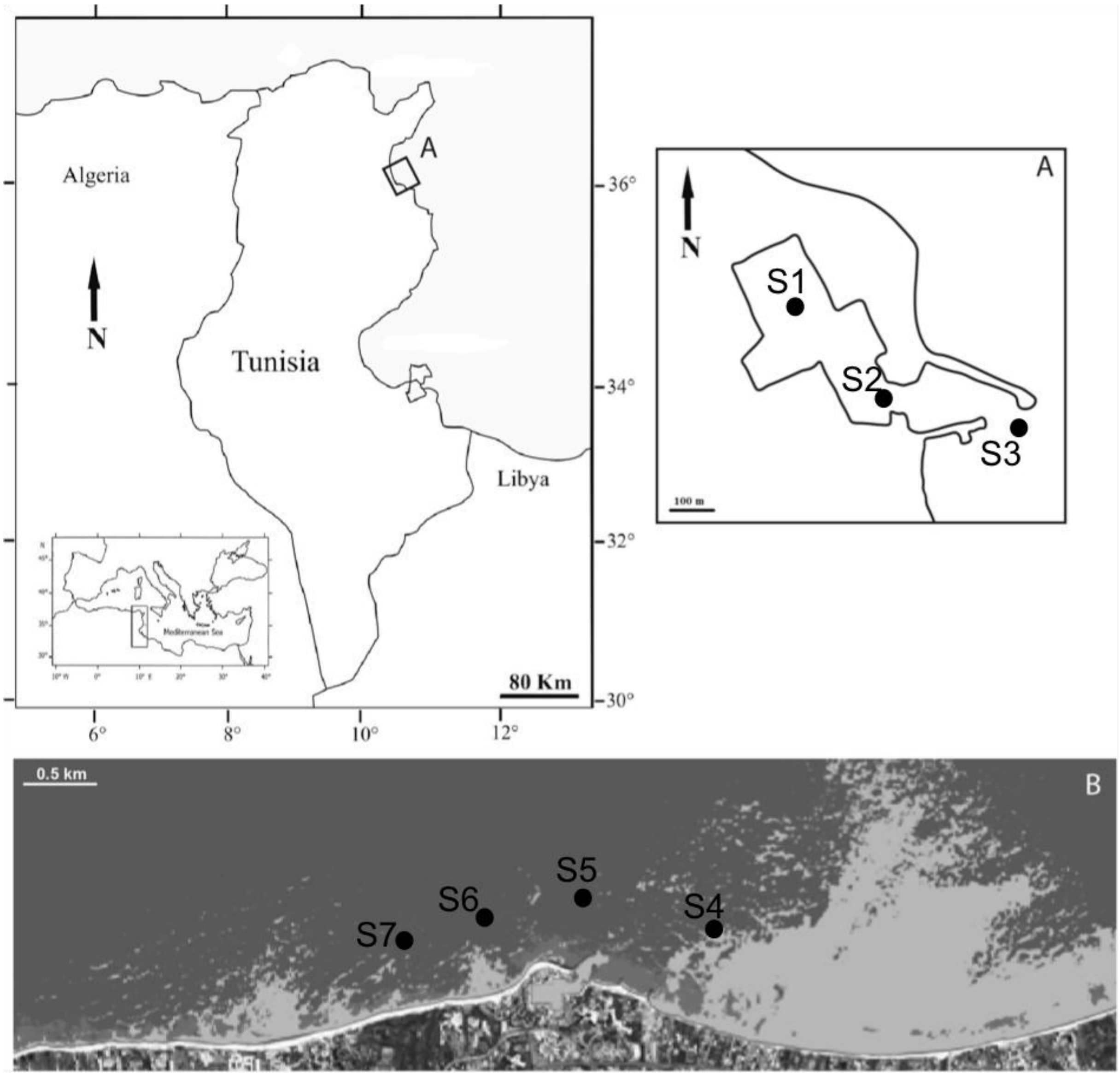

Figure 1. Location of the study area on the eastern coast of Tunisia (A), and locations of the three sampling stations (S1-S3) inside Port El Kantaoui (B); and of the four sampling stations outside the port (S4-S7).

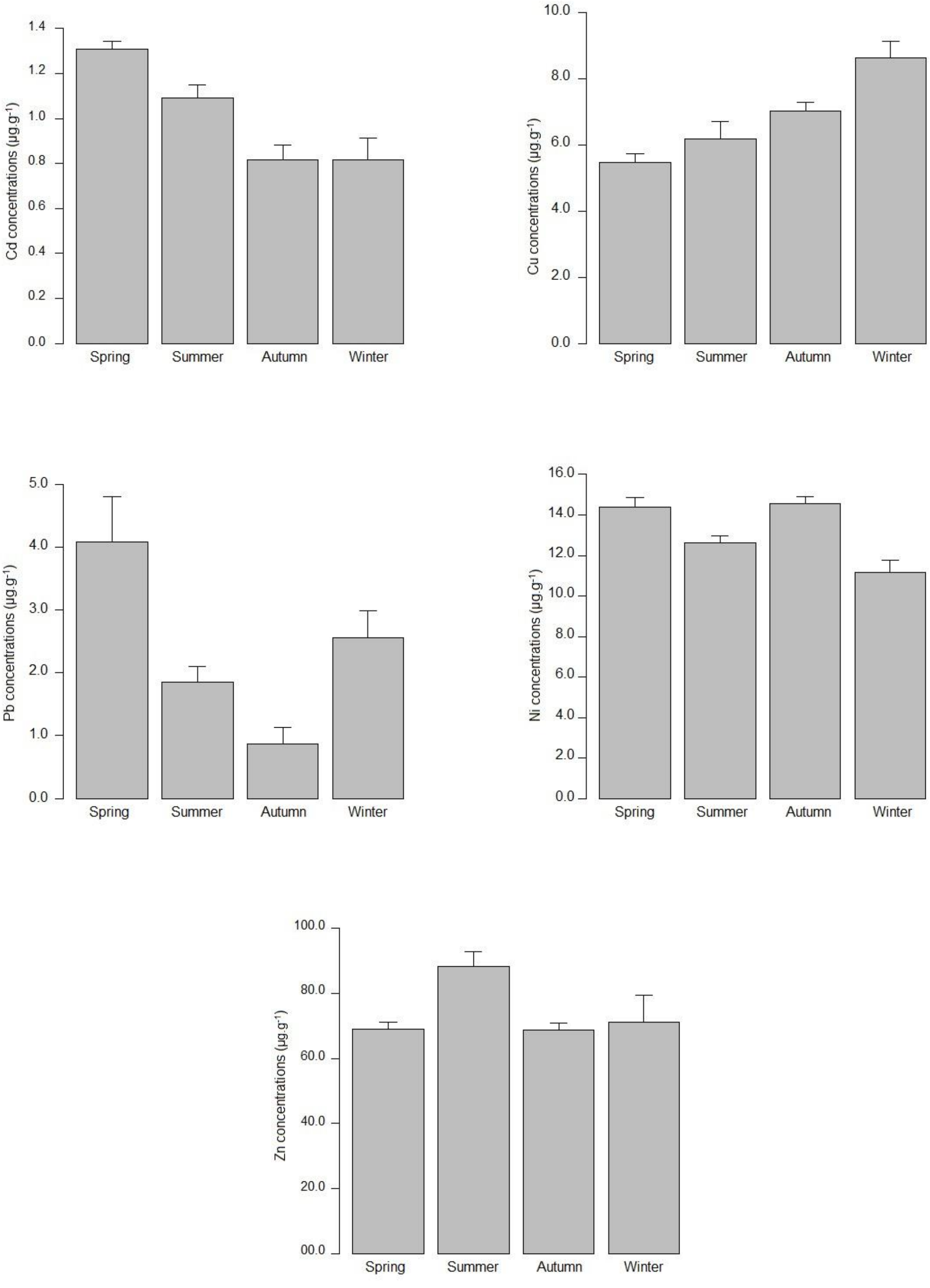

Figure 2. Mean levels ± S.E. of MTEs (µg g^{-1} dry wt in *Posidonia oceanica* adult leaves.

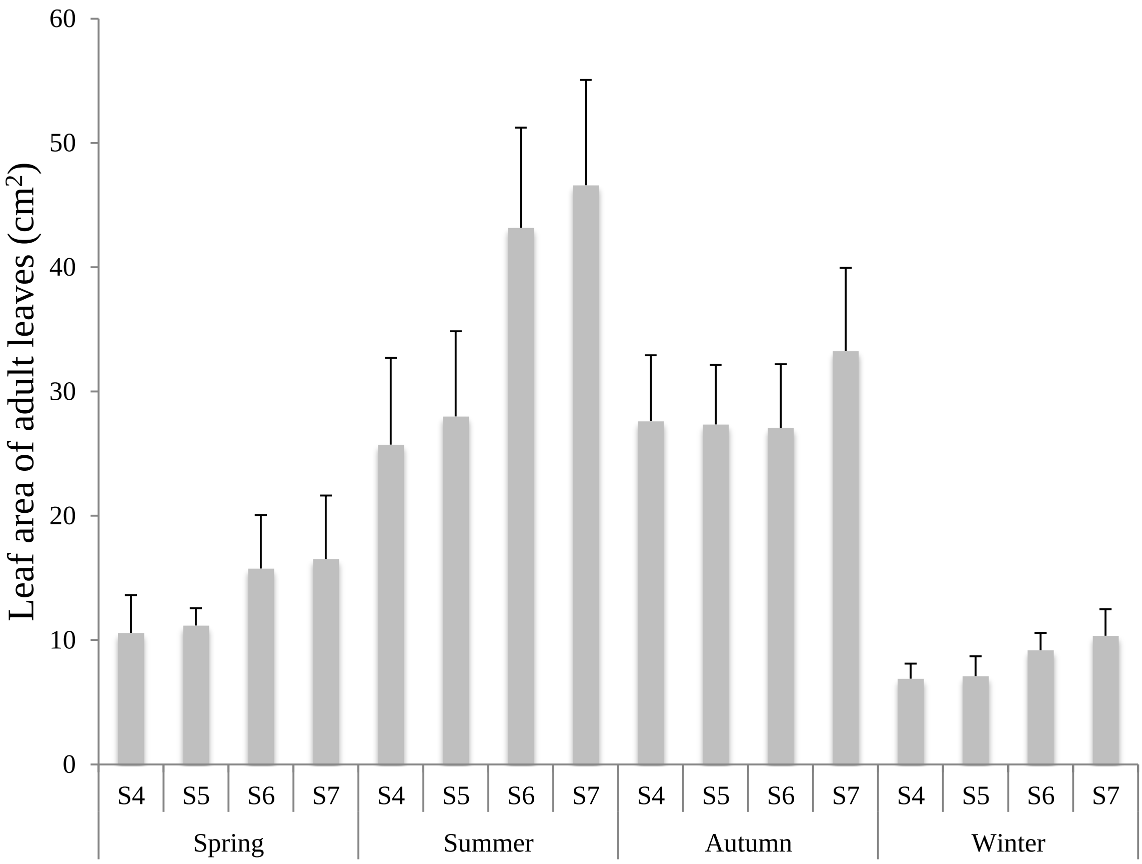

Figure 3. Spatial and seasonal variation of *P. oceanica* adult leaf area recorded from the study area.

Table 1

Geographical coordinates and water depth of the seven sampling stations.

Station	GPS location		Depth (m)
S1	35°53'37.97"N	10°35'53.49"E	2.5
S2	35°53'34.28"N	10°35'58.22"E	4
S3	35°53'32.80"N	10°36'06.59"E	3.2
S4	35°53'05.32"N	10°36'45.97"E	8-10
S5	35°53'47.80"N	10°36'34.70"E	8-10
S6	35°54'10.22"N	10°36'06.95"E	8-10
S7	35°54'40.03"N	10°35'41.35"E	8-10

Table 2

Metallic Trace Elements levels in seawater (μmg ml^{-1}) and sediment (μg g^{-1}) recorded from the study area and comparison with the EU's environmental quality standard (EQS). AA: Annual average concentration.

	Seawater (μg ml^{-1})					Sediment (μg g^{-1})				
Station	Cd	Cu	Ni	Pb	Zn	Cd	Cu	Ni	Pb	Zn
S1	<0.002	0.0116	<0.005	<0.02	0.0289	0.82	193.45	20.13	14.10	92.61
S2	<0.002	0.0155	<0.005	<0.02	0.0276	0.99	179.65	14.47	25.24	156.47
S3	<0.002	<0.005	<0.005	<0.02	0.0102	<0.142	172.12	9.43	<1.442	39.95
S4	<0.002	<0.005	<0.005	<0.02	0.0122	0.03	0.05	0.81	2.70	2.44
S5	<0.002	<0.005	<0.005	<0.02	0.0154	<0.142	<0.005	2.72	2.37	5.59
S6	<0.002	<0.005	<0.005	<0.02	<0.005	0.03	0.22	0.82	2.25	2.62
S7	<0.002	<0.005	<0.005	<0.02	0.0110	0.02	0.16	0.87	2.57	2.32
AA-EQS (EU)	0.0002	-	0.02	0.0072	-	0.3	-	30	30	-

Table 3

Mean levels of Metallic Trace Elements MTE±S.E. (μg g^{-1} dry wt) recorded from the three *P. oceanica* components (Min. average seasonal minimum value; Max. average seasonal maximum value; Mean. annual average ± S.E.). Bold numbers indicates the highest mean value.

			Cd	Cu	Pb	Ni	Zn
Adult leaves	Blade	Min	0.82±0.22	5.48±0.85	0.86±0.88	11.19±1.93	68.98±7.16
		Max	1.31±0.11	8.64±1.66	4.08±2.38	14.58±1.12	88.47±14.63
		Mean	**1.00±0.30**	6.83±1.82	**2.34±1.93**	**13.21±2.04**	**74.36±18.52**
	Petiole	Min	0.51±0.14	4.81±1.74	0.15±0.19	1.86±1.44	21.58±8.06
		Max	0.85±0.34	11.56±1.39	1.37±1.64	2.56±0.93	31.59±22.56
		Mean	0.66±0.32	8.00 ±3.25	0.86±1.38	2.10±1.18	25.77±14.96
Intermediate leaves		Min	0.63 ±0.39	5.48±0.85	0.22±0.25	7.35±1.02	43.86±5.73
		Max	1.31±0.11	14.63±1.11	4.08±2.38	14.43±1.44	115.89±54.26
		Mean	0.93±0.39	**9.00±3.79**	2.16±2.02	10.96±3.34	72.05±41.06

Table 4

Matrix showing Spearman correlation in adult blades (significant correlation at $p < 0.05$: * and at $p < 0.01$: **). L.A. : Mean Adult Leaf Area.

	Cd	Cu	Pb	Ni	Zn	L.A.
Cd	1.000					
Cu	-0.533**	1.000				
Pb	0.468**	-0.128	1.000			
Ni	0.296*	-0.141	0.129	1.000		
Zn	0.185	-0.132	0.119	0.133	1.000	
L.A.	0.254	-0.311	-0.389	0.356	0.676*	1.000

Table 5

Mean levels ± S.D. of metals (μg g^{-1} dry wt.) in leaves of *P. oceanica* recorded from different coastal areas of the Mediterranean Sea

		Cd	Cu	Pb	Ni	Zn
Present work	**Port El Kantaoui**	**1±0.30**	**6.83±1.82**	**2.34±1.93**	**13.21±2.04**	**74.36±18.52**
Catsiki and Panayotidis 1993	Saronikos, Greece		10.20±10.35		21.57±7.01	
	Cyclades, Greece		8.56±3.01		19.05±4.05	
	Lesbos, Greece		7.67±10.42		30.72±10.93	
Malea et al. 1994	Antikyra Gulf, Greece	20.80±3.00	18.00±7.50	39.50±6.60		43.40±3.00
Gosselin et al. 2006	Corsica, France	2.80±0.90	11.10±6.50	5.20±3.80	22.90±10.20	109.30±41.10
Conti et al. 2007	Sicily, Italy	5.98±1.64	31.88±15.80	2.29±1.56		213.00±47.00
Lafabrie et al. 2007	Canari, France	5.38±0.14		1.47±0.03	60.30±3.67	
	Livorno, Italy	3.39±0.12		1.40±0.25	28.90±0.65	
	Porto-Torres, Italy	2.10±0.10		1.80±0.00	27.47±1.10	
Conti et al. 2008	Sicily, Italy	2.42±1.17	11.70±4.58	1.94±1.67		70.90±31.20
Luy et al. 2012	Mediterranean French coast	2.53±0.60	11.40±3.50	3.02±1.44	39.00±5.00	107.00±22.00
Sanz-Lazaro et al. 2012	Sounion, Greece	1.19±0.19	10.90±2.00	6.12±1.60	24.50±14.00	133.00±38.00