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## Deep-water *Zostera marina* meadows in the Mediterranean

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### **Highlights**

Baseline data on the only known [Zostera marina meadows](#) on the Moroccan Mediterranean (Jbel Moussa) is provided.

Bioavailable Ni, As, Mo, Ag, Cd, Sn, Sb and U measured in sediment are mainly accumulated in *Z. marina* roots.

N is high in *Z. marina* leaves and sediment is more enriched in P.

Moroccan *Z. marina* meadows are overall in good health status and well developed compared to many places worldwide.

Implementation of measures against trawling and invasive algae are urgently needed to preserve *Z. marina* meadows in Morocco.

### **Abstract**

In Morocco, *Zostera marina* Linnaeus has disappeared from many localities where it was historically reported. The only known remaining meadows along Mediterranean coasts of Morocco, though in North Africa, are those of Belyounech bay and Oued El Mersa bay, in the marine area of 'Jbel Moussa'. An in-depth knowledge of these meadows is required for their effective conservation purpose. The *Z. marina* meadows of Jbel Moussa are deep, the lower limit being 17 m depth with patches extending down to 20 m depth. Seagrass cover of Belyounech bay meadow is continuous whereas that of Oued El Mersa is fragmented. Shoot density and aboveground biomass are higher in Belyounech meadow, with  $745 \pm 183$  shoots.m<sup>-2</sup> and  $273 \pm 40$  g<sub>DW</sub>.m<sup>-2</sup> of leaf biomass. During the survey, trawling scars and the invasive algae *Caulerpa cylindracea* Sonder were observed. Bioavailable Ni, As, Mo, Ag, Cd, Sn, Sb and U measured in the sediment are mainly accumulated in *Z. marina* roots. Nitrogen level is high in seagrass leaves and low in the sediment. Conversely, sediment is more enriched in phosphorus. Carbon levels and its isotopic ratio value are respectively higher and less negative in leaves when compared to the seagrass belowground compartments. All together, data collected during this survey allows defining the overall good health status of *Z. marina* meadows of Jbel Moussa. These Moroccan meadows, localized within the warm temperate-southern limit of the species, are well developed compared to many places worldwide. The exceptional presence of deep *Z. marina* meadows in the Mediterranean requires the implementation of measures as a major priority to ensure the conservation of these ecosystems, since seagrasses are being deeply threatened worldwide.

## **Keywords**

*Zostera marina*; Mediterranean; Morocco; Seagrass meadow description; Conservation

## **1. Introduction**

Seagrasses are important primary producers in shallow intertidal and subtidal coastal areas worldwide ([Green and Short, 2003](#)). [Seagrass meadows](#) are ranked among the most valuable ecosystems in the [biosphere](#) ([Short et al., 2007](#)) because of the important ecosystem services they provide, including spawning and hatching ground for a large diversity of marine organisms that are often endangered or commercially important ([Hughes et al., 2009](#); [Nordlund et al., 2016](#); [York et al., 2017](#)). The high species richness of these habitats relies on their structural complexity and the many [microhabitats](#), protection against predators and food resources they provide. The many other ecosystem services include hydrodynamism reduction, fine particles and detritus deposition, sediment stabilization or nutrient removal ([Gutiérrez et al., 2011](#); [Piehler and Smyth, 2011](#); [Cullen-Unsworth and Unsworth, 2013](#); [Nordlund et al., 2016](#)). Even though seagrass meadows occupy less than 0.2 % of the total ocean area, they potentially store 48–112 Tg carbon per year ([Mcleod et al., 2011](#)). Recent estimates of their economic value range from \$178,000 ha<sup>-1</sup>.year<sup>-1</sup> for enhancing fish biomass ([Blandon and Zuermgassen, 2014](#)) and up to \$13.7 billion.year<sup>-1</sup> in [carbon sequestration](#) ([Pendleton et al., 2012](#)). Despite this high value, the distribution and abundance of seagrass meadows decline worldwide and therefore requires sound monitoring and management ([Waycott et al., 2009](#); [Fourqurean et al., 2012](#)).

In the Mediterranean Sea, seagrass meadows are a significant component of coastal [marine ecosystems](#) and constitute the first most important biodiversity hotspot ([Boudouresque, 2004](#); [UNEP/MAP, 2012](#)). Despite their importance, the abundance and the distribution of seagrasses in the Mediterranean decline at alarming rates due to several threats including coastal development, water quality degradation, mechanical damage (e.g. bottom trawling, mooring and anchoring), alien species invasion and climate change (e.g. warming, ocean acidification) ([Boudouresque, 2009](#); [Pergent et al., 2014](#)). At least 21 % of the extent of seagrass habitats has disappeared from European Mediterranean coasts since 1869 ([Carmen et al., 2019](#)), while a sparsity or absence of data occurs in the eastern and southern part of that Sea ([Telesca et al., 2015](#)). Hence, it is clear that seagrass ecosystems of the Mediterranean and worldwide require sound monitoring and management to limit their regression and to ensure their perennity. Research on seagrass conservation ecology is therefore needed to increase knowledge on how these valuable habitats respond to changes over time and to address the threats they face.

[Zostera marina](#) Linnaeus (eelgrass) is one of the world's most widespread seagrass of the northern hemisphere from subtropical regions of the Pacific and Atlantic Oceans to the Arctic Circle (between 27° and 70 °N), with some locations in the Mediterranean and Black Seas ([Olesen et al., 2015](#)). Along the Mediterranean coast of Morocco, *Z. marina* has been recorded in Jbel Moussa in the 1980s ([Bitar, 1987](#)) but has disappeared from other localities such as the 'Cap des Trois Fourches' and the 'Marchica lagoon' ([Bazairi, 2015](#)). Unfortunately, historical data on their distribution is limited to little point-based information on presence/absence without any baseline datasets of past ecosystem conditions. In 2014, surveys of the marine part of the area of 'Jbel Moussa' (southern coast of the Strait of Gibraltar) identified two monospecific *Z. marina* meadows (lower limit at 17 m depth with patches extending down to 20 m depth) in Belyounech bay and Oued El Mersa bay ([PNUE-PAM-CAR/ASP, 2016](#); [Fig. 1](#)). These meadows represent the only two remaining ones along Mediterranean coasts of Morocco, and probably of North Africa. These are also the deepest ones in the entire Mediterranean. Indeed, along other coasts of the Mediterranean Sea, *Z. marina* is often restricted to shallow lagoons ([Short et al., 2010](#); [Pergent et al., 2014](#)). The only deep subtidal meadows that have been reported are found in open bays of southern Spain, in the Alboran Sea, at depths of 5–14 m ([Rueda et al., 2008](#)). Despite this special status of the Moroccan *Z. marina* meadows, these recently mapped habitats have not yet been the subject of scientific study.

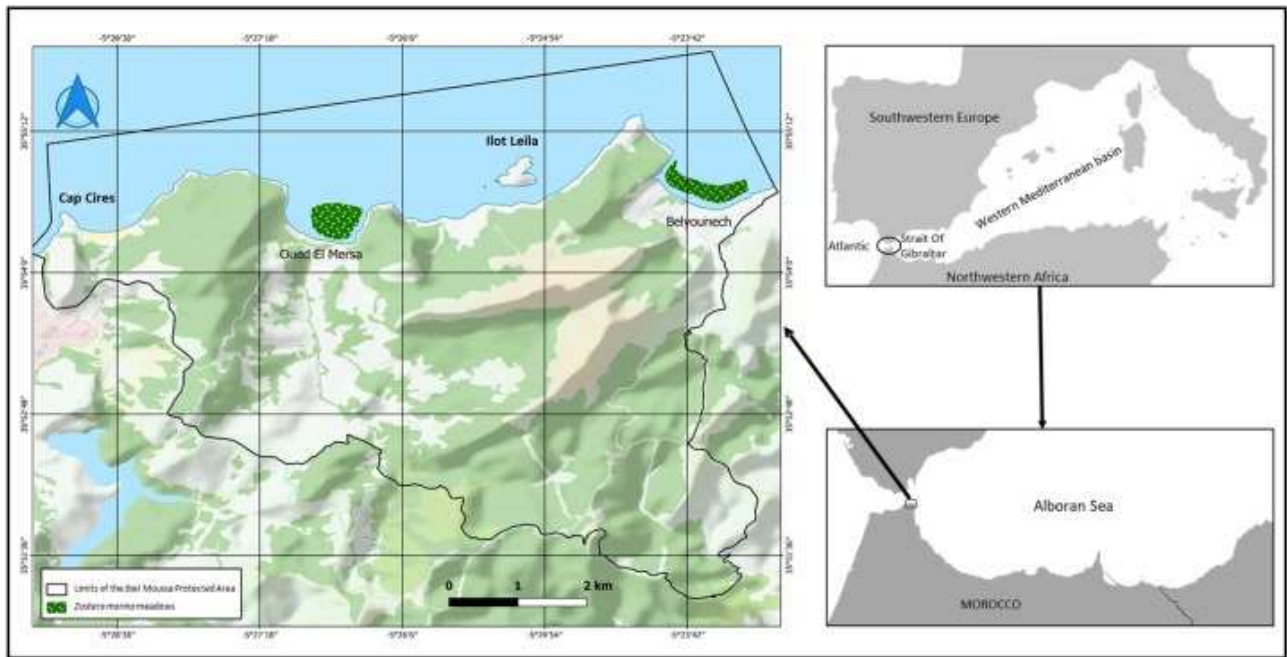


Fig. 1. Map showing the geographical position of the two studied *Zostera marina* meadows in Belyounech bay and Oued El Mersa bay, in the marine part of the area of Jbel Moussa, along the Mediterranean coast of Morocco, south of the Strait of Gibraltar (From [PNUE-PAM-CAR/ASP, 2016](#) modified).

Two of main global challenges to seagrass conservation are the unknown status and condition of many seagrass and the understanding of threatening activities at local scales to target management actions accordingly ([Unsworth et al., 2018](#)). The present work aimed at collecting baseline quantitative and qualitative data for monitoring and conservation purpose of the two *Z. marina* habitats of Jbel Moussa. To this end, a series of ecological and biochemical indicators were investigated: first, seagrass extent metrics (area extent, depth limit), seagrass density metrics (cover, shoot density, total and above-ground biomass), and presence of alien species that must be known before establishing measures of protection (e.g. establishing boundaries of a [marine protected area](#); [McKenzie et al., 2020](#)); second, seagrass shoot [morphometry](#) and biomass that are important indicators of the health status of meadows and light availability ([McMahon et al., 2013](#); [Jones and Unsworth, 2016](#)); third, major and trace element concentrations, carbon and nitrogen contents and their stable isotope ratio values that provide among others information on anthropogenic loadings into the marine environment and may help to design [eelgrass](#) restoration programs if necessary ([Ikem and Egiebor, 2005](#); [Ralph et al., 2006](#); [Jackson et al., 2013](#)).

## 2. Materials and methods

### 2.1. Study area

The area of Jbel Moussa covers about 5000 ha (with a marine area of 14.45 km<sup>2</sup> and lands covering 35.55 km<sup>2</sup>). It was identified by the Moroccan authorities as a Site of Biological and Ecological Interest ([PDAPM, 1996](#)) and it has been included (2019) in the list of [Wetlands](#) of International Importance according to the RAMSAR convention (1971). It is also included in the Mediterranean Intercontinental [Biosphere](#) Reserve (RBIM). Currently, the site is being considered for designation as a protected area.

The population of the area of Jbel Moussa is about 18,000 inhabitants, of whom 4500 live in the middle of the Site of Biological and Ecological Interest in the municipality of Belyounech. The area of Jbel Moussa is characterized by the many exploitation activities of its natural resources. The main activities are agriculture and livestock farming, artisanal fishing and tourism. In terms of artisanal fishing, the operational fleet is 81 boats of which 54 are based in Belyounech bay and 27 in Oued El Mersa bay ([SPA/RAC-ONU Environnement/PAM, 2019a](#)). Tourism and leisure activities remain limited in Jbel Moussa.

The area of Jbel Moussa is located in the southern part of the Strait of Gibraltar. Oceanic circulation in the region is characterised by exchanges of different water masses, the denser Mediterranean waters outflow at depth and less salty Atlantic waters inflow of the upper layer (García-Lafuente et al., 2017). The zonal wind has been reported as the main driving force for surface circulation (Macías et al., 2016). The entrance of Atlantic flood tidal currents to the Alboran Sea (Western Mediterranean) via the Strait of Gibraltar leads to the periodic generation of large-amplitude internal waves propagating eastward and forming turbulence, especially in shallow areas where wave overtopping occurs (Sánchez-Garrido et al., 2011). Under extreme weather conditions, waves can reach over 8 m high (Mazas and Hamm, 2011). Vargas-Yáñez et al. (2017) analysed a time series (1900–2015) of the sea surface temperature and salinity in the western part of the Alboran Sea. They reported the average annual temperature of the first 50 m of the water column oscillated between 15.02 °C (winter) and 16.45 °C (autumn) while the salinity varied from 36.82 (autumn) to 36.99 (spring). For the bottom water, the average annual temperature ranged between 13.11 °C (summer) and 13.17 °C (spring) while the salinity was fixed, equal to 38.50 in all seasons. As part of the global ocean warming, an increasing temperature trend is measured in the Strait of Gibraltar since 1995 (Sanz-Fernández et al., 2019). In the northern part of the Strait, surface temperature has increased to reach minimum monthly average of 14 °C and maximum monthly average of 23 °C in July of year 2015 (Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, 2019).

The marine part of the future protected area of Jbel Moussa encompasses the marine fringe between Cap Cires and Belyounech, including the islet of Leila/Perejil (Fig. 1). The mapping of marine key habitats of Jbel Moussa between Cap Cires and Belyounech was performed in 2015 (PNUE-PAM-CAR/ASP, 2016), leading to the discovery of the two *Zostera marina* meadows in Oued El Mersa bay and Belyounech bay (Fig. 1). Overall, the coastal zone of this site of biological and ecological interest is characterized by the dominance of rocky shores and cliffs, alternating with caves, creeks and small sandy beaches.

## 2.2. *Zostera marina* meadows description

*Zostera marina* shoot density measurement and sampling for biometry and biomass determination was performed in September 2014, in the central dense part of the two meadows of Belyounech bay and Oued El Mersa bay at depths of 13 m and 8 m, respectively. Density was determined by counting *in situ* *Z. marina* shoots inside a 20 × 20 cm randomly-thrown quadrat (n = 10). Twenty shoots were collected and stored individually in plastic bags to measure foliar biometric descriptors. Random sampling for above and belowground biomass determination was performed using a cylindrical polyvinyl chloride (PVC) corer of 0.15 m in diameter and 0.12 m long. Cores (n = 5) were sieved on site through a 0.5 mm mesh size sieve and rinsed with seawater. Plant material was separated into aboveground (leaves) and belowground (rhizomes and roots) compartments, transferred into plastic bags and stored frozen until processing in laboratory. *Z. marina* shoots were dissected for biometry. After rinsing with tap water, dissected shoot were measured for shoot height (equivalent to the length of the longest leaf) and leaf length and width by leaf category, i.e. differentiated (with sheath) and not differentiated (without sheath). The total number of leaves by shoot and the number of leaf by category were counted. From shoot density and the area sampled with the corer, the Leaf Area Index was calculated (LAI, total leaf surface per unit ground surface). Above and belowground compartments were oven dried until constant weight (minimum 48 h at 60 °C) to determine dry weight per meadow surface unit (g<sub>DW</sub>. m<sup>-2</sup>).

In September 2015, the seasonal monitoring of the covering of both *Z. marina* meadows using the photographic quadrats method (García-Gómez, 2015) was initiated. Permanent quadrats of 1 × 1 m (n = 5) were set up and fixed in the sediment with 50 cm long metal stakes, in the center and on the edge of Belyounech (at 13 m and 17 m depth, respectively) and Oued El Mersa (at 8 m and 10 m depth, respectively) meadows. Each quadrat was photographed and images were digitally analysed using Adobe Photoshop 6.0© (Adobe). A digital network of 64 squares was superimposed onto the photographs and adjusted using the distortion tool (Espinosa et al., 2014). Due to the increased development of the invasive algae *Caulerpa cylindracea* Sonder in the study area, the cover of that species was also considered to monitor its evolution and potential interactions with *Z. marina*.

### 2.3. Sediment description

The sediment sampling was performed in the central part of Belyounech meadow in September 2015. Sediment cores (n = 3) were randomly collected in the vegetated [seabed](#) using a cylindrical PVC corer of 0.15 m in diameter and 0.12 m long. Sediment cores were transferred into plastic bags and stored frozen until analysis. In the laboratory, they were oven dried until constant weight (minimum 48 h at 60 °C) and subdivided into three homogeneous subsamples to assess organic matter content, grain size partitioning and chemical composition.

Organic matter content (n = 3) was determined by the loss on ignition method ([Heiri et al., 2001](#)) into the first subsample of each sediment core. They were dried 12 h at 105 °C ( $W_d$ ), then combusted to ash and carbon dioxide during 4 h at 550 °C and weighed again ( $W_c$ ). The percentage of organic matter (OM) was calculated as followed:

$$OM = 100 * (W_d - W_c) / W_d.$$

The second subsample of each sediment core was used for the quantification of the sediment grain size fraction (gravel, sand and mud) ([Wentworth, 1922](#)). Grain size (n = 3) was measured with a laser granulometer (Malvern, Mastersizer). The third subsample of each sediment core was sifted through 0.0625 mm nylon mesh sieve for chemical analysis. The mud fraction (< 0.0625 mm) was selected because contaminants tend to associate with sediment fine particles ([Jickells and Knap, 1984](#)).

### 2.4. Chemical analyses of *Z. marina* compartments and sediment

Trace element (TE) concentrations, major element concentrations including P, C and N and the stable isotope ratio values of C and N were measured (n = 1 pooled sample of three replicates) in [seagrass](#) compartments (leaves, roots and rhizomes) and sediment mud fraction of cores collected in Belyounech *Z. marina* meadow (carbon content and  $\delta^{13}C$  not measured in carbonated sediment). Seagrass sampling (n = 3) for chemical analysis was performed concomitantly to sediment core sampling in September 2015 from the same location. Leaves, [rhizomes](#) and roots were separated on the field, transferred into plastic bags and stored frozen until analysis. In the laboratory, seagrass material was processed the same way as described in section 2. Seagrass material was oven dried until constant weight (minimum 48 h at 60 °C). Dried tissues were ground into a fine powder with a Mixer Mill Grinder (MM200, Retsch GmbH; 30 Hz, 3 min).

Grinded seagrass compartments and sediment mud fraction were mineralized in Teflon bombs using a closed microwave digestion lab station (Ethos D, Milestone Inc.). Digestion procedure was performed using nitric acid and hydrogen peroxide ( $HNO_3/H_2O_2$ ) as reagents (suprapure grade, Merck). [Digestates](#) were diluted to a volume of 50 mL with ultra-pure water (18.2 M $\Omega$ /cm Milli-Q water) prior to being analysed by [Inductively Coupled Plasma Mass Spectrometry](#) using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer Inc.) ([Richir et al., 2013](#)). ICP-MS analysis included P, four other major elements: Na, Mg, K and Ca, and 22 TEs: Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U and Bi. Hg was measured in seagrass material powder and sediment mud fraction by atomic absorption spectrometry using a Direct Mercury Analyser (DMA 80, Milestone Inc.). The accuracy of analytical methods was checked by analyzing Certified Reference Materials (CRMs) PACS-2 (Marine sediment) for sediment and BCR 60 (*Lagarosiphon major*), BCR 61 (*Platihypnidium riparioides*), GBW 07,603 (bush branches and leaves) and V463 (corn) for plant. PACS-2 mean recovery was 78 % (there was no certified value for Ba, Ti, Bi and U; partial recovery because of strong acid partial digestion for bioavailable chemical elements only, no complete digestion of sediment grain matrix). Vegetal CRM mean recovery was 103 % (there was no certified value for Sn, Ti and U). Chemical element concentrations are expressed in mg. kg<sup>-1</sup><sub>DW</sub> of sediment or seagrass compartments, except P expressed in %<sub>DW</sub>.

The analysis of C and N contents and their stable isotope ratio values ( $\delta^{13}C$  and  $\delta^{15}N$ ) were conducted via continuous flow - elemental analysis - [isotope ratio mass spectrometry](#) (CF-EA-IRMS) using a vario MICRO cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to an

IsoPrime100 mass [spectrometer](#) (Isoprime, Cheadle, United Kingdom). Sucrose (IAEA-C6;  $\delta^{13}\text{C} = -10.8 \pm 0.5 \text{ ‰}$ ) and [ammonium sulfate](#) (IAEA-N<sub>2</sub>;  $\delta^{15}\text{N} = 20.3 \pm 0.2 \text{ ‰}$ ) were used as (CRMs). Both CRMs are calibrated against international isotopic references, i.e. the Vienna Pee Dee Belemnite (VPBD) for C and Atmospheric Air for N. The standard deviations of the multi-batch replicate measurements of lab standards (amphipods) as well as Glycine (Merck, Darmstadt, Germany) interspersed among the samples were 0.1 ‰ and 0.2 ‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. C and N data are expressed in ‰<sub>DW</sub> from elemental analyses. C:N and N:P ratios were determined on an atom basis.

## 2.5. Statistical analyses

Differences in measured parameters among stations (Belyounech and Oued El Mersa bays) and zones (center and edge of meadows) were analyzed using the student test and the two-way ANOVA test. Raw or log-transformed data were tested for residual normality and for homogeneity of variance to meet the assumptions for parametric statistics. Statistical analyses were performed in RStudio version 1.1.383 ([RStudio, 2016](#)), using R's base function (R Core Team, 2017).

## 3. Results

### 3.1. *Zostera marina* meadows and sediment description

The two *Zostera marina* meadows identified in Belyounech bay and Oued El Mersa bay during the mapping of the marine key habitats of Jbel Moussa spread over 9.54 ha and 29.19 ha respectively ([PNUE-PAM-CAR/ASP, 2016](#); [Fig. 1](#)). Belyounech *Z. marina* meadow cover was continuous without any noticeable depth effect on the cover from the center (13 m depth) to the edge (17 m depth). Conversely, Oued El Mersa meadow cover was highly fragmented with a clear depth gradient but also several trawling scars ([Fig. 2](#)). Seagrass density was significantly higher in Belyounech meadow ( $p < 0.05$ ) with  $745 \pm 183 \text{ shoots.m}^{-2}$  against  $680 \pm 78 \text{ shoots.m}^{-2}$  in Oued El Mersa meadow ([Table 1](#)). The mean total number of leaves per shoot was similar in both meadows ( $5.2 \text{ leaves.shoot}^{-1}$ , [Table 1](#)). In Oued El Mersa meadow, differentiated leaves were significantly longer ( $210 \pm 71 \text{ mm}$ ) and wider ( $3.8 \pm 0.6 \text{ mm}$ ) compared to Belyounech meadow ( $156 \pm 40 \text{ mm}$  length and  $3.4 \pm 0.5 \text{ mm}$  width;  $p < 0.05$ , [Table 1](#)). As a result, the shoot mean biomass was also significantly higher in Oued El Mersa meadow ( $p < 0.05$ ), with  $125 \pm 92 \text{ mg}_{\text{DW}}.\text{shoot}^{-1}$  compared to  $64 \pm 35 \text{ mg}_{\text{DW}}.\text{shoot}^{-1}$  in Belyounech meadow ([Table 1](#)). Roots were more developed in Oued El Mersa meadow, with a biomass of  $250 \pm 105 \text{ g}_{\text{DW}}.\text{m}^{-2}$  significantly higher than that of  $121 \pm 63 \text{ g}_{\text{DW}}.\text{m}^{-2}$  in Belyounech meadow. In Oued El Mersa meadow, seagrass leaves accounted for 46 % of the total biomass. In Belyounech meadow, leaf biomass exceeded root and [rhizome](#) biomasses and accounted for 60 % of the total biomass ([Fig. 3](#)). Accordingly, the [aboveground biomass](#) to [belowground biomass](#) ratio was higher in Belyounech meadow (1.5) in comparison to Oued El Mersa meadow (0.9). Regarding belowground compartments, root biomass was more important in Oued El Mersa meadow (82 % of total belowground biomass) than in Belyounech meadow (67 %). The two *Z. marina* meadows, distant a few km only ([Fig. 1](#)), clearly differ both in terms of meadow structure and seagrass shoot characteristics.



Fig. 2. Trawl scars observed in Oued El Mersa bay near the *Zostera marina* meadow. The dark area on the right picture is a *Zostera marina* patch.

Table 1. *Zostera marina* shoot density, biomass and morphometric measurements (mean  $\pm$  standard deviation, SD) at the center of Belyounech and Oued El Mersa seagrass meadows. Bold values significantly differed ( $p < 0.05$ ) between meadows.

Stations	Belyounech		Oued El mersa	
	Mean	SD	Mean	SD
Parameters				
Shoot density (shoots. $m^{-2}$ , n = 10)	745	183	<b>680</b>	78
Total number of leaves per shoot (leaves.shoot $^{-1}$ , n = 20)	5.2	0.9	5.2	1.2
Shoot height (mm, n = 20)	<b>179</b>	40	227	71
Length of differentiated leaves (mm, n = 20)	<b>156</b>	40	210	71
Width of differentiated leaves (mm, n = 20)	<b>3.4</b>	0.5	<b>3.8</b>	0.6
Length of undifferentiated leaves (mm, n = 20)	65	49	94	80
Width of undifferentiated leaves (mm, n = 20)	3.1	0.7	3.3	1.0
Shoot weight ( $g_{DW}$ , n = 20)	<b>64</b>	35	<b>125</b>	92
Leaf biomass ( $g_{DW} \cdot m^{-2}$ , n = 5)	273	40	260	26
Rhizome biomass ( $g_{DW} \cdot m^{-2}$ , n = 5)	61	21	54	20
Root biomass ( $g_{DW} \cdot m^{-2}$ , n = 5)	<b>121</b>	63	<b>250</b>	105
Total biomass ( $g_{DW} \cdot m^{-2}$ , n = 5)	454	104	563	113
Belowground biomass ( $g_{DW} \cdot m^{-2}$ , n = 5)	181	82	303	111
Leaf Area Index ( $m^2 \cdot m^{-2}$ , n = 20)	1.4	0.5	1.9	1.2

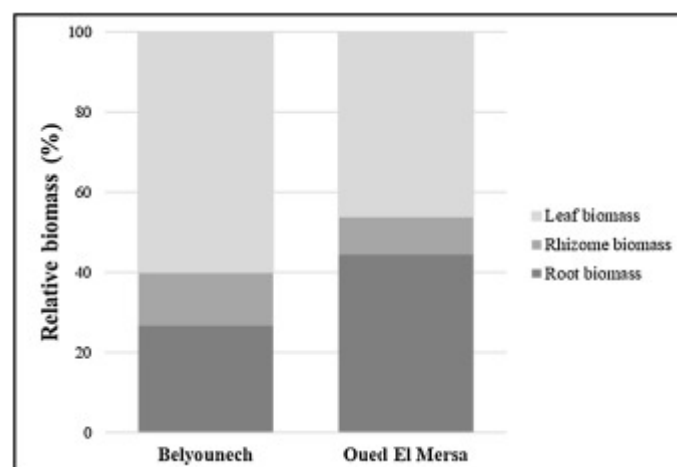


Fig. 3. Relative mean contribution (%) of leaves, rhizomes and roots to the total biomass of *Zostera marina* (n = 5) in the center of Belyounech and Oued El Mersa meadows.

The analysis of pictures of the permanent quadrats showed a *Z. marina* covering of 99.8 % in the center of both meadows and of 87.8 % and 89.6 % on the edges of Belyounech and Oued El Mersa meadows, respectively (Fig. 4). The invasive algae *Caulerpa cylindracea* was observed in both meadows. It was more abundant on the edge of Oued El Mersa meadow than Belyounech meadow, and almost absent in the center of both meadows except a few in Belyounech one (Fig. 4). For both macrophyte species, differences in cover were significant ( $p < 0.05$ ) between zones only (Supplementary material Table S1). We also reported the absence of epiphytic algae on *Z. marina* leaves at both stations.

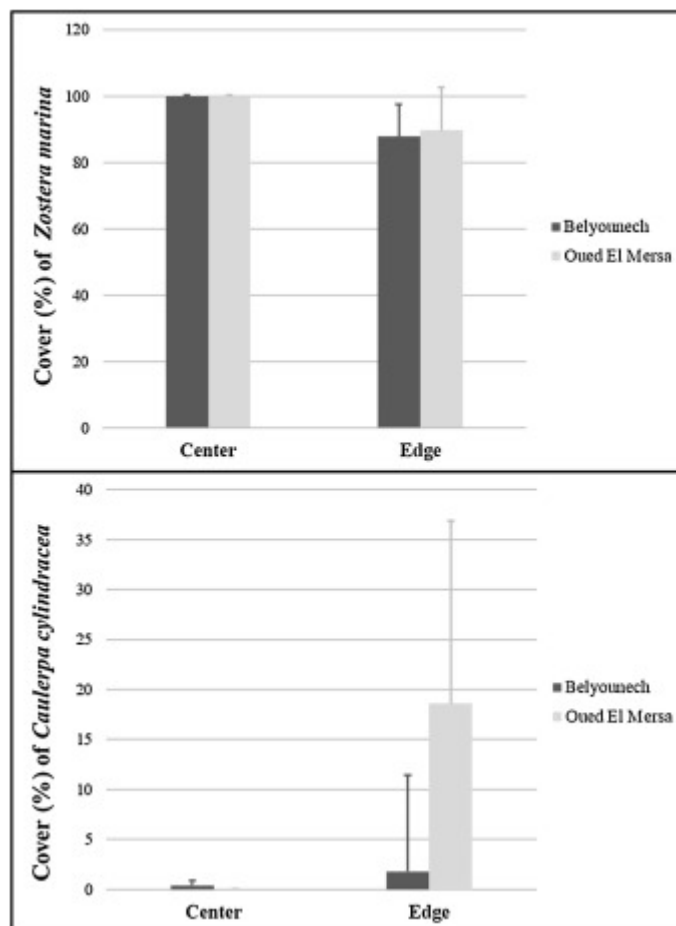


Fig. 4. *Zostera marina* and *Caulerpa cylindracea* percentage cover ( $n = 5$ ) in seagrass meadows of Belyounech bay and Oued El Mersa bay, in the center and on the edge of the meadows. Bars represent standard deviation.

The average organic matter content of sediment from Belyounech meadow was low and did not exceed 2.33 %. The sediment grain size varied from mud to very coarse sand, with 95.6 % of sand and 4.40 % of mud.

### 3.2. Chemical analyses of *Z. marina* compartments and sediment

Concentrations of major and TEs in sediment ( $< 0.0625$  mm) and in *Z. marina* compartments (leaves, roots and rhizomes) of Belyounech meadow as well as the concentration ratios between the seagrass compartments and with the sediment are given in Table 2. Most elements except a few were more concentrated in the sediment than in the seagrass compartments (the seagrass compartments over sediment concentration ratios were below 1 for Mg, Al, Ca, V, Cr, Fe, Mn, Co, Cu, Zn, Sr, Li, Ba, Ti, Pb, Bi and Hg). Conversely, Na, K, Mo, Ag and Cd were more concentrated in the seagrass compartments compared to the sediment, and Ni, As, Sn, Sb and U in roots only. Overall, chemical element concentrations decreased in *Z. marina* compartments in the following order: root > leaf > rhizome. Element distribution also differed between the three compartments of the seagrass (Table 2). Concentrations in the rhizosphere (i.e. rhizomes

and roots) ranged between 0.008 mg.kg<sup>-1</sup><sub>DW</sub> for Ni and 3.11 mg.kg<sup>-1</sup><sub>DW</sub> for K. Values of chemical element ratios rhizome/leaf varied appreciably from 0.73 for U to 6.91 for Ni; values of ratios leaf/root were the highest for K (3.52) and the lowest for Ni (0.05).

Table 2. Chemical element concentrations (in mg. kg<sup>-1</sup><sub>DW</sub>) and concentration ratios in the < 0.0625 mm sediment grain size fraction (n = 1 pooled sample) and in *Zostera marina* compartments (leaves, rhizomes, roots; n = 1 pooled sample) sampled in Belyounech bay meadow.

		Na	Mg	Al	K	Ca	V	Cr	Fe	Mn	Co
<b>Sediment</b>		15,400	23,100	18,400	4200	81,600	39.6	21.0	23,100	207	7.02
<i>Z. marina</i> compartments	<b>leaves</b>	68,100	10,400	498	33,000	8230	2.50	1.32	492	37.0	0.49
	<b>rhizomes</b>	61,100	8090	171	29,100	6410	1.10	0.32	285	6.00	0.12
	<b>roots</b>	47,100	12,800	6190	9370	29,700	21.0	7.86	6980	92.0	6.27
Ratios	<b>leaves/sediment</b>	4.42	0.45	0.03	7.86	0.10	0.06	0.06	0.02	0.18	0.07
	<b>rhizomes/sediment</b>	3.97	0.35	0.01	6.93	0.08	0.03	0.02	0.01	0.03	0.02
	<b>roots/sediment</b>	3.06	0.55	0.34	2.23	0.36	0.53	0.37	0.30	0.44	0.89
	<b>leaves/rhizomes</b>	1.11	1.29	2.91	1.13	1.28	2.27	4.13	1.73	6.17	4.08
	<b>leaves/roots</b>	1.45	0.81	0.08	3.52	0.28	0.12	0.17	0.07	0.40	0.08
	<b>rhizomes/roots</b>	1.30	0.63	0.03	3.11	0.22	0.05	0.04	0.04	0.07	0.02

Ni	Cu	Zn	Sr	Li	As	Mo	Ag	Cd	Sn	Sb	Ba	Tl	Pb	Bi	U	Hg
11.2	13.7	46.0	171	20.8	7.25	1.53	0.02	0.08	0.26	0.07	22.4	0.32	16.8	0.04	1.06	0.01
2.42	6.61	29.5	107	1.32	1.82	4.50	0.07	0.64	0.10	0.08	3.10	0.24	2.64	0.03	0.30	0.01
0.35	1.69	15.2	108	0.84	0.99	1.60	0.04	0.35	0.09	0.03	1.10	0.25	0.45	0.03	0.41	0.01
46.1	9.12	39.2	132	6.96	7.94	3.60	0.03	0.79	0.27	0.19	3.24	0.25	5.89	0.02	1.15	0.01
0.22	0.48	0.64	0.63	0.06	0.25	2.94	3.33	8.31	0.39	1.23	0.14	0.73	0.16	0.70	0.28	0.60
0.03	0.12	0.33	0.63	0.04	0.14	1.05	2.10	4.55	0.34	0.42	0.05	0.77	0.03	0.73	0.39	0.76
4.12	0.67	0.85	0.77	0.33	1.10	2.35	1.20	10.3	1.01	2.92	0.14	0.77	0.35	0.54	1.08	0.85
6.91	3.91	1.94	0.99	1.57	1.84	2.81	1.59	1.83	1.15	2.96	2.82	0.96	5.87	0.96	0.73	0.79
0.05	0.72	0.75	0.81	0.19	0.23	1.25	2.80	0.81	0.38	0.42	0.96	0.95	0.45	1.30	0.26	0.70
0.01	0.19	0.39	0.82	0.12	0.12	0.44	1.76	0.44	0.33	0.14	0.34	1.00	0.08	1.35	0.36	0.89

For elementary contents, *Z. marina* compartments from Belyounech meadow had higher N contents than sediment. Conversely, sediment was more enriched in P than *Z. marina* compartments and also showed a higher  $\delta^{15}\text{N}$  value. Organic C contents and  $\delta^{13}\text{C}$  values were respectively higher and more negative in leaves compared to rhizomes and mainly roots. The high C and low P and N contents in rhizomes resulted in higher C:N and C:P ratios than for leaves and roots. The N:P ratio was higher in leaves compared to belowground compartments ([Table 3](#)).

Table 3. Mean C and N contents (in %<sub>DW</sub>), their ratio (atom:atom) and stable isotope compositions (in ‰) in the < 0.0625 mm sediment grain size fraction (n = 1 pooled sample) and *Zostera marina* compartments (leaves, rhizomes, roots; n = 1 pooled sample) sampled in the center of Belyounech bay seagrass meadow.

	C	N	P	C:N	C:P	N:P	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<b>Sediment</b>	–	0.05	0.48	–	–	0.23	–	4.72
<b>leaves</b>	32.8	2.17	0.19	17.6	446	25.3	–12.3	2.30
<b><i>Z. marina</i> compartments</b>								
<b>rhizomes</b>	30.9	0.67	0.14	53.8	570	10.6	–11.7	1.88
<b>roots</b>	18.2	0.81	0.16	26.2	294	11.2	–11.4	2.84

#### **4. Discussion**

The decline in the extension of *Zostera marina* meadows has been observed in many localities around the Mediterranean ([Pergent et al., 2014](#)). That seagrass species remains rare and mainly occurs in intertidal and shallow subtidal lagoons and very sheltered bays ([Buia and Marzocchi, 1995](#)). Deep subtidal meadows occur at depths of 5–14 m in open bays of southern Spain in the Alboran Sea ([Rueda et al., 2008](#)). The observations of deep *Z. marina* meadows at Jbel Moussa, extending from 3 m to 17 m depth with patches extending down to 20 m depth, are probably related to the strong influence of Atlantic waters entering through the Strait of Gibraltar and the topography of the coast, as it has been pointed out for Spanish *Z. marina* meadows by [Arroyo et al. \(2006\)](#). Moroccan and Spanish *Z. marina* meadows further colonize deeper waters than in many other places worldwide where the species is present (Supplementary material Table S3).

Decreases in aboveground biomass and shoots density with increasing depth is a trend largely observed in *Z. marina* meadows ([Krause-Jensen et al., 2000](#); [Nielsen et al., 2002a](#); [Olesen et al., 2017](#)). However, lower values were measured in shallow Mediterranean waters at Grado (Italy, [Guidetti et al., 2002](#)) and Thau (France, [Laugier et al., 1999](#)) lagoons comparing to the deeper Jbel Moussa meadows. [Röhr et al. \(2018\)](#) studied *Z. marina* densities and biomasses of 54 sites in 13 countries worldwide. Leaf biomasses were much lower than measured in Moroccan meadows of Jbel Moussa and only meadows from Eastern Atlantic had slightly higher shoot densities. It has been documented that the large variability in abundance, i.e. shoot density and leaf biomass among *Z. marina* populations was affected by adaptation to local environmental factors ([Clausen et al., 2014](#); [Olesen et al., 2015](#)) such as hydrodynamism ([Krause-Jensen et al., 2003](#)), water clarity and transparency ([Krause-Jensen et al., 2005](#)), nutrients availability ([Harlin, 1993](#)), sediment characterization ([Koch, 2001](#)) or macroalgae load ([Kiparissis et al., 2011](#)). Favorable environmental conditions must therefore be wholly or partly met to enhance the exceptional development of *Z. marina* in Jbel Moussa.

The coverage of *Z. marina* at Jbel Moussa is reduced from the center to the edge of meadows, but its value is always higher than 80 %. According to [De-Jong et al. \(2004\)](#), seagrass meadows with a coverage exceeding 75 % and a high foliar coverage are considered as continuous. In Oued El Mersa bay, the meadow is fragmented by trawling scars. Bottom trawling, damaging seagrass roots and rhizomes, is

lethal to shoots and result in habitat loss ([Ardizzone et al., 2000](#)). Damage to seagrass leaves and sediment resuspension result in reduced light accessibility that can lead to a decrease of photosynthesis ([Ruiz and Romero, 2001](#)). This may explain partly the lower depth limit of Oued El Mersa meadow (10 m depth) comparing to Belyounech (17 m depth). Physical disturbances can further prevent or limit the recovery of damaged meadows and whole seagrass ecosystems. The experiment of [Valdemarsen et al. \(2010\)](#) showed that *Z. marina* seedlings exposed to physical disturbance, namely uprooting or burial through drifting macroalgae, sediment reworking and current-driven sediment resuspension encountered a threefold higher mortality rate than seedlings in unthatched zone. [Jennings et al. \(2001\)](#) measured a 75 % reduction in total faunal productivity between untrawled control sites and trawled areas. [Neckles et al. \(2005\)](#) reported that one year after the last trawl *Z. marina* shoot density and total biomass were reduced <1-3 % in comparison to the reference site. The authors estimated that an average of 10.6 years was required for *Z. marina* shoot density to match pre-trawling reference values.

In addition to trawling physical stresses, along Jbel Moussa meadows' edge that showed lower coverage than their center, the presence of *Caulerpa cylindracea* is a supplementary threat especially in Oued El Mersa bay where it covers up to 19 % of the seagrass meadow sediment. The non-indigenous seaweed *C. cylindracea* is as an important threat to the biodiversity in the Mediterranean ([Ceccherelli and Campo, 2002](#); [Ruitton et al., 2005](#)). When meadows are in bad ecological conditions, exotic invasive algae enter into competition with native seagrasses and affect the integrity of the structure of their natural communities and the functioning of the ecosystems ([Boudouresque et al., 2005, 2009](#); [Katsanevakis et al., 2010](#)). For instance, the mixed presence of seagrasses with *Caulerpa* spp. leads to: (i) the decrease of the number of leaves per seagrass shoot ([Dumay et al., 2002](#)); (ii) phytotoxicity against seagrass leaves causing chlorosis and necrosis ([Villèle Villèle and Verlaque, 1995](#)); (iii) interspecific competition for nutrient acquisition which may, in the long term, lead to nutrient shortage required for seagrass growth ([Ceccherelli and Cinelli, 1997](#)); (iv) deterioration of sediment quality by the increase of sulfate reduction rates and the enhancement of sulfide pools ([Holmer et al., 2009](#)) driving to high seagrass mortality ([Frederiksen et al., 2007](#)). No macro-effects of *C. cylindracea* presence on *Z. marina* have been observed in the two meadows of Jbel Moussa investigated in the present study. However, it has been documented that edges of dense and sparse meadows constitute very favourable environments for the development of this invasive species ([Didham et al., 2005](#); [Klein and Verlaque, 2008](#)) and that its development could in turn exacerbate the regression of seagrass meadows ([Montefalcone et al., 2010a](#); [Kiparissis et al., 2011](#)). The presence of *C. cylindracea* at the edge of *Z. marina* meadows of Jbel Moussa and observation in their center therefore requires regular monitoring of the algae presence and the vitality of the seagrass meadows.

Sediment OM content higher than 5–13 % can limit *Z. marina* growth ([Koch, 2001](#); [Krause-Jensen et al., 2011](#)). Therefore, the low OM content in Belyounech bay sediment (2.3 %) could contribute to the ability of *Z. marina* to expand into deeper water depths. Major and TE contents in Belyounech bay sediment (mud) were generally lower than reported in other studies (Supplementary material Table S4). In comparison to the Mediterranean Moroccan Nador lagoon, concentrations of Cd, Cr, Cu, Ni, Pb and Zn in Belyounech bay were similar to uncontaminated stations in this lagoon ([Maanan et al., 2014](#)). TE concentrations are comparable to levels considered as little or not enriched for semi-enclosed water bodies along the Atlantic coast of Morocco ([Boutahar et al., 2019](#)). Regarding the vegetated part, the accumulation of the studied chemicals in *Z. marina* compartments was mostly the result of root uptake (except Mo, Ag, Cd, Na and K), followed by leaves then rhizomes. Additionally, element transition from roots to rhizomes and leaves can be considered low because of the net difference in chemical accumulation between compartments. The compartmentalization leading to preferential accumulation of chemicals in roots could be a tolerance adaptation developed by the seagrass. This compartmentalization strategy in underground organs is a defensive mechanism to protect photosynthetic leaves against toxic effects of chemicals ([Gratao et al., 2005](#); [Willis et al., 2010](#)).

Our results are also in agreement with previous studies on other seagrasses species reporting that roots store generally highest concentrations of toxic elements ([Malea and Kevrekidis, 2013](#); [Richir et al., 2013](#); [Bonanno and Vymazal, 2017](#)). Studies that have reported levels of TEs in *Z. marina* in the Mediterranean are scarce, but several ones elsewhere in the world considered this species an accumulator of TEs ([Lewis and Devereux, 2009](#)). A comparison of TE concentrations in *Z. marina* from Jbel Moussa with other geographical areas is given in Supplementary material (Supplementary material Table S4). In the Thu

lagoon, Fe and Cr were also more accumulated in roots while Ni, Pb and Cu accumulated equally in roots and leaves (De Casabianca et al., 2004). In Bosphorus Strait, TE levels were higher in sediment than in macrophytes, and higher in *Z. marina* than in algae (Güven et al., 1993). Ferrat et al. (2012) reported that *Z. marina* accumulated significantly higher levels of TEs when growing on contaminant-impacted sites. These authors further reported that the accumulation of TEs was higher in below-ground compartments compared to the above-ground ones, with low transport from roots to leaves. These observations are in agreement with our results. Similar observations were also reported for seagrasses sampled from Yaquina Bay, Oregon, USA (Kaldy, 2006a). TE tissue speciation in *Z. marina* has been reported to be linked to the local characteristics of the sampling environment and TE bioavailability (De Casabianca et al., 2004), and to metabolic processes and the age of the plant (Marcías-Zamora et al., 2008).

Concentrations of N and P in *Z. marina* from Jbel Moussa were higher in leaves than in belowground compartments. This result is consistent with several studies (Pedersen and Borum, 1993, 1995; Rigollet et al., 1998; Kaldy, 2006a, b). Nutrient accumulation in *Z. marina* relies primarily on their availability in the aquatic environment (Fourqurean et al., 1992; Erftemeijer et al., 1994; Alexandre et al., 2015). According to Gerloff (1975), P can be expected to be a limiting nutrient when the leaf concentration is below 0.07 %<sub>DW</sub>; in the present study P, concentrations in *Z. marina* compartments were above 0.10 %<sub>DW</sub> (Table 3). Duarte (1990) reported that seagrass growth is N-limited when N concentration in leaves is below 1.80 %<sub>DW</sub>; in the present study, N concentrations were above that threshold. The N:P ratio reflects the overall nutrient availability to the plant; it reached 25 in leaves. N and P are thus sufficiently available in the surrounding environment. However, belowground compartments with N:P ratio values close to 11 suggested a potential N limitation (Atkinson and Smith, 1983; Abal et al., 1994; Grice et al., 1996). N levels in *Z. marina* belowground compartments from Belyounech bay meadow (0.74 %<sub>DW</sub>, average of root and rhizome values) are low when compared to other places (Supplementary material Table S5). Kim et al. (2012) reported a seasonal variation of N levels in *Z. marina* belowground compartment, with levels ranging from 5.50 %<sub>DW</sub> in winter to 0.50 %<sub>DW</sub> in summer and spring. However, the primary production of seagrass compartments can be high despite low N concentration in the plant (Pedersen and Borum, 1992; Gobert et al., 2002; Cabaco et al., 2013). The high *Z. marina* abundance and biomass measured in the Jbel Moussa meadow could therefore explain the nutrient depletion in the seagrass belowground compartments. In addition, the low level of mud in Belyounech bay sediment does not promote high levels of nutrients in porewater (Kenworthy et al., 1982; Huettel and Rusch, 2000). The low *Z. marina*  $\delta^{15}\text{N}$ , comparable to  $\delta^{15}\text{N}$  values measured along the Algerian coast in the seagrass *Posidonia oceanica* (Linnaeus) Delile (Belbachir et al., 2019), are compatible with the relatively low N content found in *Z. marina* leaves.  $\delta^{15}\text{N}$  values do not indicate any significant uptake of anthropogenic N by *Z. marina*. Indeed, anthropogenic inputs of N to the environment is often associated to elevated  $\delta^{15}\text{N}$  values in seagrass tissues (Lassauque et al., 2010).

The Belyounech bay water transparency increases light availability and can explain the deep lower limit of the meadow (Nielsen et al., 2002a, 2002b). Irradiance is considered the most important controlling factor of *Z. marina* shoot morphology, abundance and productivity (Duarte, 1991; Ralph et al., 2007). Decrease in water transparency and therefore low bottom irradiance in other Mediterranean coastal lagoons (Laugier et al., 1999; Guidetti, 2000) and in the Atlantic (Hily et al., 2003) and Pacific Oceans (Kim and Choi, 2004) may explain the occurrence of *Z. marina* with low density, low leaf biomass and shallow lower limit in comparison to Jbel Moussa. Temperature is also a relevant factor driving *Z. marina* abundance variations (Zimmerman et al., 1995). Higher leaf production rates were observed in temperate *Z. marina* beds in Spain (Marbà et al., 1996) compared to coldest regions in Greenland, suggesting that low temperatures limit production rates (Olesen et al., 2015). On the other hand, temperature difference between minimum and maximum monthly values in shallower Mediterranean *Z. marina* meadows are much greater (up to 19 °C) (Laugier et al., 1999; Guidetti, 2000) than in Gibraltar region (up to 9 °C; Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, 2019), suggesting that stable temperature throughout the year would favour seagrass meadows present in Jbel Moussa area.

All together, the environmental information collected in the *Z. marina* meadow of Belyounech bay indicates a low level of contamination by TEs and no inputs of land-derived organic matter and nutrients. These favourable conditions contribute to the overall good physiological, morphological and

biochemical state of *Z. marina* and to the high shoot density of the meadow (Cabaço et al., 2013). It has been documented that depth limit of *Z. marina* meadows is a useful bioindicator of water quality, mainly because depth limit, being largely regulated by light availability, responds predictably to eutrophication (Krause-Jensen et al., 2005). In the Water Framework Directive guidelines, the general criteria adopted in the Mediterranean and Baltic Seas to define a reference coastal area are: (i) no settlement with more than 1000 inhabitants.km<sup>-2</sup> in the next 15 km and/or more than 100 inhabitants.km<sup>-2</sup> in the next 3 km within that area (winter population); (ii) no more than 10 % of artificial coastline; (iii) no harbour (with more than 100 boats) within 3 km; (iv) no beach regeneration within 1 km; (v) no industries within 3 km; (vi) no fish farms and desalination plants within 1 km; (vii) no evidence of sensitive macrophyte regression due to other unconsidered impacts (e.g. overgrazing) (Carletti and Heiskanen, 2009; Alcoverro et al., 2013; Berov et al., 2018). Based on the above criteria, together with the environmental quality data collected in the framework of this study, we can consider that the *Z. marina* meadows of Jbel Moussa not only show a good ecological quality state but can also be considered as reference meadows and surely would require specific protective and assessment measures taken by competent authorities.

The present study describes the good health status of Jbel Moussa *Z. marina* meadows, but also highlights biological and mechanical threats, i.e. *C. cylindracea* and trawling that together could induce their regression. Another factor that may stress seagrasses is high summer temperatures above physiological critical thresholds. Moroccan *Z. marina* meadows are localized within the warm temperate-southern limit of the species, where high summer temperature is considered to set its biogeographical boundary. The average thermal amplitude of the area ranges between 14 °C and 23 °C. Several studies have reported that high water temperatures above 25 °C markedly affected *Z. marina* meadows by decreasing its growth and increasing shoot mortality (Sfriso and Ghetti, 1998; Moore and Jarvis, 2008; Bergmann et al., 2010; Lefcheck et al., 2017). The increase of water temperature summer maxima and the increase of heatwaves are therefore environmental threats that might affect greatly the perennity of Jbel Moussa *Z. marina* meadows.

## **5. Conclusion**

This study provides a first synthesis of knowledge on the environmental health status of the deepest *Zostera marina* meadows reported for the Mediterranean. Although results of this study on the health status of the meadows are positive, listed threats warn environmental managers and policy makers of the need to implement conservation measures as a major priority, considering the international commitments to the Convention on Biological Diversity to ensure the prevalence of this vulnerable ecosystem over time. The on-going designation of Jbel Moussa as protected area is a valuable proxy to the conservation and management of key marine habitats of that area. The implementation of permanent quadrats in the Jbel Moussa meadows with fixation of temperature data loggers in their vicinity will enable to continue their monitoring over time while at the same time measuring changes in environmental conditions. Take action is necessary, first against trawling to prevent degradation of these meadows. Fishers must be involved in the process to reach effective protection measures and perpetuate the nursery function for fish of these habitats. The declaration of this area as Critically Vulnerable Coastal Area and the implementation of strictly followed legislation will help in the reduction of threats and prevent further degradation of the meadows.

## **CRedit authorship contribution statement**

**Boutahar Loubna:** Conceptualization, Methodology, Investigation, Software, Writing. **Espinosa Free:** Conceptualization, Methodology, Review & Editing, Supervision. **Richir Jonathan:** Review & Editing, Validation. **Lepoint Gilles:** Resources, Validation. **Gobert Sylvie:** Resources, Validation. **Maanan Mohamed:** Resources, Validation. **Bazairi Hocein:** Conceptualization, Methodology, Review & Editing, Supervision, Project administration.

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