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Moroccan Seagrass Ecosystems:
Multi-proxy Analysis and Conservation Implications.

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FOREWORD

This study was undergone in the framework of a cotutelle agreement between the Faculty of Sciences of the University Mohammed V in Morocco, and the Faculty of Biology of the University of Seville in Spain. It was partially funded by Specially Protected Areas-Regional Activity Centre (SPA/RAC), Tunisia and the association "Action Bleu Maroc", domiciled at the Faculty of Sciences of Rabat. The thesis also benefited from a mobility grant from the University of Seville for a scientific stay at the Marine Biology Laboratory of the Faculty of Biology of Seville. It was also supported financially by the University of Nantes in France, the University of Liege in Belgium, and the University Mohammed V in Rabat for trace elements, stable isotopes and elemental content analysis.

SUMMARY

Seagrasses are valuable ecosystem service providers playing a major role in the structure and functioning of costal and marine environments. They support fisheries production, aquatic biodiversity refuge, climate change mitigation, erosion limitation, water oxygenation, and nutrient and pollutants load moderation. Yet, their accelerating loss rates due to direct and indirect human activities suggest a global crisis compromising the bundle of their vital benefits. There is strong incentive within the conservation movement to flatten the curve of seagrass decline and protect this keystone component of coastal environments throughout the world. Primary challenges for seagrass conservation consist in informing on their status and increase recognition of their importance. Although understanding of the functioning of seagrass ecosystems, their services and the way they respond to stressors has improved over the last years, they are unknown and underappreciated in Morocco with an overlooked status within the conservation plans.

The present thesis global objective was, (i) to investigate the potential use of *Zostera noltei* Hornemann and *Cymodocea nodosa* (Ucria) Ascherson in the Moroccan costal monitoring programs of trace element contamination, and (ii) to provide the first in-depth characterization of *C. nodosa* at Al Hoceima National Park and *Zostera marina* Linnaeus at Jbel Moussa with an insight into proactive measures for seagrass management and conservation.

The assessment of *Z. noltei* seagrass leaves accumulation capacity of 23 trace elements along the Atlantic latitudinal climatic gradient (Moulay Bousselham lagoon, Sidi Moussa lagoon, Oualidia lagoon, Khnifiss lagoon and Dakhla bay) revealed that *Z. noltei* leaves are a useful bioindicator of Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination in sediments. From Marchica lagoon, the only lagoon on the Mediterranean coast, *C. nodosa* leaves and roots performed equally as good bioindicators of Cu and Cd in sediment whereas leaves are considered as the best bioindicator for Zn contamination and roots for Pb load.

The structural development and the population dynamics of *C. nodosa* in Al Hoceima National Park detected a net regression of the meadows that were at the last rang values of growth parameters recorded elsewhere. At Jbel Moussa, the status of the deepest *Z. marina* meadows in the Mediterranean (lower limit at -17 m in depth with patches extending up to -20 m), shifted over only four years, from the good physiological, morphological and biochemical state to a dramatically total destruction by the combined effect of warming, trawling, and invasion by *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim. This habitat change induced a significant extinction of soft bottom amphipods communities up to 70% of the total variation.

The present work provide a proof of evidence of seagrass meadows fundamental role in supporting the costal environment health, and strengthen their widespread degradation and loss. It represents an essential synthesis to create awareness among the national managers, policymakers and the public about the importance of seagrasses to include them in the conservation agenda and management plans before that they disappear without even to be reported in our coast.

Keywords: Seagrass meadows description, transitional water biomonitoring, conservation measures, Morocco.

RESUME

Les herbiers à phanérogames marine procurent de précieux services écosystémiques et jouent un rôle clé dans la structuration et le fonctionnement des environnements côtiers et marins. Ils soutiennent la production halieutique ainsi que l'atténuation du changement climatique, mais aussi fournissent des refuges pour la biodiversité aquatique, limitent l'érosion, participent dans l'oxygénation des eaux et aussi la modération de la charge en nutriments et en polluants. Néanmoins, leur taux de disparition accéléré, dû aux activités humaines directes et indirectes, suggère une crise globale compromettant l'ensemble de leurs bénéfices vitaux. Des efforts de conservation vigoureux sont déployés dans le monde entier afin d'infléchir la courbe du déclin et protéger cette composante cruciale de l'environnement côtier et marin. Les principaux défis pour la conservation de ces écosystèmes consistent à informer sur leur statut et à sensibiliser sur leur importance. Bien que la compréhension de leur fonctionnement, leurs services écologiques et leurs réponses aux perturbateurs soit améliorée au cours des dernières années, ils demeurent méconnus et sous-estimés au Maroc avec un statut négligé dans les plans de conservation.

L'objectif global de la présente thèse était (i) d'étudier l'utilisation potentielle de *Zostera noltei* Hornemann et *Cymodocea nodosa* (Ucria) Ascherson dans les programmes de surveillance marocains de la contamination par les éléments traces, et (ii) de fournir la première caractérisation approfondie de *C. nodosa* au Parc National d'Al Hoceima et de *Zostera marina* Linnaeus à Jbel Moussa, avec un aperçu sur les mesures proactives pour leur gestion et conservation.

L'évaluation de la capacité d'accumulation de 23 éléments traces par les feuilles de *Z. noltei* au niveau de cinq écosystèmes semi-fermés le long du gradient climatique latitudinal de l'Atlantique marocain (lagune de Moulay Bousselham, lagune de Sidi Moussa, lagune de Oualidia, lagune de Khnifiss et baie de Dakhla) a révélé que les feuilles de *Z. noltei* sont un bioindicateur efficace de la contamination des sédiments par Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba et Hg. Dans la lagune de Marchica, la seule lagune de la côte méditerranéenne du Maroc, les feuilles et les racines de *C. nodosa* ont montré une performance similaire en tant que bioindicateurs du Cu et du Cd dans les sédiments, tandis que les feuilles sont considérées comme le meilleur bioindicateur de la contamination par le Zn et les racines pour la charge en Pb.

L'étude du développement structurel et de la dynamique des populations de *C. nodosa* dans le Parc National d'Al Hoceima a révélé une nette régression de ces herbiers, avec des valeurs de croissance très faibles en comparaison avec d'autres régions. A Jbel Moussa, le statut des herbiers de *Z. marina*, les plus profonds de la Méditerranée (limite inférieure à -17 m de profondeur avec des taches s'étendant jusqu'à -20 m), a changé en seulement quatre ans, passant d'un bon état physiologique, morphologique et biochimique à une destruction totale et dramatique sous l'effet combiné du réchauffement, du chalutage et de l'invasion par *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim. Ce changement d'habitat a entraîné une extinction significative des communautés d'amphipodes allant jusqu'à 70% de la variation totale.

Le présent travail confirme le rôle fondamental des herbiers marins dans le maintien du bon état de santé de l'environnement côtier et témoigne de leur vaste dégradation et disparition. Il représente une synthèse de base pour sensibiliser les gestionnaires, les décideurs politiques et le grand public à l'importance des herbiers marins afin de les intégrer dans les plans de gestion et conservation avant qu'ils ne disparaissent sans même être signalés sur nos côtes.

Mots-clés : Herbiers de phanérogames marines, biosurveillance des eaux de transition, mesures de conservation, Maroc.

RESUMEN

Las praderas de fanerógamas marinas proporcionan una gran cantidad de servicios ecosistémicos y juegan un papel clave en la estructura y funcionamiento de los ecosistemas costeros. Promueven la producción de recursos pesqueros, sirven de refugio para la biodiversidad, mitigan el cambio climático, limitan la erosión costera, contribuyen a la oxigenación del agua y a reducir los niveles de nutrientes y contaminantes. La elevada tasa de pérdida que han sufrido, directa o indirectamente causadas por actividades humanas, es indicadora de una crisis global que compromete los beneficios esenciales que ofrecen este tipo de ecosistemas. Hay una determinación dentro del movimiento conservacionista para revertir la tendencia de pérdida y proteger estos elementos clave de los ambientes costeros a lo largo del mundo. Los desafíos principales para la conservación de las praderas marinas son informar sobre su estatus de conservación y potenciar el reconocimiento de su importancia. Aunque el conocimiento del funcionamiento de estos ecosistemas, los servicios que proporcionan y la forma cómo responden a determinados estresores ha aumentado en los últimos años, aún son desconocidos y poco valorados en Marruecos, soslayando su estatus e importancia en los planes de conservación y gestión.

El objetivo principal de esta tesis ha sido, (i) investigar el uso potencial de *Zostera noltei* Hornemann y *Cymodocea nodosa* (Ucria) Ascherson en los programas de monitorización costera de diversos contaminantes en Marruecos, y (ii) proporcionar la primera caracterización detallada de *C. nodosa* en el Parque Nacional de Alhucemas y de *Zostera marina* Linnaeus en la zona de Jbel Moussa, con atención a medidas proactivas para la gestión y conservación de las praderas.

El análisis de la capacidad de acumulación de 23 elementos traza en las hojas de *Z. noltei* a lo largo de un gradiente latitudinal (laguna de Moulay Bousselham, laguna Sidi Moussa, laguna Oualidia, laguna Khnifiss y bahía de Dakhla) indicaron que las hojas de *Z. noltei* son bioindicadores útiles de contaminación por Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba y Hg en sedimentos. En la laguna de Marchica, la única laguna costera de la costa mediterránea marroquí, las hojas y raíces de *C. nodosa* también mostraron ser buenos bioindicadores de Cu y Cd en sedimento, mientras que las hojas se consideraron mejores bioindicadores de la contaminación por Zn, y las raíces de contaminación por Pb.

El desarrollo estructural y la dinámica poblacional de *C. nodosa* en el Parque Nacional de Alhucemas indicaron una regresión neta de las praderas estudiadas, que mostraron un crecimiento dentro del rango más bajo registrado a nivel mundial. En Jbel Moussa, el estatus de la pradera más profunda de *Z. marina* registrada en el Mediterráneo (límite inferior a 17 m con parches extendiéndose hasta los 20 m), varió en tan solo cuatro años desde un buen estado fisiológico, morfológico y bioquímico hasta una destrucción total debido al posible efecto combinado del calentamiento global, la pesca de arrastre y la invasión por el alga *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee y H.S. Kim. Este cambio en el hábitat provocó una extinción significativa de las comunidades de anfípodos del sedimento (hasta un 70% de variación total).

El presente trabajo proporciona una evidencia del papel que juegan las praderas de fanerógamas en mantener la salud de los ecosistemas costeros y del impacto de su pérdida y degradación. Representa también una síntesis relevante para generar conciencia entre los gestores nacionales, los políticos y el público sobre la importancia de las praderas, con el objetivo de incluirlas en la agenda de conservación y en los planes de gestión antes que desaparezcan, en algunos casos sin haber sido siquiera estudiadas o detectadas.

Palabras clave: descripción de praderas de fanerógamas, biomonitoreo de aguas de transición, medidas de conservación, Marruecos.

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GENERAL INTRODUCTION

Seagrasses are marine flowering plants, which form extensive meadows on the coasts of all continents except Antarctica (Green and Short, 2003). Because of there key ecological services in supporting the environment health and human wellbeing (Nordlund et al., 2018), seagrass meadows rank amongst the most valuable ecosystems in the biosphere with an estimated value of $1.9 trillion per year (Costanza et al., 2014). Besides, seagrasses are underappreciated compared to colourful coral reefs and mighty mangroves (Unsworth et al., 2019). However, their ecosystem service value has been estimated to be three times more than coral reefs and ten times more than tropical forests (Nordlund et al., 2016; Short et al., 2018).

Seagrass meadows are of fundamental importance to global food security supporting 20% of the world's biggest fisheries (Unsworth et al., 2018) with a total value of at least €200 million per year in the Mediterranean alone (Jackson et al. 2015). Even though they occupy less than 0.2 % of the total ocean area, they potentially store 48–112 Tg carbon per year (Mcleod et al., 2011) with an economic value up to $13.7 billion per annum (Pendleton et al., 2012). This carbon sequestration and storage capacity is suggested to contribute to the mitigation of the acute threats posed by global warming (Mazarrasa et al., 2018). Moreover, seagrass meadows provide multiple other ecosystem services including support rich biodiversity (Wong, 2018), coastal protection from erosion, flooding and storm surges (Paul, 2018), improve water quality by filtering nutrients and pollutants (Sandoval-Gil et al., 2016; Bonanno and Orlando-Bonaca, 2017) amongst others.

Despite their extensive ecosystem services, seagrass meadows are among the most threatened habitats on the planet. It has been estimated that 30% of the known seagrass areal extent disappeared since seagrass areas were initially recorded in 1879 (Waycott et al., 2009). Human disturbances such as coastal development, eutrophication, pollution and physical destruction by dredging, and anchoring, play a key role in the loss of seagrasses (Ralph et al., 2006; Salinas et al., 2020). In addition, climate change is a growing concern as rising sea levels and increasing ocean temperature may cause future seagrass losses (Fortes et al., 2018).

Faced with this situation, and in order to guarantee the seagrass meadows future sustainability, the setting up of monitoring surveys based on exhaustive characterization of natural populations has been seen, therefore, as a priority to implement proactive management and conservation measures (Unsworth et al., 2019). Although understanding of the way in which seagrass ecosystems function, the services they provide, and the way they respond to stressors improved over the last years, major gaps exist for West Africa including basic ecological and distributional knowledge (McKenzie et al., 2020).

In Morocco, there are knowledge gaps with regards to seagrass ecosystems. Unlike macroalgae, seagrasses are unknown and underappreciated. Historical reports are often limited to indicating their presence without any quantitative or qualitative information. To create greater awareness among the national managers, policymakers and the public of the seagrass meadows importance, clearer arguments about their benefits are warranted. Considering the international commitments to the Convention on Biological Diversity, major concerns have been risen with regards to assess the environmental state of costal ecosystems to ensure their prevalence over time. Monitoring investigations of the Moroccan Atlantic and Mediterranean coasts based on sediment and water quality, have documented significant threats due to the chemical contamination notably by trace elements (Bloundi, 2005; Cheggour et al., 2001; Maanan et al., 2014; Ben Omar et al., 2015; Zidane et al., 2017). However, the approach used cannot afford the compelling evidence of the possible toxicity of such contaminants on the biodiversity (Zhou et al., 2008). Seagrasses are worldwide recognized as sensitive bioindicator of coastal pollution and early sentinels of contamination transfer through the food web with consequent toxic risk at higher trophic level consumers up to humans (Govers et al., 2014; Bonanno et al., 2017). Moreover, they have been listed as one of the five biological quality elements to assess marine waters within the European Union water framework directive (Marba et al., 2012).

The global objective of the present study was therefore (i) to investigate the potential use of seagrasses in the Moroccan national monitoring programs of trace element contamination, and (ii) to provide the first deep characterization of seagrass meadows at two locations on the Moroccan Mediteranean coast to outline steps necessary for effective and proactive management and conservation initiatives.

More precisely, after a short description of seagrass state of knowledge worldwide and in Morocco **(chapter 1)**, we assessed the suitability of *Zostera noltei* Hornemann leaves as a bioindicator of trace element contamination in five semi-enclosed coastal ecosystems that are submitted to different anthropogenic pressures (urbanism, agriculture, industries and mining) along the full latitudinal climatic gradient (Mediterranean, semi-arid and arid climate) of the Moroccan Atlantic coast **(chapter 2)**. From the only lagoon on the Mediterranean coast of Morocco, we measured levels of trace elements of emerging concern in *Cymodocea nodosa* (Ucria) Ascherson tissues (leaves, rhizomes and roots), the dominant seagrass in the Marchica lagoon, and we provided a deep investigation on trace element extent in the associated sediment **(chapter 3)**. At the National Park of Al Hoceima, the assessment of the ecological quality state and the population dynamics using the reconstructing technic of two *C. nodosa* meadows was made by investigating the meadows density and biomass, the plant morphometric characteristics, the rhizome growth, the population age structure and derived population dynamics (recruitment and mortality) **(chapter 4)**. To assess the environmental status of *Zostera marina* Linnaeus meadows at Jbel Moussa (southern coasts of the Strait of Gibraltar), the only remaining ones along Mediterranean coasts of Morocco, or even of North Africa, and the deepest ones in the entire Mediterranean (lower limit at 17 m depth with patches extending up to 20 m), a series of ecological and biochemical descriptors were investigated (area extent, depth limit, density metrics, shoot morphometry and biomass, associated amphipods crustaceans species, major and trace element concentrations, carbon and nitrogen contents and the presence of alien species) **(chapter 5)**. The document ends by a general conclusion in which the main outcomes of the present study are recalled.

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CHAPTER 1 - SEAGRASS LITERATURE REVIEW

Abstract

Seagrasses are submerged aquatic vegetation that forms underwater meadows in nearshore marine environments of most continents around the world. Their complex structure creates highly productive and biologically rich habitats. They provide important ecological and economic services that greatly contribute to human well-being and the health of the world's coastal ecosystems. However, there is widespread evidence that this marine heritage is declining at an alarming rate, along with the services they supply. In Morocco, there is a significant lack of knowledge on the distribution, structure, and ecological status of seagrass ecosystems, which remains neglected in conservation agendas.

Keywords: Seagrasses, ecosystem services, worldwide decline, Morocco, knowledge gaps.

1. Seagrass distribution worldwide

Seagrasses are a unique group of monocotyledonous angiosperms that recolonized the marine environment 60–90 million years ago (Les et al., 1997). Ascherson (1871) was probably the first to use the term “seagrass” to refer to marine plants in the scientific literature (Kuo and Hartog, 2001). Consisting of a small number of species (72) seagrasses have developed adaptive characteristics to the submerged marine lifestyle which has influenced their speciation and worldwide geographic distribution (Fig. 1, Olsen et al., 2016). They form diverse meadows across thousands of kilometres shorelines of 103 countries/territories with estimated cover between 160,387 km^2 and 266,562 km^2 globally (McKenzie et al., 2020). Seagrass meadows extent is estimated to be higher than mangrove and kelp forests, but marginally lower than coral reefs. Unlike these habitats, a key feature of seagrass is their occurrence also into temperate and polar latitudes (McKenzie et al., 2020).

Fig. 1: Seagrass distribution worldwide (from Short et al., 2018).

2. Taxonomy, structure and reproduction of seagrasses

Seagrasses are represented by 13 genera belonging to six families: Zosteraceae (two genera), Posidoniaceae (one genus), Cymodoceaceae (five genera), Ruppiaceae (one genus), Zannichelliaceae (one genus), and three genera in the family Hydrocharitaceae (Den Hartog and Kuo, 2007; Short et al., 2018). Around 50% of species are found within just three genera: Halophila, Zostera and Posidonia (Table 1).

Table 1: List of the seagrass species of the world (from Den Hartog and Kuo, 2007 and Short et al.,

A List of the Seagrass Species of the World

Zosteraceae

Zostera Linnaeus
Zostera subgenus Zostera
1. *Zostera marina* Linnaeus
2. *Zostera caespitosa* Miki
3. *Zostera caulescens* Miki
4. *Zostera asiatica* Miki
5. *Zostera tasmanica* G.Martens ex Ascherson, 1868
6. *Zostera polychlamys* (J.Kuo) S.W.L.Jacobs & D.H.Les, 2009
7. *Zostera nigricaulis* (J.Kuo) S.W.L.Jacobs & D.H.Les, 2009
8. *Zostera chilensis (J.Kuo)* S.W.L.Jacobs & D.H.Les, 2009

Zostera subgenus Zosterella (Ascherson) Ostenfeld
9. *Zostera noltii* Hornemann
10. *Zostera japonica* Ascherson et Graebner
11. *Zostera capensis* Setchell
12. *Zostera capricorni* Ascherson
13. *Zostera muelleri* Irmisch ex Ascherson
14. *Zostera mucronata* den Hartog
15. *Zostera novazelandica* Setchell

Phyllospadix W.J. Hooker
16. *Phyllospadix scouleri* W.J. Hooker
17. *Phyllospadix torreyi* S. Watson
18. *Phyllospadix serrulatus* Ruprecht ex Ascherson
19. *Phyllospadix iwatensis* Makino
20. *Phyllospadix japonicus* Makino

Cymodoceaceae

Halodule Endlicher
21. *Halodule uninervis* (Forssk*al) Ascherson
22. *Halodule beaudettei* (den Hartog) den Hartog
23. *Halodule wrightii* Ascherson
24. *Halodule bermudensis* den Hartog
25. *Halodule ciliata* den Hartog
26. *Halodule pinifolia* (Miki) den Hartog
27. *Halodule emarginata* den Hartog

Cymodocea Konig in Konig et Sims
28. *Cymodocea nodosa* (Ucria) Ascherson
29. *Cymodocea rotundata* Ehrenberg et Hemprich ex Ascherson
30. *Cymodocea serrulata* (R. Brown) Ascherson et Magnus
31. *Cymodocea angustata* Ostenfeld

Syringodium Kutzing in Hohenacker
32. *Syringodium filiforme* Kutzing in Hohenacker
33. *Syringodium isoetifolium* (Ascherson) Dandy

Thalassodendron den Hartog
34. *Thalassodendron ciliatum* (Forssk* al) den Hartog
35. *Thalassodendron pachyrhizum* den Hartog

Amphibolis C. Agardh
36. *Amphibolis antarctica* (Labillardiere) Sonder et Ascherson
37. *Amphibolis griffithii* (J.M. Black) den Hartog

Posidoniaceae

Posidonia
38. *Posidonia oceanica* (Linnaeus) Delile
39. *Posidonia australis* J.D. Hooker
40. *Posidonia sinuosa* Cambridge et Kuo
41. *Posidonia angustifolia* Cambridge et Kuo
42. *Posidonia ostenfeldii* den Hartog
43. *Posidonia robertsoniae* Kuo et Cambridge
44. *Posidonia coriacea* Cambridge et Kuo
45. *Posidonia denhartogii* Kuo et Cambridge
46. *Posidonia kirkmanii* Kuo et Cambridge

Hydrocharitaceae

Vallisnerioideae
Enhalus L.C. Richard
47. *Enhalus acoroides* (Linnaeus *f.*) Royle

Thalassioideae
Thalassia Banks ex König in König et Sims
48. *Thalassia hemprichii* (Ehrenberg) Ascherson in Petermann
49. *Thalassia testudinum* Banks ex König in König et Sims

Halophiloideae
Halophila Du Petit Thouars
Halophila sect. **Halophila**
50. *Halophila ovalis* (R. Brown) J.D. Hooker
ssp. *ovalis*
ssp. *bullosa* (Setchell) den Hartog
ssp. *linearis* (Den Hartog) den Hartog
ssp. *ramamurthiana* Ravikumar et Ganesan
51. *Halophila ovata* Gaudichaud in Freycinet
52. *Halophila minor* (Zollinger) den Hartog
53. *Halophila australis* Doty et Stone
54. *Halophila hawaiiana* Doty et Stone
55. *Halophila madagascariensis* Steudel ex Doty et Stone
56. *Halophila johnsonii* Eiseman in Eiseman et McMillan
57. *Halophila stipulacea* (Forssk* al) Ascherson
58. *Halophila decipiens* Ostenfeld
59. *Halophila capricorni* Larkum

Halophila sect. **Microhalophila** Ascherson
60. *Halophila beccarii* Ascherson

Halophila sect. **Spinulosae** Ostenfeld
61. *Halophila spinulosa* (R. Brown) Ascherson

Halophila sect. **Tricostatae** Greenway
62. *Halophila tricostata* Greenway

Halophila sect. **Americanae** Ostenfeld
63. *Halophila engelmanni* Ascherson
64. *Halophila baillonii* Ascherson ex Dickie in J.D. Hooker

Ruppiaceae

Ruppia Linnaeus
65. *Ruppia* aff. *tuberosa* den Hartog
66. *Ruppia cirrhosa* (Petagna) Grande
67. *Ruppia filifolia* Philippi
68. *Ruppia maritima* Linnaeus
69. *Ruppia megacarpa* Mason
70. *Ruppia polycarpaa* R.Mason

Zannichelliaceae

Lepilaena Drummond ex Harvey
71. *Lepilaena marina* E.L. Robertson in Womersley
72. *Lepilaena australis* J.Drumm. ex Harv.

Seagrasses are differentiated into distinct segments: roots, rhizomes and leaves, and vary in morphology and size, ranging from the eelgrass *Zostera caulescens* with strap-like leaves that reach 7 m in height to shorter ovate leaved spoongrass *Halophila minor* that grows to less than 1.5 cm tall (Fig. 2).

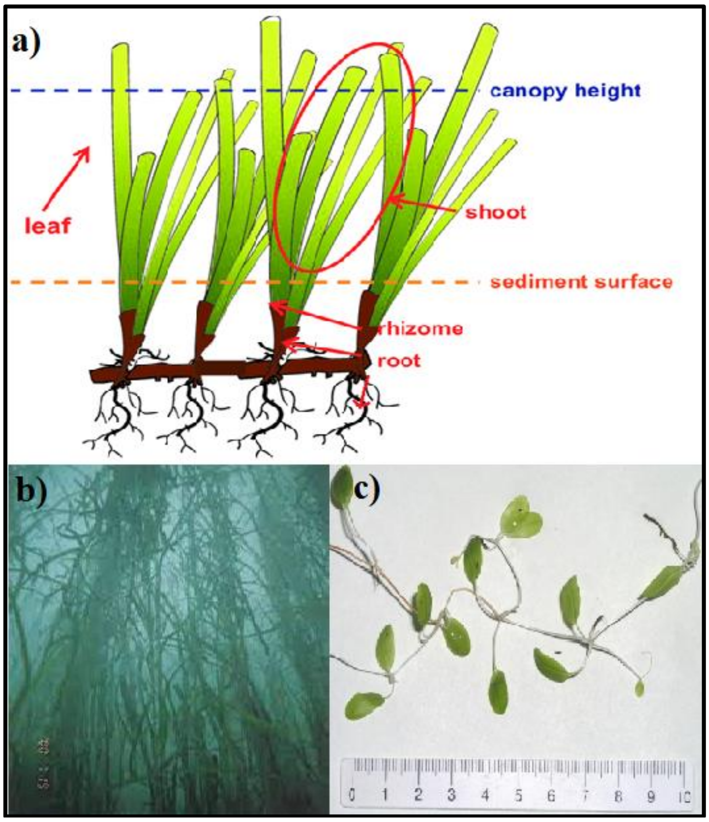

Fig. 2: a) Marine magnoliophyte general morphology. b) *Zostera caulescens* c) *Halophila minor*.

Seagrasses distribution is a product of combined plant sexual reproduction and asexual growth. Successful sexual reproduction is limited owing to low seed dispersal and survival rates (Table 2, Larkum et al., 2006). However, sexual recruitment is the only way to maintain the genetic diversity of a seagrass population (Reynolds et al., 2013). All seagrass species are capable of asexual reproduction through horizontal rhizome growth. The amounts of both sexual and asexual reproduction differ relying on environmental conditions (Xu et al., 2018).

Table 2: Reproductive characters in some seagrass gener (from Larkum et al., 2006).

Genus	Dicliny	Max. seed density (m^{-2})	Max. % germinated seeds	seeding survival (%)
Zostera	Monoecious	9000	50–90	0–40
Cymodocea	dioecious	1300	2–5	10–20
Halodule	dioecious	20000	2	<2
Posidonia	Bisexual	450	50–90	0–67
Halophila	Monoecious and dioecious	70000	12–63	---
Thalassia	dioecious	230	---	11

3. Seagrass ecosystem services

As autogenic and allogenic engineers, seagrasses are the basis of one of the most productive coastal ecosystems on Earth (York et al., 2015; Nordlund et al., 2018). They are commonly known to host an extensive array of biodiverse fauna than bare sediments (Wong, 2018; Barnes and Hamylton, 2016) including cyanobacteria (Novak, 1984), diatoms (Thursby and Davis, 1984), epiphytic algae (Tsioli et al., 2021), and sessile as well as mobile epifauna. This is due to the microhabitats formed by leaf canopy and the belowground architectural structure (Romero et al., 2014). Seagrasses provide shelter, feeding and nursery grounds for demersal juvenile fish while adult fish undergo daily migrations between coral reefs and seagrasses (Honda et al., 2016). In the North Atlantic, 297 species of fish are documented to utilize seagrass meadows, 486 in Australasia, and 746 in the Indo-Pacific (Unsworth et al., 2018). In the Mediterranean, even though seagrass meadows occupy less than 2 % of the sea floor, their associated fish and invertebrate species comprise 30%–40% of the total value of commercial landed fisheries (Jackson et al., 2015). Shallow water fishery, including invertebrate gleaning activities (i.e., invertebrate harvesting by hand and on foot), provide the major source of protein for millions of people in tropical and subtropical developing regions and 79% are seagrass associated (Fig. 3; Unsworth et al., 2018).

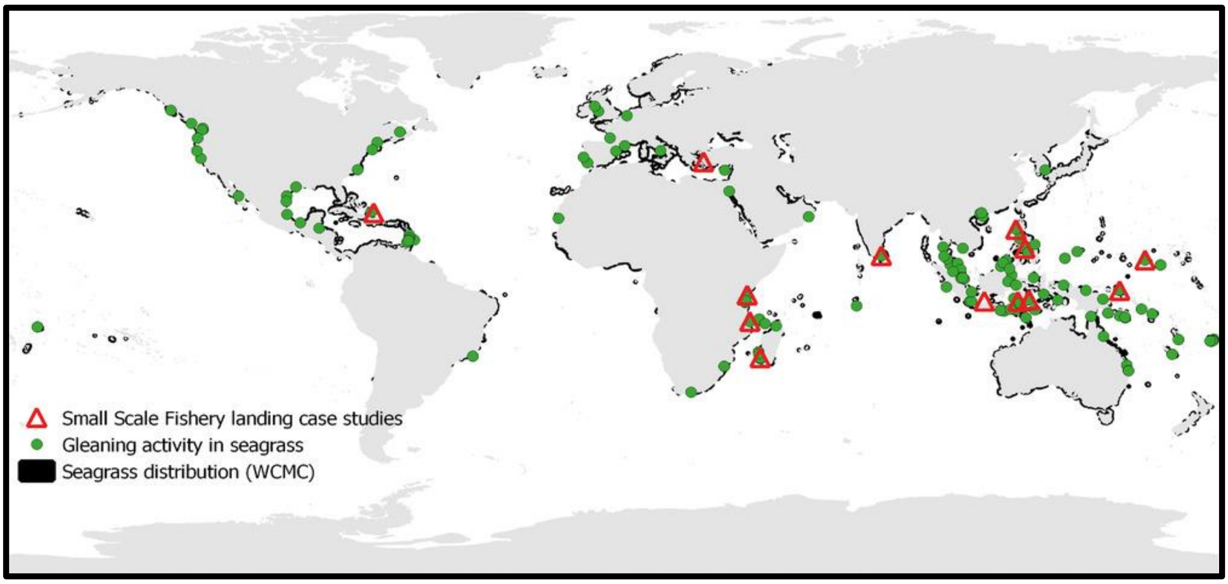

Fig. 3: Locations of small-scale fishery and known gleaning activity in seagrass meadows for invertebrates and fish (from Unsworth et al., 2018).

Seagrass meadows have been highlighted as efficient carbon sinks (Mazarrasa et al., 2018). Although seagrasses represent only a small area (0.2% of the oceans surface), it is estimated that they store 20% of oceanic blue carbon (Fourqurean et al., 2012). The large quantities of dissolved inorganic carbon taken up during photosynthesis can also buffer ocean acidification, thus contributing to the resilience of calcifying organisms, such as corals (Manzello et al., 2012) and shellfish (Wahl et al., 2017) living within or adjacent to seagrasses (Koweek et al., 2018).

Moreover, seagrasses act as the first line of defence along coasts by protecting people from the increasing floods and storms risk (Paul, 2018). Their leaves reduce flow velocity and decrease wave energy by about 40% and their extensive root and rhizome systems stabilise the sand (Maun, 2009; Ondiviela et al., 2014). The velocity reduction promotes settling of finer particles that enhance light availability for the meadow (Adams et al., 2016). The coastal protection service that seagrass meadows provide will be particularly relevant under climate change scenarios, considering that the strength of waves and storm surges are expected to increase (Dreier and Fröhle, 2015; Houston, 2015).

Seagrasses can also improve water quality by filtering, cycling and storing nutrients and pollutants. For instance, they can filter the ammonium produced by intensive oyster culture (Sandoval-Gil et al. 2016) and accumulate contaminants such as trace metals, which they can store in the sediment for millennia (for example, *Posidonia oceanica* in the Mediterranean Sea) (Pergent-Martini, 1998). Thanks to their markedly respond to environmental degradation, seagrasses can be seen as bioindicators of ecosystem health (Marba et al., 2012).

4. Threats to seagrasses

One billion people inhabit areas located within 100 km from the ocean with the highest population density occurring within the closest 10 km (Nicholls and Small, 2002). The expanding densely-populated nearshore areas is transforming both coastal land and marine environments (Cullen-Unsworth and Unsworth, 2018). Seagrass losses have been accelerating from a median of 0.9% year^{-1} before 1940 to 7% year^{-1} since 1990 (Waycott et al., 2009). These habitats are under increasing pressure from variable human activities related to direct removal during coastal development (e.g., harbors, marinas, and channels) (Grech et al., 2012), run-off of nutrients and other pollutants from land-based sources (Orth et al., 2006), destructive fishing methods (such as trawling), aquaculture (Skinner et al., 2013; Cullain et al., 2018). Climate change is a growing concern for seagrass ecosystems (Fortes et al., 2018). Extreme events such as heatwaves (Campbell et al., 2006), severe storms (Short et al., 2016), extreme rain events (Björk et al., 1997), can strongly affect seagrasses.

5. Seagrass knowledge state in Morocco

In Morocco, seagrasses are unknown or neglected compared to macroalgae. There is no scientific study on the seagrass diversity, distribution and extent. Scarce historical reports are often limited to indicating their presence without characterisation. *Zostera noltii* Hornemann occur in vast meadows in the sheltered area of the five semi-enclosed coastal systems of the Atlantic coast (Moulay Bousselham lagoon, Sidi Moussa lagoon, Oualidia lagoon, Khnifiss lagoon and Dakhla bay). It is also recorded in Smir from the Mediterranean coast (Bazairi, 2015). *Cymodocea nodosa* (Ucria) Ascherson show large distribution in the Mediterranean side and have been signalled in Marchica lagoon, Tres Forcas Cape, Cabo Negro and Ceuta, while it disappeared from Dalia bay (Bazairi, 2015). Its presence is also likely along the Atlantic coast at depths >3m and in intertidal zone at Dakhla bay associated with *Z. noltei. Zostera marina* Linnaeus has disappeared from many localities where it was historically cited (e.g. Cap des Trois Fourches, Marchica lagoon) while the presence *of Posidonia oceanica* (Linnaeus) Delile in the Marchica lagoon, seem to be a systematic confusion (Bazairi, 2015). Unfortunately, many losses are unknown due to lack of a reliable baseline information.

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CHAPTER 2 - BIOMONITORING ENVIRONMENTAL STATUS IN SEMI-ENCLOSED COASTAL ECOSYSTEMS USING *ZOSTERA NOLTEI* MEADOWS.

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Abstract

Semi enclosed waters, such as estuaries and lagoons, are vulnerable ecosystems that are experiencing persistent trace element (TE) contamination. Seagrasses have been reported worldwide as valuable bioindicator species for coastal contamination monitoring purpose. Here we monitored the TE contamination of semi-enclosed ecosystems for the first time along the full latitudinal gradient of the Moroccan Atlantic coast. The dominant seagrass species in these ecosystems is *Zostera noltei*. 23 TEs (Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U, Bi and Hg) and four major elements (Na, Mg, K, Ca) were measured in sediment and seagrass leaf samples collected upstream and downstream of five semi-enclosed areas. These contrasted in both climatic conditions and by levels of environmental contamination. From chemical element concentrations in the samples, the Trace Element Pollution Index (TEPI) and the Trace Element Spatial Variation Index (TESVI) were calculated. Of the five semi-enclosed areas, sediments from Sidi Moussa lagoon were the most contaminated (TEPI = 1.18). The TESVI differed highly between chemical elements among the five water bodies for both sediments and seagrass leaves, the highest spatial variability being for Ag (TESVI = 72.01 and 21.05 respectively). For *Z. noltei* leaves, a latitudinal gradient of TE accumulation was recorded. High bioconcentration factor (BCF > 1) for Cd, Mo, Sb, Ag, Zn and U indicated their efficient uptake by the seagrass from sediments. Significant correlations ($p < 0.05$) between levels of Cd, Ag, Fe, Al, Ba, Hg, Mn and Zn in sediments and in *Z. noltei* leaves indicated similar contamination occurrence in both environmental matrices and their bioavailability to seagrasses. Overall, leaf TE bioconcentration among and within the study sites resulted from differences in both element bioavailability and environmental conditions (climatic context, hydrological conditions and human impact). Ultimately, *Z. noltei* is a useful bioindicator of Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination in sediments.

Keywords: *Zostera noltei*, bioindicator, transitional waters, trace and major elements, climatic latitudinal gradient, Atlantic, Morocco.

1. Introduction

Over the last century, most of semi-enclosed coastal ecosystems have been significantly impacted by a wide range of anthropogenic contaminants, mainly as a consequence of increased human activities (Halpern et al., 2008; Maanan, 2008; Affian et al., 2009; Waycott et al., 2009; Anthony et al., 2014; Mendoza-Carranza et al., 2016). Among these pollutants, trace elements (TEs) that accumulate in aquatic organisms and even bio-magnified through the food chain and posing therefore threats to the aquatic ecosystem and potential harmful effects on human health (Richir and Gobert, 2014; Wei et al., 2016). Mining and industrial uses are increasing worldwide, and their runoff from natural and anthropogenic sources can dramatically increase their environmental occurrence (Islam and Tanaka, 2004; Norgate et al., 2007).

An assessment of the contamination level of TEs in aquatic ecosystems is a key step to the maintenance of the food security and public health concerns (Godwill et al., 2015). Chemical analysis of the environmental matrices such as water and sediment remained the most direct approach for detecting the levels of TEs in the environment. However, this approach cannot afford the compelling evidence of the integrated influence and possible toxicity of such contaminants on the organisms (Zhou et al., 2008). Biomonitoring, based on bioindicators, can provide direct information on the biologically available fraction of TEs in aquatic ecosystems and provides an early warning of the potential for influences at higher levels as a result of trophic interactions (Bonanno and Di Martino, 2017; Farias et al., 2018).

Seagrasses are marine angiosperms that play important roles in coastal ecosystems where they fulfill important trophic and structural functions (Duffy et al., 2006; Short et al., 2007; Gutiérrez et al., 2011). They have the ability to uptake TEs and major elements from overlying water through leaf surfaces and from the sediment through their roots and rhizomes (Llagostera et al., 2011). Additionally, as they contribute significantly to the primary production of aquatic ecosystem, they are expected to be indicative of contaminants at higher trophic levels (Govers et al., 2014). Consequently, the interest in seagrasses as bioindicators of TE contamination has increased in the past few decades (Brix et al., 1983; Pergent-Martini, 1998; Gosselin et al., 2006; Richir and Gobert, 2014; Lin et al., 2016; Bonanno et al., 2017; Sanchez-Quiles et al., 2017; Wilkes et al., 2017). Nevertheless, monitoring TEs contamination using seagrasses have mainly been used along the Mediterranean coasts (Hu et al., 2019).

The dwarf eelgrass *Zostera noltei* Hornemann, 1832, is one of the world predominant species living in intertidal zones, representing the land-sea interface (Short et al., 2001). *Z. noltei* extends its distribution from the eastern Atlantic shores from Mauritania to southern Norway/Kattegat Sea and throughout the Mediterranean, Black, Azov, Caspian, Aral Seas and the Canary Islands (Green and Short, 2003, Moore and Short, 2006, Diekmann et al., 2010). In semi-enclosed coastal areas, this species is particularly vulnerable to climate change derived effects and to anthropogenic pressures (Cabaço & Santos, 2012). Compared to other seagrasses, a limited number of studies evaluated coastal TE contamination using *Z. noltei*, and concluded that the species can be used as good bioindigator to monitor some coastal waters (Wilkes et al., 2017, Sanchiz et al., 2000, Bat et al., 2016). In the temperate coastal semi-enclosed areas along the Atlantic coast of Morocco, where this species is the dominant seagrass, no similar study has been conducted. Therefore, further evidence is necessary to support the employment of dwarf eelgrass as bioindicator for TE contamination in such ecosystems.

To achieve coastal ecosystems persistence, monitoring programs are a crucial step. Therefore, development and validation of assessment tools are very important, both from a scientific and stakeholder point of view. The aim of the present study was to assess for the first time the suitability of *Z. noltei* leaves as bioindicator of TE contamination along the full latitudinal gradient of the Moroccan Atlantic coast. To achieve this, the approach followed was: (1) to study 23 TE distribution in seagrass leaves and sediments along the latitudinal climatic gradient (Mediterranean, semi-arid and arid climate) of Moroccan Atlantic coasts, (2) to compare downstream and upstream stations in the study sites, (3) to determine TE Bioconcentration Factor (BCF) to *Z. noltei* leaves from sediments and (4) to test the application of two pollution indices, the Trace Element Spatial Variation Index (TESVI) and the Trace Element Pollution Index (TEPI).

2. Material and methods

2.1. Study sites

The five study sites are located along the Atlantic coast of Morocco: i) Moulay Bousselham lagoon, ii) Sidi Moussa lagoon, iii) Oualidia lagoon, iv) Khnifiss lagoon and v) Dakhla bay (Fig. 1). The sampling sites represent particularly interesting study cases with good knowledge of their functioning and their numerous common and specific characteristics. They are submitted to different stressors: Industrial effluents (Sidi Moussa and Dakhla), domestic wastewaters (Oualidia), agriculture (Moulay Bousselham, Oualidia and Sidi Moussa), harbour activities (Dakhla bay) and mining activities (Sidi Moussa, Oualidia and Khnifiss). Together, these five semi-enclosed water bodies thus cover many sources of potential TE contamination along a latitudinal climatic gradient from the desert to the Mediterranean.

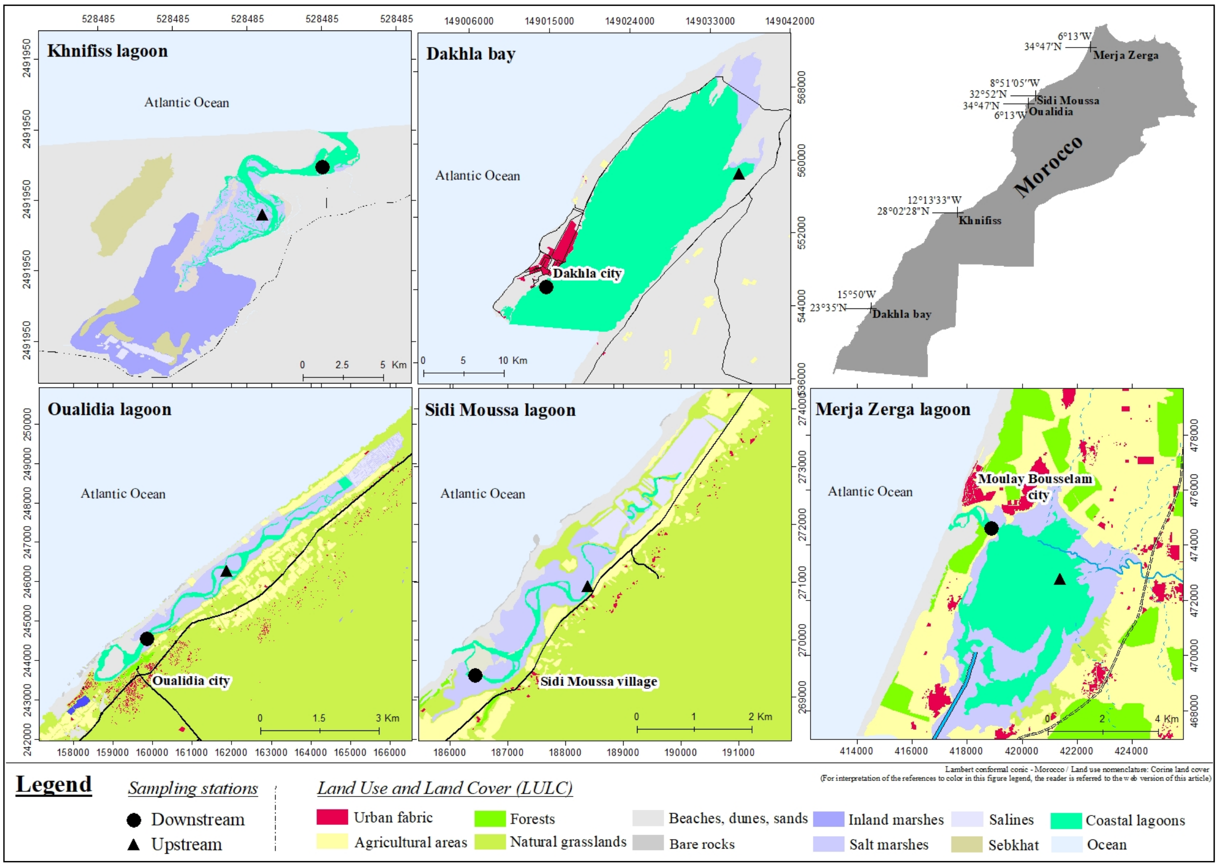


Fig. 1: Maps of the five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. In each site one upstream (U) station and one downstream (D) station were sampled for *Zostera noltei* and sediments.

The Moulay Bousselham lagoon (34°47′N–6°13′W and 34°52′N–6°14′W), commonly known as Merja Zerga, covers 45 km^2 with an average depth of 1.5 m. In addition to its tidal inflow, the lagoon receives freshwater from the Oued Drader, the Canal de Nador and the underlying water-table. As a result of the large exchanges with the Atlantic, the salinity of the downstream part is close to that of sea water with 34 while it doesn't exceed 2.34 in the southern sector away from the ‘‘gullet’’ (Touhami et al., 2017). The spatial evolution of the water temperature showed also a slight decreasing gradient from the downstream to the upstream of the lagoon (Touhami et al., 2017). It represents important social and economic activities, *e.g.*, tourism and traditional fisheries of fishes and molluscs. Lands around the lagoon and its watershed are experiencing modern intensive agriculture and rapid urbanization (Maanan et al., 2013).

The lagoon complex of Sidi Moussa and Oualidia is located in the central part of the Atlantic coast of Morocco. The surrounding area is considered as the second largest industrial zone in Morocco because of the development of its agricultural, tourist and industrial activities. These activities can affect the exploitation of the lagoon resources (fishing, exploitation of algae, oyster farming, etc.) and may have an impact on its environmental quality. Sidi Moussa lagoon (32°52′0″ N–8°51′05″W) covers an area of 4.2 km^2 with a maximum depth of 5 m (Carruesco, 1989). Freshwater inputs to the lagoon are relatively small, emanating from rainfall (run-off from the surrounding lands), the water-table which has a net flow seawards and subterranean resurgences (Cheggour et al., 2001). The water salinity and temperature range from 29.2 to 34.8 and 14.4 to 26.1 °C, respectively (Maanan 2008). Industries in the area produce agricultural and medicinal products, phosphor-chemicals and ferrous metals (El Himer et al., 2013). In the lagoon, many activities like fishing remain traditional and seasonal, whilst aquaculture is developing (Maanan 2008). In Oualidia lagoon (34°47'N-6°13'W and 34°52'N-6°14'W), the maximum depth during flood tides does not exceed 5 m (Carruesco, 1989) and the water temperature and salinity are 13.9–21.7 °C and 30.1–34.7, respectively (Maanan 2008). A rapid urbanization has occurred in recent years (Maanan et al., 2014). Agriculture that uses agro-chemicals and cattle rearing are mainly concentrated around the lagoon. The lagoon provides basic resources for the livelihood of local fishermen. Aquaculture activities, in particular oyster farming, are very important. During summer, there are number of tourism activities such as boating, bathing and camping. Wastewaters from rural and urban centers discharged directly into coastal areas without pre-treatment and water runoff from the watershed are expected to cause additional contamination.

The National Park of Khnifiss is located south of Morocco in the coastal Sahara (28° 02' 28" N 12° 13' 33" W). It is the largest wetland in the desert bioclimatic zone (Beaubrun 1976). The lagoon (20 km long, 65 km^2 surface area and maximum depth of 8.7 m) and adjacent depression of Guelta El Aouina are included in the 60,000 ha making up an integral natural reserve (Dakki and Ligny, 1988). Water temperature and salinity increase from downstream to upstream, ranging from 16.1 to 17.2 °C and from 34 to 44.1, respectively (Lakhdar et al., 2004). The high salinity levels in the upper part of the lagoon is due to the aridity of the area and the existence of a salt lake (Lakhdar et al., 2004). This reserve shelters a diversity of desert fauna and flora. It offers extensive feeding biotopes and roosting grounds for migratory birds such as the wintering waders along the East Atlantic flyway and was designated a Ramsar site (Qninba et al., 2006). The National Park of Khnifiss is also the subject of increased exploitation, namely, shellfish aquaculture, fishing and nature tourism (Lakhdar et al., 2004; Lefrere et al., 2015).

Dakhla Bay (23°35'N 15°50'W) is 37 km long and 12 km wide with depth ranging from 6 to 20 m. It is separated from the ocean on its southern extremity by a 13 km wide pass (Hilmi et al., 2017). Water temperature in the bay ranged from 15 °C in winter (December to February) to 26 °C in summer (May and October) and salinity from 35 to 40 (Zidane et al. 2008). The desert climate, the cold water Canaries current and high subtropical pressure make this area a very productive natural system with outstanding ecological, biological and socio-economic values (Berraho, 2006). The overexploitation of its large natural reservoir of shellfish such as clams (*Ruditapes decussatus*), mussels (*Perna perna*), cockles (*C. edule*) and razors (*Solen marginatus*) is exhausting the site which now requires the establishment of an aquaculture management plan (Zidane et al., 2008). Currently, Dakhla Bay is the biggest national shellfish center in Morocco (Zidane et al., 2017). Harbor activities, tourism, urbanization and shellfish farming development have led to severe environmental problems within the Bay (Saad et al., 2013).

2.2. Sample collection

Zostera noltei and adjacent sediments were sampled during winter 2015. In each semi-enclosed water body, two stations, one upstream and one downstream, were surveyed (Fig. 1). In each station, three replicates were sampled for both seagrass and sediments. Each seagrass replicate consisted of at least five *Z. noltei* shoots handpicked from monospecific stands and thoroughly rinsed on site with seawater to remove remaining inorganic particles. Three 0.15 m long and 0.12 m diameter sediment cores were sampled within the seagrass bed using a PVC hand corer. Each sediment core was subdivided into three homogeneous subsamples. All seagrass and sediment samples were stored in plastic bags and frozen until preparation for analysis.

2.3. Sample preparation and analysis

Seagrass leaves were cleaned of their epiphytes using a glass slide (Dauby and Poulicek, 1995), then rinsed gently with distilled water. Sediment and leaf samples were oven-dried at 60°C to constant weight. Dried leaves were grinded using a Mixer Mill (Retsch GmbH). One of the three sediment core subsamples was sifted through nylon mesh to recover the mud fraction (< 0.0625 mm; Wentworth, 1922) for TE analysis, since this fraction provides the greatest surface area for TE adsorption (Jickells and Knap 1984).

A second sediments core subsample was used for the determination of the different fraction ratios (gravel, sand and mud; Wentworth, 1922). Grain size was measured with a laser granulometer (Malvern, Mastersizer) at the “Littoral, Environnement, Télédétection, Géomatique” (LETG, UMR 6554, University of Nantes).

Organic matter content was determined by the loss on ignition method (Heiri et al., 2001) in the third sediment core subsample. Subsamples were dried at 105°C up to a constant weight (DW105), then combusted to ash and carbon dioxide for 4 h at 550°C and weighed again (DW550). The percentage of organic matter (OM) was calculated as follows:

LOI550 = ((DW105–DW550)/DW105)*100

Ground seagrass leaves and sediment mud fractions were digested in Teflon bombs in a closed microwave digestion lab station (Ethos D, Milestone Inc.). Nitric acid and hydrogen peroxide (4ml HNO_3/ 1ml H_2O_2 for leaves and 2ml HNO_3/ 1ml H_2O_2 for sediment) were used as reagents (’Suprapur’ grade, Merck). This partial digestion procedure extracts from sediments the adsorbed and organic fractions of chemicals bioavailable to organisms without digesting the sediment mineral matrix (Khrisnamurty et al., 1976; U.S. EPA., 1996). Digestates were diluted to a volume of 50 ml using milli-Q water prior to being analysed. Chemical elements were analysed by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). Analyses included four major elements : Na, Mg, K, 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. Concentrations of Hg were determined by atomic absorption spectrometry using a Direct Mercury Analyzer (DMA 80, Milestone Inc.). The accuracy of analytical methods was checked by analyzing Certified Reference Materials (CRMs): PACS-2 (marine sediments) from the National Research Council Canada, BCR 60 (*Lagarosiphon major*) and BCR 61 (*Platihypnidium riparioides*) from the JCR’s Institute for Reference Materials and Measurements, GBW 07603 (bush branches and leaves) from the Chinese Institute of Geophysical and Geochemical Exploration and V463 (maize) from the French National Institute for Agricultural Research. PACS-2 mean recovery was 78% (partial digestion procedure; no certified value for Ba, Tl, Bi and U). Vegetal CRM mean recovery was 103% (no certified value for Sn, Tl and U). Limit of detection (minimum detectable value, LOD) and limit of quantification (minimum quantifiable value, LOQ) were determined by measuring 11 blank samples as follows:

LOD = mblanc + 3 Sblanc

LOQ = mblanc + 10 Sblanc,

where mblanc and Sblanc are the mean and the standard deviation of the blank samples.

Chemical element concentrations are reported in mg $kg^{-1}{}_{DW}$ of sediments or leaves. Ti in sediments and Bi, Ti and Sn in *Z. noltei* leaves, below LOD for the majority of analyzed samples, were not considered.

2.4. Data analysis

2.4.1. Index calculation

To compare chemical elements according to the overall spatial variability of their environmental levels along the Moroccan Atlantic Coast (upstream and downstream transitional water stations included) using *Z. noltei* and sediments, the Trace Element Spatial Variation Index (TESVI; Richir and Gobert, 2014) was calculated, for each element, as follows:

$$TESVI= [(x_{max}/ x_{min})/(\Sigma (x_{max}/x_i)/n)] * SD.$$

where x_{max} and x_{min} are the maximum and minimum mean concentrations recorded among the n stations, x_i are the mean concentrations recorded in each of the n stations, and SD is the standard deviation of the mean ratio $\Sigma(x_{max}/x_i)/n$. The higher the index value for a given element, the more its environmental levels globally vary along the Moroccan Atlantic Coast.

To compare global TE contamination levels among the monitored stations using either sediments or *Z. noltei*, the Trace Element Pollution Index (TEPI; Richir and Gobert, 2014) was calculated, for each of the 10 stations, as follows:

$$TEPI = (Cf_1 * Cf_2 \dots Cf_n)^{1/n}$$

where Cf_n is the mean concentration of the TE_n in a given monitored station. The higher the index value is, the more contaminated the monitored station. As this index calculates the contamination rate by trace elements considered as potential environmental contaminants, the major elements Na, Mg, K, and Ca were not included in its calculation.

The bioconcentration factor (BCF; Lewis et al, 2007) of each element from sediments to *Z. noltei* was calculated as follows:

BCF = mean concentration in a seagrass compartment (in mg kg^{-1}_{DW}) / mean concentration in sediments (in mg kg^{-1}_{DW}).

Low BCF values are indicative of low accumulation by *Z. noltei* whereas high values indicate active uptake.

2.4.2. Statistical analyses

Significant differences between stations mean element concentrations were tested using one-way analysis of variance (one-way ANOVAs) followed by Tukey HSD pairwise comparison test of means ($p < 0.05$), after testing for normality of residual distribution and homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric analysis of variance (Kruskal-Wallis test) was performed when assumptions prior to ANOVAs (normality and/or homoscedasticity) were not achieved, followed by Dunn pairwise comparison test of means ($p < 0.05$).

Multivariate statistical analyses (Principal component analysis, PCA, and cluster analysis, CA) were performed on matrices of centered and reduced data. For the sediments matrix, sampling stations were the objects (rows) and TEs, major elements, TEPI values, organic matter (OM), sand and mud contents were the variables (columns). For the *Z. noltei* matrix, sampling stations were the objects (row) and TEs and TEPI value were the variables. Samples were clustered using Ward's method (average Euclidean distance between objects as measure of similarity).

Spearman's rank correlation coefficient analyses (r) were performed to detect correlations between TE concentrations in *Z. noltei* leaves and TEPI values, and correlations between chemical element concentrations, TEPI values, organic matter, sand and mud content for sediment samples. To further identify correlations between TE concentrations and TEPI values in sediments and *Z. noltei* leaves, a third Spearman's correlation coefficient analysis was performed on matrices of *Z. noltei* and sediment data, followed by linear regression for significant correlations after testing for model assumptions.

Statistical analyses were performed in R version 3.4.2 (R Core Team 2017).

3. Results

3.1. TEs in Sediment

The sediment granulometry of the five semi-enclosed water bodies varied from finer silt to coarse sand, with no gravel (Table 1 and Fig 2). The sediments of the sampling stations ranged from sand to muddy-sand without downstream-upstream variation except for Oualidia and Dakhla. Organic matter contents fluctuated from 1.91% in Dakhla upstream to 11.95% recorded for Merja zerga downstream.

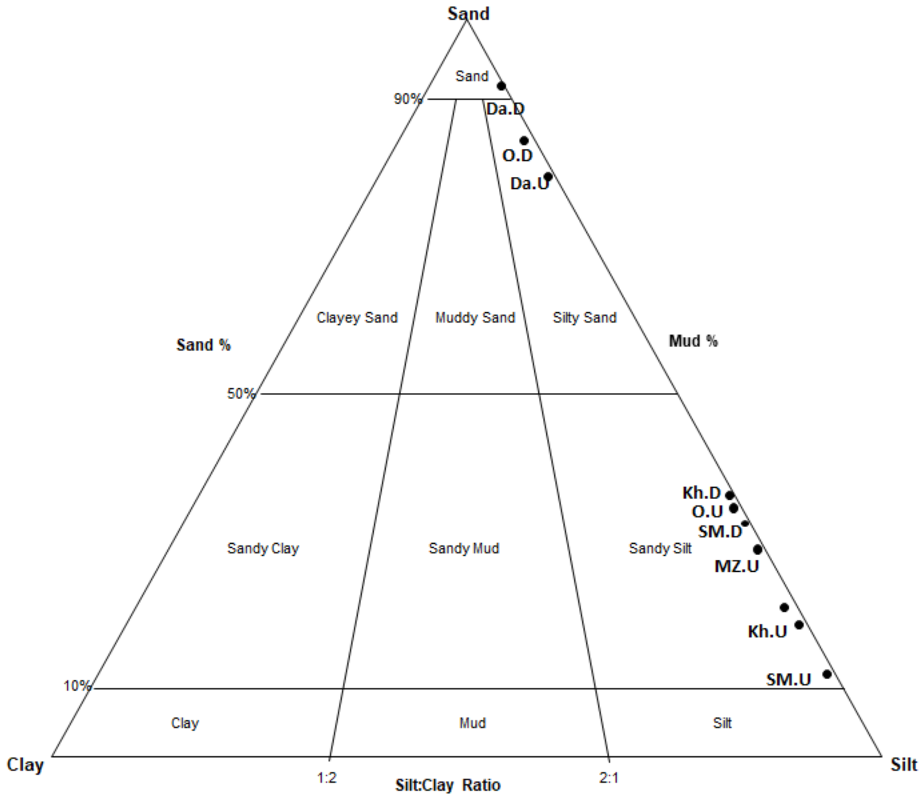


Fig. 2: Textural classification (mean. in %) of sediments (n = 3) sampled from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the Atlantic coast of Morocco. MZ: Merja Zerga. SM: Sidi Moussa. O: Oualidia. Kh: Khnifiss. Da: Dakhla.

Table 1: Granulometry characteristics (mean, in %) of sediments (n = 3) sampled from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the Atlantic coast of Morocco. MZ: Merja Zerga, SM: Sidi Moussa, O: Oualidia, Kh: Khnifiss, Da: Dakhla. V. is for very.

	MZ-D	**MZ-U**	**SM-D**	**SM-U**	**O-D**	**O-U**	**Kh-D**	**Kh-U**	**Da-D**	**Da-U**
% Organic matter	5.10	8.18	10.34	11.95	3.72	11.72	8.64	7.140	2.01	1.91
% Gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Sand	20.20	29.30	32.00	11.60	85.00	33.50	34.70	18.20	92.00	80.30
% Mud	79.80	70.70	68.00	88.40	15.00	66.50	65.30	81.80	8.00	19.700
% V. Coarse gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Coarse gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Medium gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
% Fine gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% V. Fine gravel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% V. Coarse sand	0.00	0.00	0.00	0.00	0.00	0.90	0.00	0.40	0.00	1.70
% Coarse sand	1.70	0.60	7.10	0.70	6.50	2.80	1.70	2.60	17.60	6.70
% Medium sand	5.00	2.30	10.60	0.90	40.50	4.30	9.00	2.00	51.40	15.50
% Fine sand	6.20	8.20	5.40	1.90	33.60	9.50	12.00	3.40	22.30	29.50
% V. Fine sand	7.30	18.20	8.80	8.10	4.40	16.10	12.00	9.80	0.60	27.00
% V. Coarse silt	7.70	12.00	16.00	19.80	2.80	18.10	18.50	18.50	2.20	8.30
% Coarse silt	10.40	8.50	18.50	25.80	3.60	16.90	18.20	23.30	2.00	3.50
% Medium silt	17.50	14.80	15.00	20.00	3.10	13.30	12.40	18.40	1.60	3.60
% Fine silt	23.00	19.50	10.80	13.80	3.00	10.30	9.00	12.40	1.30	2.40
% V. Fine silt	17.20	13.40	6.30	7.60	1.90	6.50	5.80	7.40	0.70	1.40
% Clay	4.00	2.50	1.50	1.40	0.50	1.50	1.50	1.70	0.20	0.40
Textural group	Sandy Mud	Sandy Mud	Sandy Mud	Sandy Mud	Muddy Sand	Sandy Mud	Sandy Mud	Sandy Mud	Sand	Muddy Sand
Sediment type	Very Fine Sandy Fine Silt	Very Fine Sandy Fine Silt	Medium Sandy Coarse Silt	Very Fine Sandy Coarse Silt	Coarse Silty Medium Sand	Very Fine Sandy Very Coarse Silt	Fine Sandy Very Coarse Silt	Very Fine Sandy Coarse Silt	Poorly Sorted Medium Sand	Very Coarse Silty Fine Sand

Table 2 shows the mean concentrations of the 27 chemical elements concentrations in sediments from the sampling sites. Considering all stations, the average concentrations decreased in the following order: Ca > Al > Fe > Mg > Na > K > Sr > Mn > Cr > Ba > Zn > V> Cu > Ni > Li > Pb > As > U > Co > Mo > Ag> Cd > Sn > Sb > Bi > Hg. The spatial variation index values (TESVI) fluctuated from 0.84 for Cu to 64.10 and 72.01 for Ca and Ag respectively (Table 2). TEPI values ranged between 1.09 (Dakhla upstream) to 1.18 (Sidi Moussa downstream and upstream) and were positively correlated to Cr, Pb, Bi, Zn, Hg, Ag, Ba, Al, V, Sn and Sr ($p < 0.05$, $0.65 < r < 0.98$; Table 3). According to TEPI index values, the level of the total contamination of the five transitional water decreased in the following order: Sidi moussa lagoon > Oualidia lagoon > Merja Zerga lagoon > Khnifiss lagoon > Dakhla bay.

PCA multivariate analysis showed that 78.41% of the total variance was explained by the first two principal components (Fig. 3). TEPI values, concentrations of Pb, Zn, Bi, K, Cr, Li, Mn, Ba, Mg, Al, Ni, Ca, V, Hg, As, Ag, Co and Fe in addition to OM, mud and sand contents were the dominating features in the first PC (PC1) that explained 54% of the total variance of the data set (loading values of respectively 0.97, 0.97, 0.96, 0.96, 0.96, 0.91, 0.87, 0.82, 0.82, 0.81, 0.79, 0.79, 0.78, 0.73, 0.72, 0.68, 0.67, 0.66, 0.63, 0.78, 0.73 and -0.73). The second PC (PC2) had 24.41% of the total variance of the data set and was weighted by Cd (0.81), Sn (0.71), Sb (0.70), U (0.70), Hg (0.64), As (-0.69), Co (-0.75) and Fe (-0.77).

Table 2: Chemical element (major and trace elements) concentrations (mean ± SD, in mg $kg^{-1}{}_{DW}$, n = 3) in *Zostera noltei* leaves and sediments sampled from downstream (D) and upstream (U) stations in five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. MZ: Merja Zerga, SM: Sidi Moussa, O: Oualidia, Kh: Khnifiss, Da: Dakhla. Z.n. is for *Zostera noltei* leaves. Trace Element Spatial Variation Index (TESVI), Trace Element Pollution Index (TEPI) and Bioconcentratiob factor (BCF) values were calculated for each station in leaves and sediment concentrations of the 27 chemical elements (major elements Na, Mg, K, and Ca were not included in TEPI calculation). LOD and LOQ represent limits of detection and quantification, respectively. BLD: Below Limit of Detection.

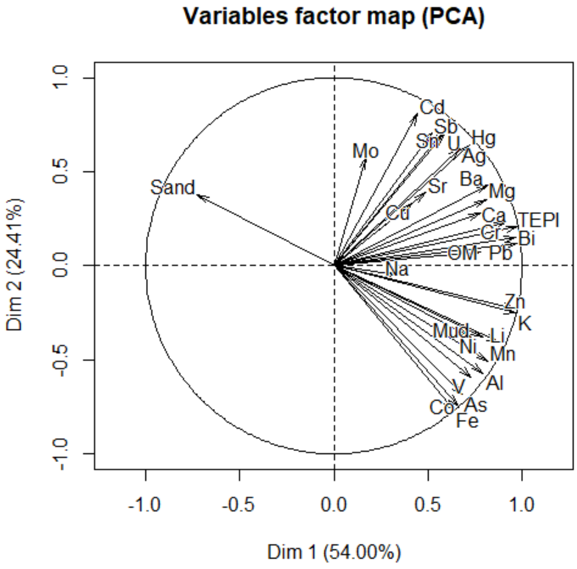
Chemical elements	Compartment	Sites										LOD	LOQ	TESVI	BCF
		MZ		SM		O		Kh		Da					
		D	U	D	U	D	U	D	U	D	U				
Na	Sediments	17429±585	9737±1188	11712±3930	9980±854	11801±705	7997±1527	6971±1907	6067±906	16111±12524	3878±1279	1.60	5.20	2.26	0.68±0.79
	Z.n.	6632±1981	5827±1682	1354±463	1673±258	2407±145	2675±1291	4806±965	5027±3121	10685±2874	10936±1523	3.80	13.0	5.90	
Mg	Sediments	14036 ±303	11475.3±623	21772±1571	22685±2425	21889±3033	20515±1658	18265±949	15473±1973	12498±270	4887±946	0.50	1.60	2.99	0.72±0.67
	Z.n.	10932±654	10147±1444	4128±535	5169±596	8452±988	6219±1678	10533±260	8273±2841	10159±333	12138±1033	0.300	1.00	1.18	
Ca	Sediments	103040±8321	107084±8893	163004±20810	140559±17665	180263±10239	143155±9166	88340±3379	62034±611	85496±1244	5704±601	0.500	1.80	64.10	0.40±0.65
	Z.n.	18037±3179	13599±1869	25314±3939	29355±2637	19128±1087	24650±2752	21142±1330	26115±3940	16803±3106	12747±1304	0.600	1.90	0.67	
K	Sediments	10794±9523	7815±1392	9385±1162	9960±1064	7337±1582	8469±1101	8025±1238	7185±1854	3948±551	2164±554	1.10	3.80	3.40	0.16±0.18
	Z.n.	1151±375	1196±494	193±116	382±75.3	387±103	610±470	652±128	647±253	1656±469	1224±271	1.40	4.80	6.49	
Fe	Sediments	41577±1377	35285±2089	18478±1777	25511±1869	18688±2800	22707±2975	27051±1974	24812±3292	9165±1057	6158±1387	5.30	18.0	4.94	0.10±0.05
	Z.n.	5045±1024	3638±833	466±14.1	1522±567	832±118	839±145	2935±456	3372±721	1268±110	1185±328	1.90	6.20	8.19	
Al	Sediments	45744 ±4156	33348±5511	27582±3328	30719±3351	21090±46882	26153±2817	25721±4100	23098±5833	11714±3435	7846±2048	0.002	0.006	3.73	0.03±0.02
	Z.n.	2221±667	1256±388	244±113	520±92.6	382±108	317±44	903±219	831±201	628±138	439±101	0.002	0.005	5.36	
V	Sediments	113±8.10	87.5±10.9	71.8±7.28	67.0±6.44	48.8±8.56	56.6±6.30	61.2±7.03	56.0±10.3	33.2±4.36	20.7±4.52	0.007	0.023	3.16	0.40±0.33
	Z.n.	26.7±7.31	15.9±6.86	7.16±0.791	8.34±1.14	6.41±1.63	8.26±2.81	46.2±5.47	46.8±7.65	19.1±9.02	18.4±1.80	0.006	0.021	4.69	
Cr	Sediments	95.2±5.78	73.5±9.01	131±19.0	123±11.8	89.3±50.4	73.8±12.8	49.6±5.68	43.5±8.46	35.6±7.60	21.2±6.79	0.600	1.90	4.09	0.03±0.02
	Z.n.	4.08±1.25	2.41±0.632	1.49±0.448	2.27±0.435	1.35±0.342	1.15±0.087	1.67±0.283	1.48±0.250	1.88±0.259	1.35±0.380	2.90	9.70	1.12	
Mn	Sediments	304±25.3	255±17.7	206±10.9	231.5±18.1	244.9±31.5	236±16.3	236±11.02	210±26.5	95.3±6.32	40.6±3.42	0.007	0.024	7.12	0.80±0.56
	Z.n.	439±417	281±195	62.7±19.3	172±65.3	107.7±66.9	132±16.1	78.3±13.8	167±65.2	28.9±3.64	80.2±24.1	0.025	0.085	12.85	
Co	Sediments	10.7±0.088	9.01±0.283	5.45±0.443	6.83±0.501	5.88±1.22	6.56±0.849	7.85±0.378	6.77±0.980	3.04±0.341	2.14±0.307	0.006	0.019	2.99	0.16±0.15
	Z.n.	2.62±1.79	1.36±0.621	0.263±0.091	0.683±0.269	0.264±0.126	0.465±0.010	0.756±0.118	1.13±0.317	0.444±0.036	1.15±0.139	0.007	0.023	6.89	
Ni	Sediments	33.7±0.756	27.1±1.51	25.4±2.72	28.0±1.92	34.9±23.3	26.7±5.05	28.5±1.99	24.7±3.80	11.0±2.38	7.97±0.675	0.018	0.060	2.80	0.11±0.08
	Z.n.	4.78±2.28	3.20±1.23	1.83±0.396	2.19±0.563	1.44±0.530	0.970±0.089	2.33±0.226	2.11±0.405	2.12±0.069	2.26±0.962	0.023	0.078	2.18	
	Sediments	38±4.02	31.1±4.70	63.3±5.46	72.5±7.30	54.5±8.78	58.0±6.22	76.4±9.32	75.4±14.5	46.6±5.17	35.7±4.57	0.070	0.230	0.84	

Cu	Z.n.	10.6±3.68	10.1±4.48	5.15±1.26	6.72±1.45	3.62±0.301	3.64±0.620	4.74±0.593	5.70±1.12	6.09±0.269	3.96±1.02	0.078	0.260	1.02	0.13±0.09
Zn	Sediments	95.4±2.07	78.7±5.27	87.8±10.1	92.9±8.51	60.7±10.6	77.2±8.98	69.2±3.97	60.6±9.03	40.6±5.33	24.4±3.90	0.041	0.140	2.13	1.44±0.64
	Z.n.	68.8±31.1	62.3±28.4	192±22.4	180±21.0	99.0±27.6	126±22.7	87.2±11.2	93.5±30.2	56.9±26.9	31.4±5.67	0.085	0.280	3.63	
Sr	Sediments	356±43	347±19.6	627±73.6	522±75.2	936±69	762±82.5	281±14.2	190±14.9	400±18.7	132±28.8	0.012	0.040	4.70	0.91±0.59
	Z.n.	330±42.8	284±29.3	228±22.4	323±44	283±12.6	277±17.0	325±7.94	291±24.5	344±27.0	287±14.5	0.007	0.023	0.19	
Li	Sediments	31.7±2.00	25.9±3.80	24.2±0.980	30.1±1.60	21.4±4.11	29.-±2.85	26.9±2.20	26.6±4.66	12.1±4.21	6.10±0.985	0.010	0.034	3.90	0.04±0.02
	Z.n.	1.41±0.498	0.821±0.192	0.271±0.057	0.499±0.050	0.387±0.090	0.402±0.068	0.879±0.194	0.858±0.110	0.761±0.208	0.536±0.117	0.011	0.037	2.59	
As	Sediments	14.7±1.05	12.1±0.549	8.64±1.25	8.50±0.263	7.94±0.129	7.37±0.877	9.88±1.20	8.87±0.829	4.03±0.456	2.49±0.364	4.70	16.0	3.92	0.28±0.24
	Z.n.	3.54±1.58	3.94±1.93	0.601±0.081	1.04±0.252	1.01±0.104	0.746±0.070	2.86±0.444	1.84±0.286	1.93±0.156	2.17±0.275	5.3	18	4.28	
Mo	Sediments	1.60±0.363	1.15±0.173	4.86±1.28	2.11±0.137	1.62±1.07	2.92±0.640	3.97±0.338	2.69±0.559	2.88±0.518	2.28±0.510	0.009	0.028	1.85	5.60±2.05
	Z.n.	7.58±2.01	7.66±1.90	16.6±2.32	8.25±1.09	12.1±1.99	10.6±2.04	23.6±1.87	19.6±7.09	10.8±0.699	21.7±2.61	0.014	0.047	1.26	
Ag	Sediments	0.082±0.010	0.073±0.009	2.48±0.828	2.92±0.214	0.44980.164	0.752±0.146	0.0730.003	0.0630.009	0.116±0.112	0.037±0.005	0.027	0.088	72.01	1.94±1.64
	Z.n.	0.178±0.107	0.146±0.058	1.86±0.717	2.47±0.846	2.20±0.393	0.468±0.256	0.122±0.037	0.157±0.037	0.940±0.014	0.092±0.030	0.013	0.042	21.05	
Cd	Sediments	0.116±0.001	0.97±0.013	1.17±0.251	1.47±0.186	0.523±0.016	0.515±0.154	0.238±0.045	0.215±0.013	0.770±0.067	0.292±0.027	0.006	0.020	13.23	7.44±3.01
	Z.n.	0.803±0.201	0.779±0.208	11.9±0.538	2.52±5.92	6.53±1.24	2.78±0.157	1.12±0.346	1.10±0.133	2.15±0.50	3.01±0.330	0.008	0.026	12.87	
Sn	Sediments	0.329±0.077	0.169±0.114	0.760±0.056	0.686±0.050	0.538±0.057	0.342±0.039	0.245±0.082	0.263±0.075	0.550±0.0768	0.108±0.022	0.005	0.015	4.91	---
	Z.n.	0.077±0.027	0.052±0.014	BLD	0.046±0.002	BLD	BLD	0.043±0.010	0.040±0.005	0.086±0.013	0.032±0.005	0.004	0.013	---	
Sb	Sediments	0.043±0.007	0.031±0.056	0.598±0.111	0.225±0.026	0.131±0.008	0.071±0.09	0.062±0.007	0.060±0.009	0.083±0.011	0.053±0.036	0.015	0.051	11.60	4.87±2.92
	Z.n.	0.318±0.069	0.258±0.077	0.430±0.062	0.279±0.053	0.306±0.035	0.273±0.058	0.489±0.018	0.354±0.025	0.273±0.053	0.416±0.015	0.026	0.085	0.40	
Ba	Sediments	68.0±8.27	54.7±10.1	1045±10.6	82.9±7.26	86.2±9.90	76.9±14.8	68.1±9.95	71.2±16.3	62.2±16.5	32.0±4.80	0.013	0.044	1.27	0.23±0.05
	Z.n.	12.8±2.54	10.4±0.594	27±2.63	21.1±2.16	12.75±2.07	16.9±4.82	18.2±2.53	20.9±9.03	10.2±4.87	9.63±1.24	0.006	0.019	0.96	
Ti	Sediments	0.238±0.024	0.194±0.032	0.245±0.032	0.412±0.015	0.178±0.031	0.238±0.0333	0.238±0.033	0.227±0.043	BLD	BLD	0.006	0.021	---	---
	Z.n.	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	0.004	0.012	---	
Pb	Sediments	12.6±0.73	10.2±1.13	14.98±0.866	17.3±0.530	9.43±1.16	12.5±1.26	9.54±0.529	8.56±0.829	7.04±1.25	2.47±0.389	0.100	0.330	5.62	0.19±0.09
	Z.n.	1.73±0.56	1.22±0.192	2.31±0.247	2.40±0.024	1.42±0.69	1.24±0.344	1.20±0.257	1.43±0.2199	2.21±0.114	0.972±0.231	0.070	0.240	0.78	
Bi	Sediments	0.127±0.005	0.112±0.015	0.166±0.015	0.186±0.009	0.095±0.015	0.130±0.014	0.105±0.004	0.091±0.014	0.076±0.025	0.023±0.006	0.020	0.065	7.11	1.35±1.14
	Z.n.	0.026±0.007	0.016±0.002	0.012±0.001	0.017±0.001	0.012±0.003	BLOD	0.022±0.006	0.019±0.003	0.021±0.002	0.011±0.004	0.054	0.180	---	
U	Sediments	1.20±0.161	0.998±0.130	32.1±7.65	16.3±1.94	5.18±0.39	4.22±1.17	2.18±0.197	1.94±0.315	2.31±0.109	1.92±0.129	0.014	0.046	23.64	1.35±1.14
	Z.n.	2.35±1.34	1.50±0.508	1.78±0.209	1.33.±0.161	0.939±0.221	1.34±0.571	6.01±1.29	5.16±1.79	2.86±0.924	5.23±0.756	0.007	0.026	3.74	

Hg	Sediments	0.020±0.002	0.017±0.003	0.107±0.016	0.096±0.001	0.032±0.004	0.038±0.007	0.015±0.001	0.015±0.002	0.020±0.004	0.005±0.001	---	---	19.22	0.87±0.48
	Z.n.	0.027±0.008	0.020±0.005	0.037±0.002	0.023±0.001	0.027±0.003	0.012±0.002	0.011±0.001	0.020±0.007	0.015±0.001	0.008±0.000	---	---	2.18	
TEPI	Sediments	1.15	1.14	1.18	1.18	1.15	1.15	1.14	1.14	1.13	1.09	---	---	---	---
	Z.n.	1.18	1.16	1.16	1.17	1.15	1.14	1.17	1.17	1.15	1.15	---	---	---	

Table 3: Correlation matrix of non-parametric Spearman's rank correlation coefficients between the mean chemical element concentrations, Trace Element Pollution Index (TEPI) values, organic matter (OM), mud and sand content for sediments sampled from downstream and upstream stations of five semi-enclosed water bodies along the Atlantic coast of Morocco. Correlation coefficients significant at $p < 0.05$ are underlined in bold.

	Na	Mg	Ca	K	Fe	Al	V	Cr	Mn	Co	Ni	Cu	Zn	Sr	Li	As	Mo	Ag	Cd	Sn	Sb	Ba	Pb	Bi	U	Hg	TEPI	OM	Sand	Mud
Na	1.00																													
Mg	0.24	1																												
Ca	0.47	**0.75**	1.00																											
K	0.43	0.54	0.55	1																										
Fe	0.15	0.07	0.10	0.62	1.00																									
Al	0.36	0.21	0.41	**0.87**	**0.81**	1																								
V	0.36	0.18	0.38	**0.84**	**0.79**	**0.98**	1.00																							
Cr	0.53	**0.73**	**0.82**	**0.87**	0.35	**0.71**	**0.70**	1																						
Mn	0.37	0.16	0.47	0.55	**0.81**	**0.68**	**0.65**	0.44	1.00																					
Co	0.15	0.07	0.10	0.62	1.00	**0.81**	**0.79**	0.35	**0.81**	1																				
Ni	0.44	0.52	0.59	0.60	**0.66**	0.52	0.52	0.59	**0.83**	**0.66**	1.00																			
Cu	-0.25	**0.65**	0.09	0.24	0.09	-0.04	-0.01	0.20	-0.18	0.09	0.14	1																		
Zn	0.47	0.43	0.55	**0.96**	**0.68**	**0.95**	**0.93**	**0.87**	0.61	**0.68**	0.59	0.07	1.00																	
Sr	0.60	**0.72**	**0.90**	0.42	-0.12	0.19	0.13	**0.67**	0.30	-0.12	0.43	0.08	0.37	1																
Li	0.15	0.38	0.21	**0.83**	**0.81**	**0.77**	**0.70**	0.53	0.61	**0.81**	0.55	0.36	**0.77**	0.13	1															
As	0.19	0.01	0.09	0.56	**0.89**	**0.77**	**0.84**	0.38	**0.67**	**0.89**	0.55	0.12	**0.65**	-0.19	0.61	1														
Mo	-0.25	0.25	-0.01	-0.03	-0.44	-0.32	-0.24	-0.04	-0.56	-0.44	-0.38	0.62	-0.21	0.09	-0.13	-0.30	1													
Ag	0.56	**0.77**	**0.81**	0.61	-0.03	0.38	0.31	**0.79**	0.14	-0.03	0.31	0.20	0.58	**0.88**	0.30	-0.12	0.15	1												
Cd	0.25	**0.64**	0.41	0.10	-0.58	-0.25	-0.28	0.36	-0.50	-0.58	-0.13	0.33	0.01	0.59	-0.19	-0.60	0.44	**0.73**	1.00											
Sn	0.62	**0.72**	0.61	0.43	-0.22	0.15	0.13	**0.66**	-0.13	-0.22	0.13	0.33	0.37	**0.77**	0.12	-0.18	0.32	**0.89**	**0.82**	1										
Sb	0.28	**0.79**	0.56	0.20	-0.45	-0.16	-0.16	0.49	-0.36	-0.45	0.04	0.52	0.09	**0.68**	-0.10	-0.41	0.53	**0.77**	**0.94**	**0.88**	1.00									
Ba	0.25	**0.94**	**0.77**	0.48	-0.05	0.18	0.18	**0.75**	0.09	-0.05	0.38	0.61	0.38	**0.73**	0.25	0.02	0.36	**0.73**	0.60	**0.76**	**0.81**	1								
Pb	0.37	0.61	**0.65**	**0.95**	0.52	**0.85**	**0.82**	**0.92**	0.44	0.52	0.49	0.24	**0.95**	0.49	**0.72**	0.47	-0.01	**0.73**	0.25	0.53	0.33	0.56	1							
Bi	0.30	**0.65**	**0.68**	**0.92**	0.45	**0.81**	**0.76**	**0.89**	0.41	0.45	0.44	0.27	**0.90**	0.54	**0.70**	0.38	0.07	**0.77**	0.30	0.54	0.38	0.60	**0.99**	1						
U	0.22	**0.84**	0.62	0.26	-0.42	-0.12	-0.13	0.54	-0.30	-0.42	0.08	0.54	0.14	**0.72**	-0.03	-0.39	0.54	**0.79**	**0.92**	**0.85**	**0.99**	**0.85**	0.39	0.45	1.00					
Hg	0.58	**0.75**	**0.83**	0.60	-0.08	0.37	0.32	0.81	0.12	-0.08	0.27	0.19	0.56	**0.89**	0.24	-0.10	0.22	**0.99**	**0.72**	**0.90**	**0.78**	**0.76**	**0.72**	**0.76**	**0.81**	1				
TEPI	0.45	0.76	0.77	0.92	0.35	**0.70**	**0.68**	**0.98**	0.39	0.35	0.55	0.32	**0.87**	**0.65**	0.61	0.36	0.12	**0.79**	0.38	**0.66**	0.52	**0.76**	**0.94**	**0.93**	0.58	**0.81**	1.00			
OM	-0.08	**0.65**	0.52	**0.71**	0.41	0.61	0.56	**0.64**	0.24	0.41	0.27	0.55	**0.66**	0.37	**0.66**	0.30	0.30	0.61	0.27	0.39	0.38	0.58	**0.82**	**0.88**	0.47	0.59	**0.72**	1		
Sand	0.09	-0.24	-0.04	-0.60	**-0.64**	**-0.72**	**-0.66**	-0.47	-0.30	**-0.64**	-0.18	-0.26	**-0.66**	0.15	**-0.72**	-0.60	0.26	-0.15	0.19	-0.05	0.14	-0.20	**-0.64**	-0.60	0.09	-0.10	-0.47	-0.59	1	
Mud	-0.09	0.24	0.04	0.60	**0.64**	**0.72**	**0.66**	0.47	0.30	**0.64**	0.18	0.26	**0.66**	-0.15	**0.72**	0.60	-0.26	0.15	-0.19	0.05	-0.14	0.20	**0.64**	0.60	-0.09	0.10	0.47	0.59	**-1**	**1**



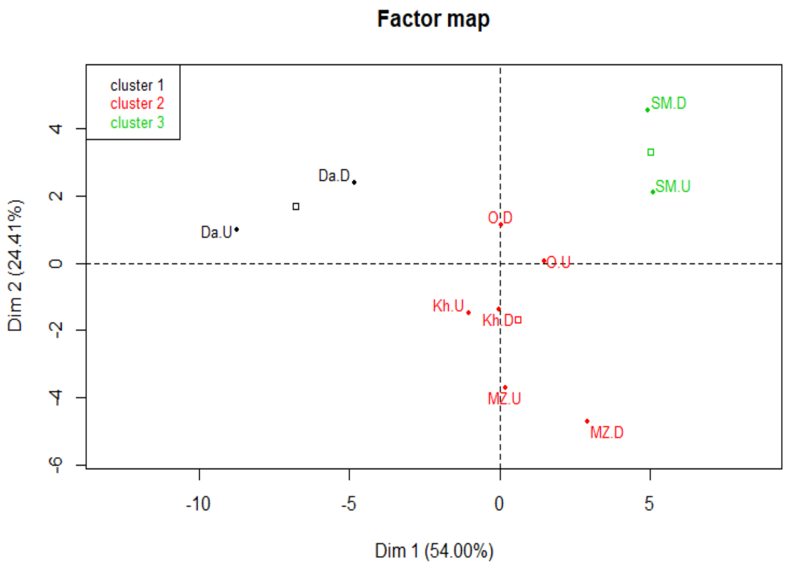


Fig. 3: Factor loading plots for the first two principal components identified in the PCA of chemical element concentrations and Trace Element Pollution Index (TEPI) values for sediments. Samples were collected from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the Atlantic coast of Morocco. MZ: Merja Zerga. SM: Sidi Moussa. O: Oualidia. Kh: Khnifiss. Da: Dakhla. The ten stations are color-grouped according to their dendrographic classification after CA at a linkage distance of 3.8.

According to Cluster Analysis (Fig. 3), the first cluster grouped Dakhla downstream and upstream stations, characterized by a higher level of sand content and the lowest concentrations of V, Mg, Bi, Pb, Fe, Al, As, Co, Zn, K, Li, Ni and Mn, as well as the lowest TEPI, OM and mud values. The second cluster included Oualidia, Merja Zerga and Khnifiss downstream and upstream stations. They were characterized by low concentrations of Cd and high values of Co, Mn and Ni. The final cluster was from Sidi Moussa downstream and upstream stations that displayed high TEPI values and high levels of nine TEs: Ag, Hg, U, Cd, Sb, Sn, Cr, Bi, Zn and Pb.

Additionally, the ANOVA performed to the five water bodies showed that Hg, Ba, Ag, Sr and Ca sediment levels were also low in the Dakhla bay and did not differ significantly ($p > 0.05$) from Merja Zerga - Khnifiss lagoons. The Oualidia lagoon displayed the higher level of Sr without significant difference with Sidi Moussa lagoon. Li and Zn were also higher in Sidi moussa lagoon without significant difference ($p > 0.05$) with Merja zerga lagoon.

For the chemical elements and sediment properties determining the clusters of stations, significant ($p < 0.05$) negative correlations were observed between sand and seven TEs: V, Pb, Fe, Al, Co, Zn and Li ($-0.72 < r < -0.64$) and positive correlations between Co-Mn-Ni ($0.66 < r < 0.83$) (Table 3). Significant ($p < 0.05$) positive correlations were also observed between Cd, U, Sn, Sb, Hg and Ag ($0.72 < r < 0.99$), between Cr, Pb, Bi and Ag ($0.73 < r < 0.99$) and between Cr and Sr ($r = 0.67$) (Table 3).

Regarding the individual analysis of each body water, no significant differences ($p > 0.05$) were observed between downstream and upstream stations of Khnifiss for chemical element concentrations in sediments. At the Merja Zerga lagoon, Al, V, Fe and As concentrations were significantly ($p < 0.05$) higher in the downstream station than in the upstream station. In Oualidia lagoon, the downstream station showed significantly ($p < 0.05$) higher Ca, Sr and Sn levels and lower Pb levels compared to the upstream station. Dakhla bay, downstream station showed significantly higher concentrations ($p < 0.05$) of Mg, Ca, Mn, Sr, Sn, Pb and Bi compared to the upstream station. In Sidi Moussa lagoon, the downstream station showed significantly ($p < 0.05$) higher Sb, U and Mo levels and lower Fe levels compared to the upstream station.

3.2. TEs in *Zostera noltei* leaves

The 27 chemical elements average concentrations in *Z. noltei* leaves (Table 2), considering all stations, were as follows: Ca > Mg > Na > Fe > K > Al > Sr > Mn > Zn > V > Ba > Mo > Cu > Cd > U > Ni > As > Cr > Pb > Co > Ag > Li > Sb > Hg. The spatial variation index values (TESVI) ranged between 0.19 for Sr and 21.05 for Ag. TEPI values ranged between 1.14 for Oualidia upstream to 1.18 for Merja Zerga downstream. Only Cr, Fe, Al and Ni were correlated with TEPI values (Table 4).

The two first principal components PC1 and PC2 resulting from the PCA analysis explained 69.57% of the total variance (Fig. 4). The dominating features in the first PC (PC1) explaining 45.71% of the total variance of the data set were As, Li, Al, Co, Fe, Ni, Mg, K, Cr, Cu, Mn, Na, Ba, Ag, Zn and Cd concentrations (loading values of respectively 0.93, 0.92, 0.88, 0.86, 0.85, 0.84, 0.83, 0.75, 0.70, 0.70, 0.66, 0.65, -0.63, -0.74, -0.78 and -0.78). The second PC (PC2) explained 23.86% of the total variance and was weighted by Hg (0.77), Cr (0.67), Mn (0.65), Cu (0.64) TEPI (0.68) and Mo (-0.64).

CA (Fig. 4) showed a clear latitudinal gradient of chemical elements accumulated by *Z. noltei* leaves. The first cluster (Merja Zerga downstream and upstream stations) showed the highest contamination levels of Cu, Mn, Al, Ni, Cr, Co, As, Fe and Li. The second cluster (Sidi Moussa downstream and upstream; Oualidia downstream and upstream stations) were characterized by the highest Ag, Zn and Cd concentrations and the lowest levels of Fe, U, V, Li, K, Na, As and Mg. The final cluster (Dakhla downstream and upstream; Khnifiss downstream and upstream stations) was characterized by the highest concentrations of U, Mo, V and Na. For the chemical elements determining these clusters of stations, significant ($p < 0.05$) positive correlations were observed between Ag and Zn ($r = 0.88$), V and U ($r = 0.81$), and between Li-Al-Fe-Co-Ni-As ($0.70 < r < 0.96$), Cu-Al-Cr-Fe-Ni ($0.79 < r < 0.96$) and Mn-Fe-Co ($0.66 < r < 0.85$; Table 4).

Regarding the individual analysis of each inland body of water, Cr and Li concentrations were significantly ($p < 0.05$) higher in Merja Zerga lagoon downstream station than in the upstream station. The downstream station at Sidi Moussa lagoon showed significantly ($p < 0.05$) higher levels of Mo, Sb and Hg and lower concentrations of Sr than the upstream station. In Oualidia lagoon, Ag and Hg concentrations were significantly ($p < 0.05$) higher downstream. Dakhla downstream showed high Pb concentrations while the upstream station had high Mo concentrations. For Khnifiss, there were no significant differences (p > 0.05) between downstream and upstream stations.

Table 4: Correlation matrix of non-parametric Spearman's rank correlation coefficients between mean chemical element concentrations and Trace Element Pollution Index (TEPI) values for *Zostera noltei* leaves sampled from downstream and upstream stations of five semi-enclosed water bodies along the Atlantic coast of Morocco. Correlations significant at $p < 0.05$ are underlined in bold.

	Na																								
Na	1	**Mg**																							
Mg	**0.85**	1	**Ca**																						
Ca	**-0.78**	**-0.79**	1	**K**																					
K	**0.96**	**0.83**	**-0.82**	1	**Fe**																				
Fe	0.48	0.44	-0.15	0.47	1	**Al**																			
Al	0.54	0.58	-0.30	0.56	**0.96**	1	**V**																		
V	0.60	0.54	-0.09	0.56	**0.75**	**0.75**	1	**Cr**																	
Cr	0.14	0.16	-0.09	0.21	**0.70**	**0.73**	0.31	1	**Mn**																
Mn	0.04	0.02	0.08	-0.05	**0.66**	0.52	0.12	0.42	1	**Co**															
Co	**0.64**	0.62	-0.39	0.55	**0.85**	**0.79**	0.62	0.42	**0.68**	1	**Ni**														
Ni	0.54	**0.66**	-0.45	0.55	**0.79**	**0.84**	0.56	**0.75**	0.41	**0.81**	1	**Cu**													
Cu	0.26	0.10	-0.04	0.27	**0.76**	**0.71**	0.42	**0.92**	0.52	0.54	**0.70**	1	**Zn**												
Zn	**-0.96**	**-0.89**	**0.87**	**-0.98**	-0.44	-0.55	-0.53	-0.18	0.03	-0.59	-0.59	-0.21	1	**Sr**											
Sr	0.55	0.56	-0.18	0.58	**0.64**	**0.71**	**0.73**	0.58	0.05	0.41	0.60	0.54	-0.53	1	**Li**										
Li	0.61	**0.65**	-0.25	0.60	**0.92**	**0.94**	**0.90**	0.54	0.38	**0.79**	**0.77**	0.56	-0.58	**0.75**	1	**As**									
As	**0.72**	**0.78**	-0.62	**0.76**	**0.83**	**0.90**	**0.64**	0.60	0.38	**0.84**	**0.92**	0.56	**-0.78**	0.60	**0.84**	1	**Mo**								
Mo	-0.01	0.13	0.07	-0.02	-0.37	-0.30	0.22	-0.59	**-0.67**	-0.26	-0.24	-0.62	-0.05	-0.10	-0.08	-0.19	1	**Ag**							
Ag	**-0.85**	**-0.76**	**0.70**	**-0.89**	-0.30	-0.39	-0.62	0.02	0.32	-0.43	-0.44	-0.05	**0.88**	-0.42	-0.53	-0.62	-0.38	1	**Cd**						
Cd	-0.60	-0.52	0.42	-0.62	**-0.79**	**-0.82**	**-0.66**	-0.41	-0.41	**-0.71**	-0.56	-0.47	0.58	-0.37	**-0.82**	**-0.77**	0.22	0.53	1	**Sb**					
Sb	-0.10	0.18	0.18	-0.19	-0.14	-0.08	0.30	-0.18	-0.39	-0.05	0.10	-0.25	0.09	0.04	0.12	-0.07	**0.75**	-0.15	0.19	1	**Ba**				
Ba	**-0.81**	**-0.75**	**0.93**	**-0.83**	-0.10	-0.22	-0.08	0.05	0.07	-0.32	-0.27	0.07	**0.87**	-0.24	-0.19	-0.52	0.08	**0.65**	0.33	0.35	1	**Pb**			
Pb	-0.49	-0.44	0.61	-0.43	-0.07	-0.05	0.02	0.43	-0.25	-0.44	-0.04	0.33	0.50	0.33	-0.08	-0.31	-0.05	0.41	0.39	0.24	**0.65**	1	**U**		
U	0.59	0.6	-0.27	0.56	0.32	0.38	**0.81**	-0.01	-0.35	0.38	0.43	0.07	-0.56	0.47	0.61	0.47	0.61	**-0.82**	-0.41	0.61	-0.16	-0.07	1	**Hg**	
Hg	-0.58	-0.52	0.33	-0.60	-0.14	-0.14	-0.49	0.36	0.24	-0.32	-0.15	0.26	0.56	-0.28	-0.35	-0.33	-0.44	**0.75**	0.24	-0.05	0.43	0.42	-0.62	1	**TEPI**
TEPI	-0.14	0.07	0.32	- 0.19	**-0.67**	**0.64**	0.42	**0.70**	0.56	0.49	**0.64**	0.62	0.14	0.42	0.56	0.42	-0.21	0.25	-0.24	0.31	0.45	0.41	0.10	0.33	1

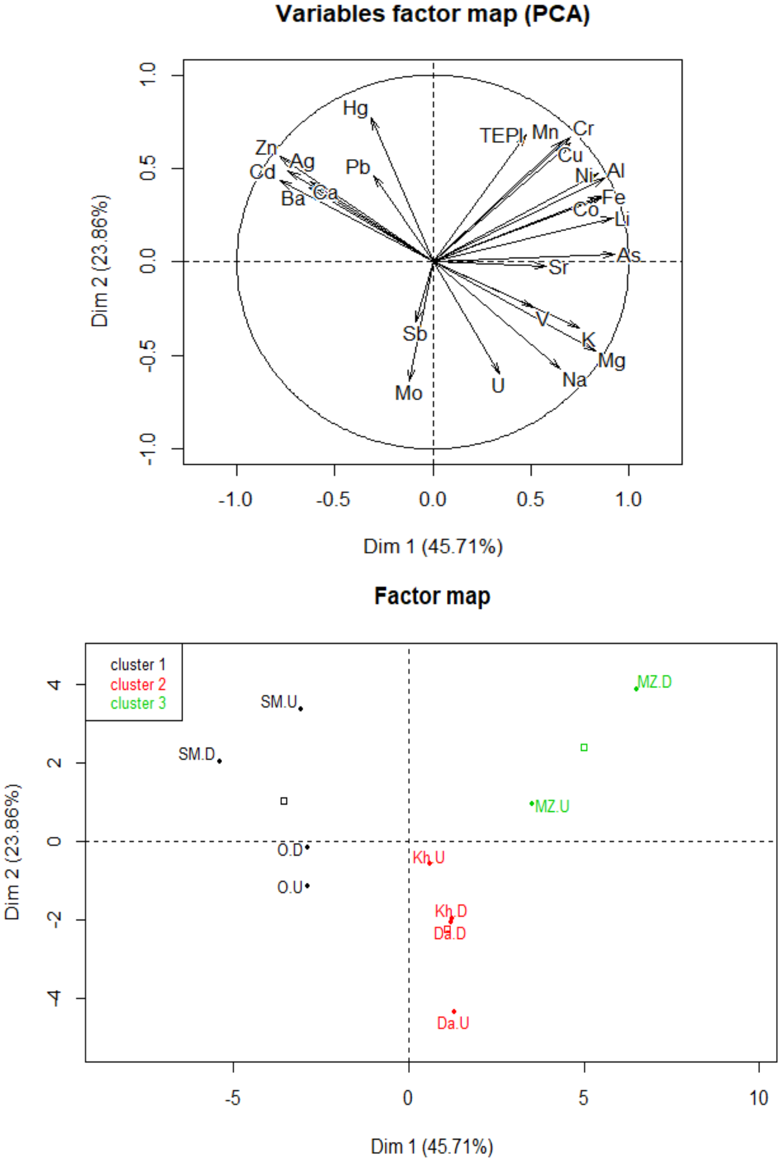


Fig. 4: Factor loading plots for the first two principal components identified in the PCA of chemical element concentrations and Trace Element Pollution Index (TEPI) values for *Zostera noltei* leaves. Samples were collected from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the Atlantic coast of Morocco. MZ: Merja Zerga. SM: Sidi Moussa. O: Oualidia. Kh: Khnifiss. Da: Dakhla. The ten stations are color-grouped according to their dendrographic classification after CA at a linkage distance of 3.

3.3. Relationships between chemical elements in sediments and *Zostera noltei*

When comparing the spatial variability of chemical element levels in sediments and *Z. noltei* leaves among the 10 stations (Five sites x upstream and downstream stations), TESVI values displayed the same trends for Ag, Cd , Mn, Fe, Al, V, As, Zn, Li, Ni, Mo, Mg, Cu and Ba in both compartments, were higher in sediments for Ca, U, Hg, Sb, Pb, Sr and Cr and higher in leaves for Co, K and Na.

Spearman's rank correlation tests performed on chemical elements and TEPI variables for *Z. noltei* leaves and sediments showed significant positive relationships ($p < 0.05$) for Al (r = 0.70), Fe (r = 0.80), Mn (r = 0.65), Zn (r = 0.65), Ag (r = 0.80), Cd (r = 0.91), Ba (r = 0.77) and Hg (r = 0.66) and a significant negative correlation for Mg (r = -0.81) (Table 5). Concentrations of these nine elements in seagrass leaves are plotted against sediment concentrations in Fig. 5.

Table 5: Non-parametric Spearman's rank correlation coefficients between the mean chemical element concentrations and Trace Element Pollution Index (TEPI) values for *Zostera noltei* leaves and sediments sampled from downstream and upstream stations of five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. Correlations significant at $p < 0.05$ are in bold.

Na	**Mg**	**Al**	**K**	**Ca**	**V**	**Cr**	**Fe**	**Mn**	**Co**	**Ni**	**Cu**	**Zn**
0.03	**-0.81**	**0.70**	-0.57	0.47	0.06	0.28	**0.80**	**0.65**	0.59	0.20	-0.45	**0.65**
Sr	**Li**	**As**	**Mo**	**Ag**	**Cd**	**Sb**	**Ba**	**Pb**	**U**	**Hg**	**TEPI**	
-0.34	0.26	0.51	0.57	**0.80**	**0.91**	0.29	**0.77**	0.60	-0.36	**0.66**	0.35	

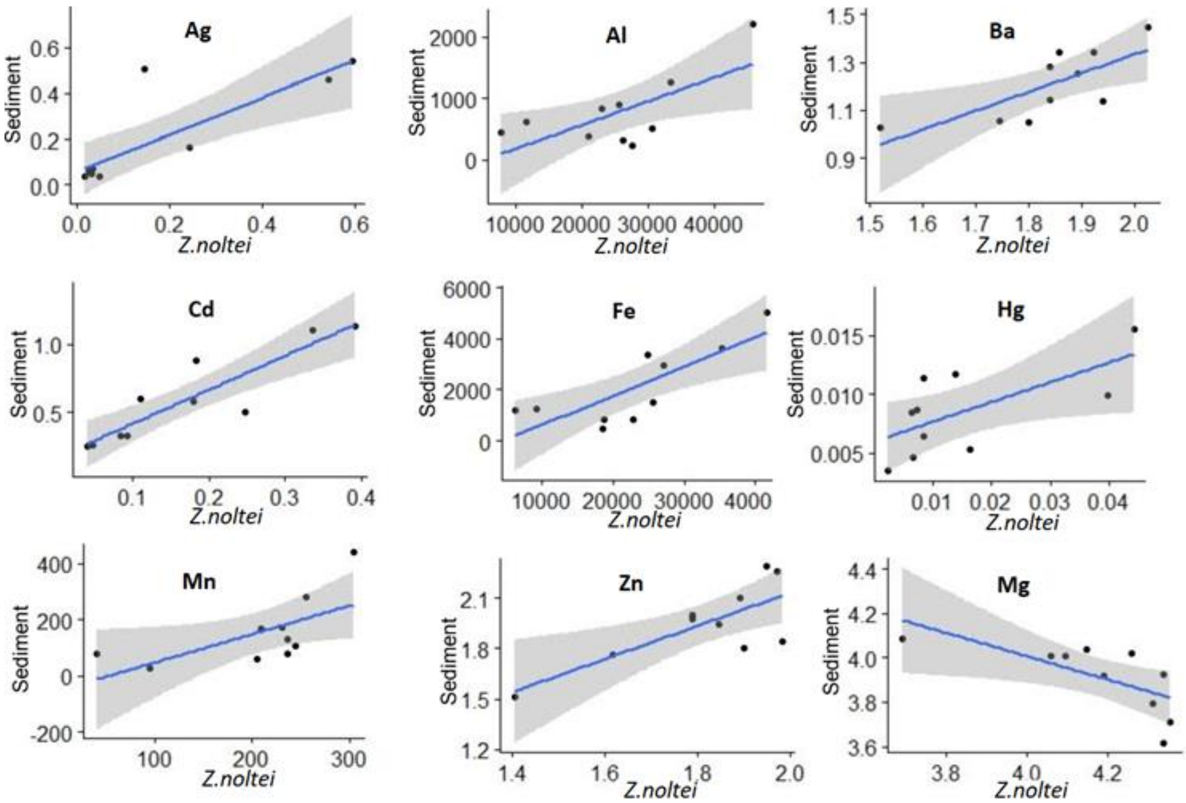


Fig. 5: Mean concentrations of Ag, Al, Ba, Cd, Fe, Hg, Mn Zn and Mg in *Zostera noltei* leaves (in mg kg^{-1}_{DW}) plotted against mean concentrations measured in sediments (in mg kg^{-1}_{DW}). Samples were collected from downstream and upstream stations of five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. The blue lines are the least square best fit lines. Grey shaded area 95% confidence intervals of best fit lines.

Bioconcentration factors (BCF) of major and TEs from sediments to *Z. noltei* leaves are reported in Table 2 and Fig. 6. They differ greatly between elements. High BCF (> 1) were calculated for Cd, Mo, Sb, Ag, Zn and U with values of 7.44, 5.60, 4.87, 1.94, 1.44 and 1.35 respectively.

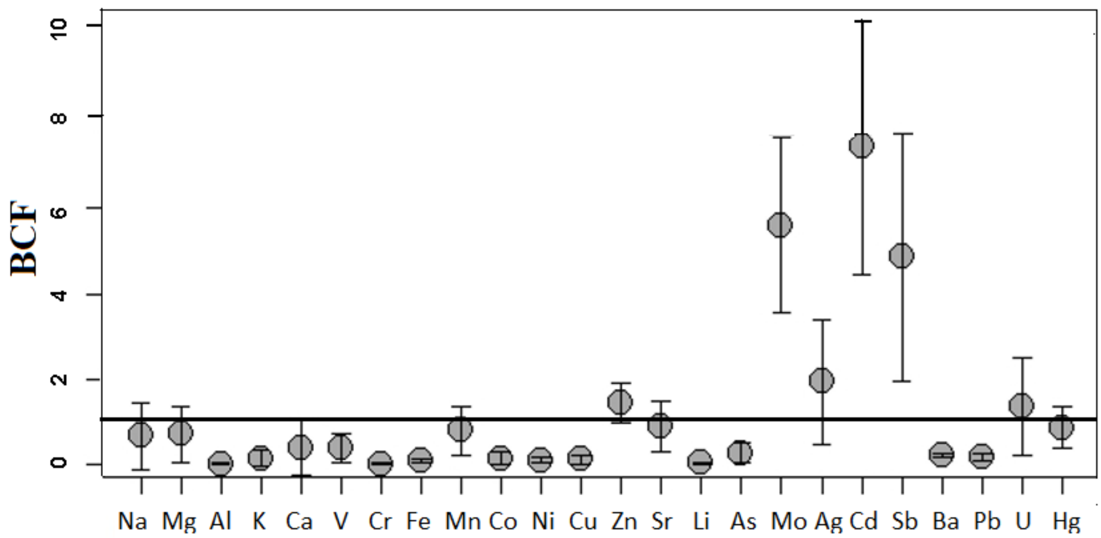


Fig. 6: Mean (n = 10) bioconcentration factor (BCF) from sediments to *Zostera noltei* leaves for chemical elements monitored in downstream and upstream stations of five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. Bars symbolize SD. Solid black line corresponds to the BAF value equal to 1.

4. Discussion

4.1. Distribution and accumulation of TEs in *Zostera noltei* leaves and sediment

Chemical elements concentrations for both sediments and *Z. noltei* leaves showed inter-site and intra-site (downstream/upstream) variability, presumably related to environmental conditions and the anthropic pressure in each case study. The high TESVI values recorded for many elements in seagrass leaves highlight the great variability of their bioavailability among the five water bodies.

The five coastal semi-enclosed water systems are subjected to different human pressures: urbanism (Oualidia), harbour activities (Dakhla bay), agriculture (Merja Zerga, Sidi Moussa and Oualidia), industries (Sidi Moussa) and mining (Sidi Moussa, Oualidia and Khnifiss). In addition, they are located along a wide latitudinal gradient and have therefore different climatic (Mediterranean, semi-arid and arid climates) and hydrologic (e.g., currentology, temperature, salinity, etc.) conditions. The clustering of water systems according to chemical element concentrations and TEPI values of seagrass leaves further highlighted clearly this spatial distribution along the North-South latitudinal gradient of the Atlantic coast of Morocco (Fig. 4). Differences in anthropic pressures and environmental conditions exist not only between sites but also between upstream and downstream stations within transitional water systems. The hydrodynamic situation resulting from small intra-lagoon currents remained the main factor differentiating between the both zones. This upstream-downstream variability further contributed to the high TESVI values calculated for many elements in seagrass leaves. Thus, significant differences ($p < 0.05$) in leaf TE concentrations observed between downstream and upstream stations in Merga Zerga for Cr and Li, in Sidi Moussa for Mo, Sb and Hg, in Dakhla for Pb and in Oualidia for Ag and Hg were not observed for sediments. This indicates that the uptake of chemical elements in leaves is also determined by hydrological conditions of water bodies in addition to sediment characteristics. This hypothesis is consolidated by the absence of variation in leaf chemical element concentrations between the two stations of Khnifiss lagoon where no hydrological differences occur. These findings are in agreement with those from previous studies (Greger, 1999; Yang and Ye, 2009).

While the global seagrass leaf TE contamination estimated by the Trace Element Pollution Index (TEPI) values varied little among Moroccan Atlantic semi-enclosed water bodies, the opposite was observed for the sediment.

The TEPI showed that sediment from Sidi Moussa lagoon were the most contaminated as a result of the high level of Cr, Ag, Hg, Bi, Sn, Zn, Pb and Sr content. The lagoon showed also a high level of Sb, U, Cd, Li and Cu. Comparing to the result of 2001 given by Maanan et al. (2004, Table 6), Cr, Zn and Cu has been increased by 1.31, 1.82 and 2.23% respectively.

Table 6: Average TEs concentrations recorded in sediments from some lagoons of the world (values reported in $mg.kg^{-1}$), SS: surface sediment, LGB: local geographical background.

Sites	References	Sample type	Cr	Zn	Cu	Cd	Mn	Ni	Hg
Sidi Moussa lagoon	Maanan et al., 2004	LGB	45.00	45.00	30.00	0.20			
	Maanan et al., 2005	SS	96.90	49.80	30.40				
Oualidia lagoon	Maanan et al., 2013	LGB	38.40		26.60	0.30			
Merja zerga lagoon	Maanan et al., 2012	LGB	23.6	33.60	19.30	0	210.3	12.8	0
	Maanan et al., 2013	SS	47.00						

According to the natural geochemical background noise of the area (Maanan et al., 2004, Table 6), Cr, Cd, Zn and Cu concentrations of this study were more elevated. This means that the main source of these elements is anthropogenic. The positive correlations found between the 13 TEs elevated in this lagoon indicate their same anthropogenic sources. Industrial effluents from the phosphate processing plants located 15 km northeast of the lagoon at Jorf Lasfar are the most important sources of Cr, Cd, Cu, Zn and other elements in the lagoon (Chafik et al., 2001; Cheggour et al., 1999a, Kaimoussi, 2001). In addition, agricultural activities near the lagoon use heavy machinery and can contribute to the Cr, Cd, Zn and Cu sediment enrichment (OFEFP, 1991; Cheggour et al., 2001). The high number of motorized fishing boats and the significant tourist influx are also associated to the contamination of the lagoon sediments with TEs. In addition, these factors of contamination are magnified by the low resistance to pollution of the hydrogeological basin of the lagoon (El Himer et al., 2013).

Oualidia lagoon was the second most contaminated site. Cr, Cd and Cu concentrations were almost double their preindustrial background levels (Maanan et al., 2013, Table 6). Agricultural drainage water from the sub-watershed was the likely contamination source of sediments for the upstream station (Maanan et al., 2013) while urban sewage seemed to be the main source of sediment contamination for the downstream station (Maanan et al., 2013).

Merja Zerga lagoon exhibited the 3rd highest global contamination level. Levels of Zn, Cu, Mn, Ni, Cr, Cd and Hg exceeded the average shale values (local natural background levels; Maanan et al., 2012, Table 6). The lagoon contamination by these elements can therefore be related to anthropogenic activities. Lower Cr sediment concentration of 1.79% was recorded in 2010 (Maanan et al., 2013) compared to that in our study. The level of As is 44 times higher than that reported in 2004 (0.3 $mg.kg^{-1}{}_{DW}$; Alaoui et al., 2010). Downstream contamination mainly results from the presence of the largest urbanized center of the lagoon area, of two jetties and a campsite. The increase of sediment-bound TEs in the lagoon further results from modern intensive agriculture, mainly in the lagoon catchment area and at the lagoon outlet that uses agro-chemicals fertilizers extensively. In addition, the Oued Drader and the Nador Canal feed the north part of the lagoon with freshwater drained from the Loukkos and the Rharb regions, the two most important agricultural plains in Morocco. The intensive use of herbicides is one of the main sources of As contamination (Reese 1998). The Nador Canal also receives domestic waste and sewage from an urbanized area. Consequently, previous studies (Alaoui et al., 2010; Maanan et al., 2012) that reported high pollution rates along the Nador canal considered this as the probable main source of contamination for Merja Zerga. Furthermore, Al and Fe enrichment in Merja Zerga downstream sediments was modestly significant to very strongly correlated to the higher V and As levels of that area, indicating the terrigenous origin of these elements (Zourarah et al., 2007). On the other hand, the significant positive correlations of Al and V to Zn, Mn, Cr and TEPI indicate their additional anthropogenic sources. Therefore, natural processes and anthropogenic inputs are both responsible of TE contamination in Merja Zerga lagoon, accentuated by the confinement effect in the lagoon.

Khnifiss lagoon exhibited the second lowest global contamination level. Sediment individual element concentrations in this lagoon did not significantly differ ($p > 0.05$) between stations; this found can be related to the strong hydrodynamic conditions ensuring the homogenization of water masses and their fast renewal rate together with the absence of continental freshwater inputs (Lakhdar et al., 2004) maintain a general good environmental quality of the lagoon. Our results are in agreement with Lefrere et al. (2015) who reported a good environmental status of the lagoon using benthic macrofauna as biondicators.

Dakhla bay was the last contaminated site and no chemical element levels were significantly higher in this water body compared to the other sites. However, this bay shared the highest level of Cd content with Sidi Moussa and Oualidia lagoons. The high level of Cd in Dakhla bay was previously considered marine in origin from metal and nutrient-rich water upwellings (Zidane et al., 2017). Our results showed modest to strong correlations between Cd and Ag, Sn, Sb, U and Hg indicating supplementary industrial sources for this element. Overall, the downstream station was more contaminated than the upstream station mostly due to liquid and solid waste inputs from over 30 industrial centers and harbor activities concentrated in this area.

Modest to very strong significant positive correlations between *Z. noltei* leaf and sediment TEs concentrations for Al, Fe, Mn, Zn, Ag, Cd, Ba and Hg indicated that their accumulation by the seagrass was at least partly determined by their availability in sediment. Similar results were found between leaf TEs bioaccumulation and sediments in other species of marine magnoliophytes (Bonanno et al., 2017; Richir et al., 2013; Malea et al., 2013). High BCF values were calculated for Cd, Mo, Sb, Ag, Zn and U, respectively. These > 1.00 BCF values indicated that the seagrass efficiently bioconcentrated these TEs from sediments. However, when sediments were highly contaminated with U in Sidi Moussa and Oualidia, with Ag in Sidi Moussa, and with Sb in Sidi Moussa downstream, the BCF decreased to below one. This suggests that the accumulation of the three TEs can be slowed down or stopped when levels of environmental contamination are too high. In contrast, BCF values of Cd and to a lesser extent, Zn increased together with the augmentation of their concentrations in sediments. Zn is a micronutrient involved in protein synthesis (Malea et al., 1995) and is required for *Z. noltei* photosynthesis and growth (Kabata-Pendias, 2011; Memon et al., 2001). This may explain its efficient accumulation and retention in seagrass leaves. As for Cd, known for its toxicity, in seagrasses it can also induce the synthesis of metal-biomolecules such as phytochelatin and metallothionein that can reduce oxidative stress caused by metals (Alvarez-Legorreta et al., 2008; Wang et al., 2010).

Comparing to the few studies conducted on this species, in Ireland where an ecological status of HIGH or GOOD has been assessed at the *Z.noltei* beds (Wilkes et al., 2017), Co, Ni, Mo, Pb and Cu concentrations were higher than levels reported here semi-enclosed water systems of the Atlantic coast of Morocco (up to 1.5%, 1.1%, 5.6%, 2.8% and 1.3% respectively; Wilkes et al., 2017) while Ag and especially V, Fe and Zn were lower (up to 2%, 5.3%, 1.9% and 2.9% respectively; Wilkes et al., 2017). Levels of Zn, Pb, Cu, Fe and Cu displayed the same trend as concentrations in sediment and both were higher in Moroccan coastal semi-enclosed waters comparing to Turkish coast of the Black Sea (Bat et al., 2016). In contrast, As value was higher in Turkish sediment while similar concentrations were reported for *Z. noltei* from Morocco.

4.2. Performance of *Zostera noltei* as bioindicator

There's currently enough scientific evidence that supports the suitability of seagrasses as bioindicators of TEs contamination (Bonnano and Orlando-Bonaca, 2018). For example, *Posidonia oceanica* and *Cymodocea nodosa* can reflect the level of wide range of TEs in the environment, thus acting as a sensitive bioindicator (Sanchiz et al., 2000; Lafabrie et al., 2007; Bonanno and Di Martino, 2016; Bonanno and Raccuia, 2018). According to Bonanno and Raccuia (2018), *Halophila stipulacea* can act as a promising bioindicator of As, Cd, Cu, Mn, Ni and Zn in sediments. Moreover, tropical seagrasses (*Thalassia hemprichii*, *Enhalus acoroides* and *Cymodocea rotundata*) are potential bioindicators to Cd contents in sediments (Li and Huang, 2012). Regarding eelgrasses, some scholars have demonstrated the capacity of *Zostera capricorni* (Birch et al., 2018), *Z. muelleri* (Farias et al., 2018) and *Z. marina* (Hu et al., 2019) to monitor TEs in other coastal waters. Regarding *Zostera noltei*, despite its wide global distribution, few works has been conducted on it highlighting its capacity to monitor TEs in Ireland, Mediterranean Spanish coast and Turkish coast of the Black Sea (Sanchiz et al., 2000; Bat et al., 2016; Wilkes et al., 2017). Furthermore, this study was necessary to understand the response of dwarf eelgrass to different anthropogenic pressures and climate change of the temperate waters of Morocco.

Ideal bioindicators should show primordial characteristics: (1) they should live in a sedentary style to definitely reflect the local contamination, (2) are abundant enough and have wide distribution for repetitious sampling and comparison, (3) are easy to identify, (4) are easy to sample and easy raise in the laboratory, and (5) occupy an important position in the food chain (Zhou et al., 2008; Polechońska et al., 2018) and has to accumulate high levels of contaminants without death (Zhou et al., 2008). In this study, being widespread and abundant species in coastal semi-enclosed ecosystems along the Atlantic coast of Morocco, *Z. noltei* fit most of the abovementioned characteristics.

Additionally, ideal bioindicators are sensitive to change in the surrounding environment. Specifically, bioindicators should show a significant correlation between the levels of contaminants in their tissues and in the surrounding environments (Ward, 1987; Bonanno and Orlando-Bonaca, 2018). According to the present results, significant correlations ($p < 0.05$) between levels of Cd, Ag, Fe, Al, Ba, Hg, Mn and Zn in sediments and in *Z. noltei* leaves indicated similar contamination occurrence in both environmental matrices and their bioavailability to seagrasses.

Generally, the BCF of TEs in seagrasses reflect the capability of the plants to accumulate them from sediments (Zhang et al., 2011). In this study, high bioconcentration factor (BCF > 1) for Cd, Mo, Sb, Ag, Zn and U indicated their efficient uptake by *Z. noltei* from sediment.

Consequently, this study corroborated seagrass *Z. noltei* leaves as a useful bioindicator of Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination in sediments in semi-enclosed coastal water of Morocco. The periodical regeneration of seagrass leaves led several authors to investigate which seagrass organs are more suitable for short or long-term monitoring. Current studies claim that the leaves of seagrasses (*e.g. P. oceanica*) should be considered as effective short-term bioindicators able to provide accurate information on the presence of trace elements in the marine environment over short time periods, e.g. months (Richir et al., 2013). On the contrary, permanent organs such as roots and rhizomes of seagrasses, whose element concentrations can reflect multiyear inputs of trace elements, seem more appropriate in case of long term biomonitoring, but with the limitation that these organs are usually less sensitive to element variations in the environment compared to leaves (Gosselin et al., 2006).

Ultimately, this first attempt on assessing the suitability of the *Zostera notei* as bioindicator of TEs contamination should be considered as a step towards more comprehensive and robust evaluation. Several studies showed that the levels of TEs vary seasonally in seagrasses, with higher concentrations in the dormant period than in the growing season (Schlacher-Hoenlinger and Schlacher, 1998). Additionally, the degree of TEs accumulation from sediment to seagrass belowground tissues depend on element speciation, plant phenology and numerous environmental factors, such as sediment organic matter and clay content and the spatial distribution of physical properties (pH, redox potential, temperature, salinity…) (Yang and Ye, 2009; Bonanno and Cirelli, 2017). Spatio-temporal distribution of these parameters should then be taking in account for farther investigations.

5. Conclusion

The present study was carried out at ten different seagrass beds (5 sites and two up- and downstream stations by site) that are submitted to different to anthropogenic pressures along a latitudinal climatic gradient from the desert to the Mediterranean. The results of the present study suggest that leaves of *Z. noltei* can be used as a bioindicator for Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination. Therefore, the present study includes the proof of principle that the seagrass system is of high application value. Further investigations are needed to better understand the physiological and phenotypic responses of *Z. noltei* seagrasses to different contaminants as well as the effect of metal speciation and environmental factors on the TEs translocation from rhizosphere sediments to different plant tissues.

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Appendix A.

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Biomonitoring environmental status in semi-enclosed coastal ecosystems using *Zostera noltei* meadows

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A R T I C L E I N F O

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Atlantic
Morocco

A B S T R A C T

Semi enclosed waters, such as estuaries and lagoons, are vulnerable ecosystems that are experiencing persistent trace element (TE) contamination. Seagrasses have been reported worldwide as valuable bioindicator species for coastal contamination monitoring purpose. This is, to our knowledge, the first time the TE contamination of semi-enclosed ecosystems has been monitored along the full latitudinal gradient of the Moroccan Atlantic coast. In these ecosystems, the dominant seagrass species is *Zostera noltei.* 23 TEs (Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U, Bi and Hg) and four major elements (Na, Mg, K, Ca) were measured in sediments and seagrass leaf samples were collected upstream and downstream of five semi-enclosed areas. They contrasted in both climatic conditions and levels of environmental contamination. The Trace Element Pollution Index (TEPI) and the Trace Element Spatial Variation Index (TESVI) were calculated from chemical element concentrations in the samples. Of the five semi-enclosed areas, Sidi Moussa lagoon's sediments were the most contaminated (TEPI = 1.18). The TESVI differed highly between chemical elements among the five water bodies for sediments and seagrass leaves, the highest spatial variability being for Ag (TESVI = 72.01 and 21.05 respectively). For *Z. noltei* leaves, a latitudinal gradient of TE accumulation was recorded. A high bioconcentration factor (BCF > 1) for Cd, Mo, Sb, Ag, Zn and U indicated that the sediments were efficiently uptaken by the seagrass. Significant correlations ($p < 0.05$) between levels of Cd, Ag, Fe, Al, Ba, Hg, Mn and Zn in sediments and in *Z. noltei* leaves indicated similar contamination occurrences in both environmental matrices and their bioavailability for seagrasses. Overall, leaf TE bioconcentration among and within the study sites resulted from differences in element bioavailability and environmental conditions (climatic context, hydrological conditions and human impact). Ultimately, *Z. noltei* is a useful bioindicator of Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination in sediments.

1. Introduction

Over the last century, most semi-enclosed coastal ecosystems have been significantly impacted by a wide range of anthropogenic contaminants, mainly as a consequence of increased human activities (Halpern et al., 2008; Maanan, 2008; Affian et al., 2009; Waycott et al., 2009; Anthony et al., 2014; Mendoza-Carranza et al., 2016). Among these pollutants, trace elements (TEs) are one of the most concerning. They may accumulate in aquatic organisms and even bio-magnify through the food chain, thus threatening the aquatic ecosystem and potentially have harmful effects on human health (Wei et al., 2016). Mining and industrial uses are increasing worldwide, and their runoff from natural and anthropogenic sources can dramatically increase their environmental occurrence (Islam and Tanaka, 2004; Norgate et al., 2007).

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CHAPTER 3 - TRACE ELEMENT BIOACCUMULATION IN THE SEAGRASS *CYMODOCEA NODOSA* FROM A POLLUTED COASTAL LAGOON: BIOMONITORING IMPLICATIONS.

This chapter was treated as an article published in the journal 'Marine Pollution Bulletin' indexed in 'Scopus' and 'Web of Science' databases:

Boutahar, L., Espinosa, F., Sempere-Valverde, J., Selfati, M., Bazairi, H., 2021. Trace element bioaccumulation in the seagrass *Cymodocea nodosa* from a polluted coastal lagoon: Biomonitoring implications. Mar. Pollut. Bull. 166, 112209. https://doi.org/10.1016/j.marpolbul.2021.112209

Abstract

This is the first investigation of the potential for using *Cymodocea nodosa* to biomonitor trace element (TE) contamination in Marchica lagoon (Morocco), a Mediterranean pollution hotspot. We measured concentrations of seven TEs in seagrass tissues (leaf-rhizome-root) and sediments. Single and multi-element indices confirmed that sediments near illegal discharges were heavily polluted and we predict risks of frequent adverse biological effects in these areas. Four of the TEs increased concentrations in *C. nodosa* leaf and root along sediment pollution gradient. Leaves and roots were both good indicators of Cu and Cd contamination in sediment, whereas leaves are the best indicator of Zn and roots for Pb. This seagrass was not a bioindicator of Al, Cr and Ni contamination. These results show the bioaccumulation patterns of TEs in *C. nodosa*, and can be used to design biomonitoring programs.

Keywords: Pollution, Sediment ecological risk assessment, Bioavailability, Bioindicator, Marchica lagoon, Mediterranean.

1. Introduction

Pollution of coastal ecosystems by trace elements (TEs) has becoming a major environmental problem due to their toxicity, persistence, and bioaccumulation into the food chain which lead to potential threat to aquatic biota and human health (Zhuang and Gao, 2014; Suresh et al., 2015; Abreu et al., 2016; Ihedioha et al., 2017; Ali and Khan, 2018).

The entry of TEs within the aquatic environments arises from natural processes (i.e. geological weathering and soil erosion) and anthropogenic activities (i.e. urban sewage discharge, industrial wastewaters, fertilizer leaching from adjacent agricultural lands and riverine fluxes) (Vikas and Dwarakish, 2015; Duodu et al., 2017a; Boutahar et al., 2019). Then, they are accumulated in bottom sediment to levels significantly higher compared with the concentration of the overlying water (Benson et al., 2016; Simpson and Spadaro, 2016; Matache et al., 2018).

Various sediment quality indices have been widely applied to quantify the degree of TE contamination and evaluate their biological adverse risk in aquatic ecosystems (Duodu et al., 2016; Birch, 2018). However, high TE concentration in the sediment does not indicate their high bioavailability to the living organisms (Ralph et al., 2006). Accordingly, when determining the ecotoxicological relevance of TEs, it is critical to monitor their bioavailable form (Bradly et al., 2016; Duodu et al, 2017a). The worldwide adopted procedures to extract the active fraction of TEs in sediment use single step extraction by dilute acids and chelating agents (Sahuquillo et al., 2003; Hu et al., 2011) or multiple-sequential extraction methods (Tessier et al., 1979; Rauret, 1998; Cuong and Obbard, 2006). Although these approaches have brought significant knowledge on the interactions between TEs and sediment components, their ability to provide a robust evidence on the fraction interacting with organisms is limited (Mourier et al., 2011; Bradly et al., 2016; Liu et al., 2018). Because of this deficiency, biological indicators have been identified as the useful tool to assess the availability of TEs through the trophic levels (Farias et al., 2018).

Worldwide, seagrasses have been recommended as efficient biomonitor and bioindicator species to assess the contamination of the marine environment by TEs (Bonanno and Raccuia, 2018; Boutahar et al. 2019; Boutahar et al., 2020; Gopi et al., 2020; Jeong et al., 2021). As primary producers, seagrasses may be used as early detectors of contamination by TEs because they react more rapidly to the presence of contaminants than organisms from higher stages in the food web (Rainbow, 1995; Lafabrie et al., 2007; Govers et al., 2014; Bonanno and DI

Martino, 2016). Thus, seagrasses appears to be more tolerant to chemical contaminations than other marine flora (macroalgae, mangroves, kelp and saltmarsh plants; Lewis and Devereux, 2009). Moreover, seagrasses are able to properly reflect sediment pollution at volcanic marine seeps analogous to future ocean acidification conditions (Mishra et al., 2020a). The principal uptake path of TEs into seagrasses are acropetal translocation from sediment interstitial water into roots to rhizomes and leaves (Jackson, 1998), from overlying water to leaves then rhizomes (Ralph et al., 2006; Harguinteguy et al., 2016) or directly from sediments to photosynthetic tissues during low tides, when the leaves are lying on the sediment (Wasserman, 1990). However, it is widely proved that sediment act as a primary source of TEs for seagrasses (Govers et al., 2014; Harguinteguy et al., 2016).

The Marchica lagoon is the second largest lagoon on the Northern African coast (Ruiz et al., 2006). This ecosystem has major socioeconomic, cultural and ecological values (Najih et al., 2015; Selfati et al., 2019). With the rise in industrial and agriculture activities and urban development, the lagoon has been increasingly exposed to metal pollutants and thereby was classified as an environmental hotspot of pollution on the Mediterranean coast (UNEP/ EEA Report No 4, 2006; UNEP/MAP, 2012). Elevated levels of TEs in the sediment with the potential to pose moderate to relatively high ecological risk have been revealed previously in this area (Maanan et al., 2015). However, to the best of our knowledge, no investigation combining TE loads in sediment and their accumulation by leaving organisms has been performed in the lagoon until now.

In the studied area, the dominant seagrass species is *Cymodocea nodosa* (Ucria) Asch. This tropical origin phanerogam occurs mainly in the Mediterranean Sea and some locations in the North Atlantic, from southern Portugal and Spain to Senegal, and around Madeira and the Canary Islands (Green and Short, 2003; OSPAR Commission, 2010). *C. nodosa* is considered a pioneer species with high capacity to adapt to environmental variability; it colonises sandy to muddy pristine, as well as degraded coastal environments (Orfanidis et al. 2010). The usefulness of *C. nodosa* as a bioindicator of coastal TE contamination has been evaluated and proved in the Mediterranean Sea (Marin-Guirao et al., 2005; Malea and Kevrekidis 2013; Bonanno and Di Martino 2016; Serrano et al., 2019; Zakhama-Sraieb et al., 2019; Bonanno et al., 2020).

Regarding the metal pollution level of the Marchica lagoon, it is meaningful to carry out continuous monitoring and management of contaminants effects for conservation of aquatic organisms and the whole ecosystem. The present study aimed to be the first to determine if *C. nodosa* could serve as bioindicator of TE contaminations in the Marchica lagoon, and provide deep investigation on TE extent using both environmental and biological compartments as complementary approaches. For this purpose, several aims have been assessed: (i) the concentration of seven TEs in *C. nodosa* tissues (leaves, rhizomes and roots) and sediment; (ii) the degree of pollution and ecological risk level using a range of sediment pollution indices and numerical sediment quality guidelines (SQGs); (iii) the TE Bioconcentration Factor (BCF) to *C. nodosa* tissues from sediment and Translocation Factor (TF) within the seagrass tissues; (iv) the *C. nodosa* tissue that is more suitable as bioindicator of TE contamination.

2. Material and methods

2.1. Study area and sampling procedures

The Marchica lagoon (Fig. 1), commonly called lagoon of Nador or Sebkha Bou Areg (34°54'N–02°10'W and 35°17'N–03°05'W) is the largest lagoon of Morocco and second largest one in the southern shore of the Mediterranean Sea with an area of 115 km^2 for a depth not exceeding 8 m (Ruiz et al., 2006). It is oriented NW–SE and separated from the sea by a 25 km long sandy barrier. The communication with the sea is insured by an artificial entrance (300 m wide and 6 m deep) allowing the renewal of the water in the lagoon (Hilmi et al., 2015). Freshwater inputs to the lagoon are emanating from subterranean resurgences surface waters from the periodic flow of 10 small rivers (Maanan et al., 2015). The Marchica lagoon is wintering and stopover site for more than 150 species of birds (Dakki, 2003), a nursery area for commercial fish species and hosts several endemic and endangered species (Bocci et al., 2016; Selfati, 2020). It was declared by the Moroccan Ministry of Waters and Forests as a Site of Ecological and Biological Interest (AEFCS, 1996) and it has been included (2005) in the list of Wetlands of International Importance according to the RAMSAR convention Ramsar site (Dakki et al., 2011). In the addition to its ecological values, the lagoon provides important ecosystem services such as fishing, tourism, and agriculture (Najih et al., 2015).

However, these activities developed in the terrestrial and marine parts enhanced the human pressures within the lagoon resulting in high impact on its environmental quality (Maanan et al., 2015). The main sources of contamination are related to: (i) wastewaters from two cities and a village bordering the lagoon, namely Beni Ensar on the northwestern side, Nador on the southwestern side, and Kariat Arekmane on the eastern side; (ii) liquid effluents and solid waste runoff from the irrigation channels and oueds draining the southern border of the lagoon covered by agricultural fields; (iii) industrial waste discharges specially those of the village of Selouane dumped in the Oued Selouane and (iv) water runoff from the old iron mine in the Atalayoun promontory, and (v) port activities at Beni Ensar.

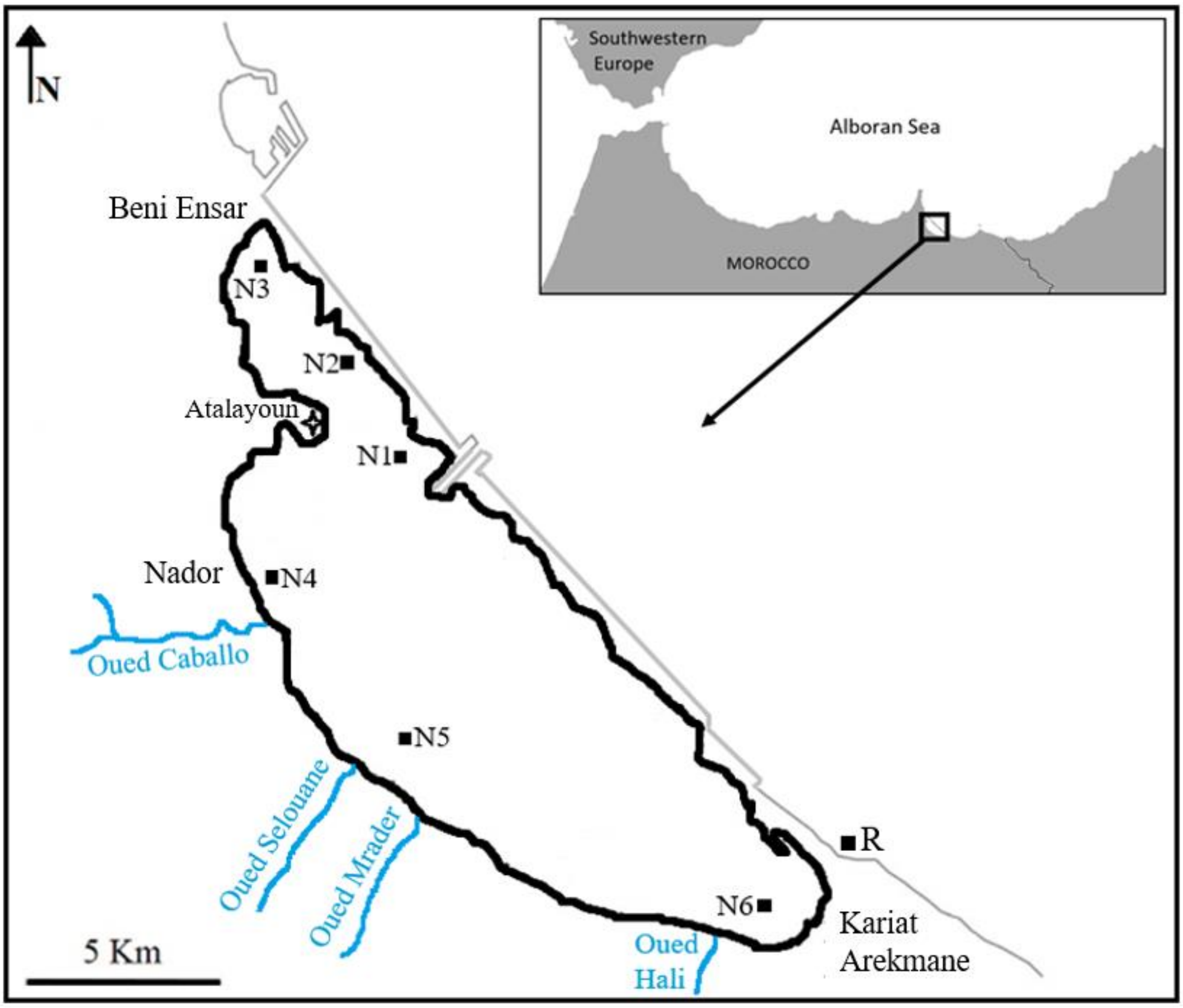

Fig. 1: Map of the six sampled stations in the Marchica lagoon (N1 to N6) and one reference station in the open sea (R). Stations were sampled for *C. nodosa* tissues (leaves, rhizomes and roots) and sediment.

Sampling was conducted by the end of September 2015 from monospecific dense *Cymodocea nodosa* meadows based on capturing all potential sources of pollution. Six sampling stations have been chosen within the lagoon: N1 near the pass, N2 from sandbar border between the pass and Beni Ensar, N3 at Beni Ensar city, N4 at Nador city, N5 near mouths of Oued Selouane, and N6 at Kariat Arekmane village. In addition, one station distant in the open sea (R) has been sampled to serve as a reference (Fig. 1). These stations encompassed depths from 0.5 m to 3.5 m.

At each station, random sampling (n = 3) of *C. nodosa* was performed using a cylindrical polyvinyl chloride (PVC) corer of 0.15 m in diameter and 0.12 m long. Cores were sieved through a 0.5 mm mesh size sieve and rinsed with lagoon water to eliminate residual sediment. Plant material was then separated into leaves, rhizomes and roots. Simultaneously, at each station, sediment was randomly sampled (n =3) in the vegetated seabed through a cylindrical PVC corer (0.15 m x 0.12 m). Each sediment core was subdivided into three homogeneous subsamples. All seagrass and sediment samples were transferred into plastic bags, stored on ice, transported to the laboratory and frozen until further analysis.

2.2. Samples preparation and analysis

In the laboratory, epiphytic algae were removed from *C. nodosa* leaves, rhizomes and roots by scraping with a glass slide (Dauby and Poulicek, 1995). Plant tissues and sediment were oven dried until constant weight (minimum 48 h at 50 °C). All samples were ground into a fine powder with an agate mortar and a pestle.

The first subsample of each sediment core was used for the determination of grain-size partitioning (gravel, sand and mud; Wentworth, 1922) using Afnor sieves (63 to 2000 µm).

Organic matter content was determined by the loss on ignition method (Heiri et al., 2001) into the second sediment core subsample. They were dried 12 h at 105 °C (DW_{105}), combusted during 4 h at 550 °C and weighed again (DW_{550}). The percentage of organic matter (OM) was defined by the following equation:

$$OM = ((DW_{105} – DW_{550})/DW_{105})*100$$

The third sediment core subsample was sifted through 63 µm mesh sieve for TE analysis. The mud fraction (< 63 µm) was selected because it provides the greatest surface area for contaminants adsorption (Jickells and Knap, 1984; Dassenakis et al. 1995).

Concentrations of cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), chromium (Cr) and aluminum (Al) in grinded seagrass samples and sediment mud fraction were measured at the UATRS-CNRST (Unité d'Appui Technique à la Recherche Scientifique-Centre National pour la Recherche Scientifique et Technique) laboratory, using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Jobin Yvan ULTIMA 2). Acid solution (H_2O_2/HNO_3, 2:3 ratio; Brix et al., 1983) was used as reagent. The digestates were filtred directly into 50 ml volumetric flasks through acid-washed filters (Whatman GF/C) and the final volume adjusted with distilled deionized water. It is recognized that total TE concentrations measured using strong acids as HF is generally not considered a good indicator of TE bioavailability and does not reflect their posed toxic effects (Shikazono et al., 2012; Brady et al., 2016). Instead, the use of partial digestion procedure extracts the adsorbed and organic fractions of metals bioavailable to organisms from sediment without digesting their mineral matrix (Brix et al., 1983). Standard reference materials were used to ensure the data validation. The analytical results of the quality control samples showed good agreement with the certified values. TE concentrations are reported in $mg.kg^{-1}{}_{DW}$ of sediment mud or seagrass tissues. The average and standard deviation of the TE concentrations formed the basis for further data analyses.

2.3. Data analysis

2.3.1. Sediment pollution assessment

Single and multi-element sediment indices were employed to assess the pollution level of the Marchica lagoon sediment. The selected indices are defined in the following.

Geo-accumulation index (I_{geo})

The I_{geo} method evaluate TE pollution in sediment with corresponding natural background level as reference (Varol, 2011). The I_{geo} index is calculated as (Muller, 1969):

$$I_{geo} = \log_2 [C^i / 1.5\ B^i]$$

where C^i and B^i are the measured concentration of TE i in the sediment sample and its geochemical background value respectively. The background values of Ni, Cd, Pb, Cu, Cr, Zn and Al in sediment of Marchica lagoon are 20.7, 0.3, 20, 37.5, 55, 70 ($mg.kg^{-1}{}_{DW}$), and 8 (%) respectively (Maanan et al., 2015). Factor 1.5 is the background matrix correction factor due to lithospheric (Zhuang and Gao, 2014), and anthropogenic (Goher et al., 2014) effects. The seven classes of the geo-accumlation index are (Muller 1981): unpolluted ($I_{geo} \leq 0$), slightly polluted ($0 < I_{geo} \leq 1$), moderately polluted ($1 < I_{geo} \leq 2$), moderately to strongly polluted ($2 < I_{geo} \leq 3$), strongly polluted ($3 < I_{geo} \leq 4$), strongly to extremely polluted ($4 < I_{geo} \leq 5$), and extremely polluted ($5 < I_{geo}$).

Enrichment factor index (EF)

The EF index is used to determine TE sources of anthropogenic or natural origin (Qingjie et al. 2008; Hu et al., 2013). This involves a geochemical normalization to reduce TE variability caused by grain size and mineralogy of sediment (Zhang and Shan, 2008). This is conducted with respect to reference elements such as Fe and Al (Zhang et al., 2007), Li and Cs (Pereira et al., 2007) or Ti, Mn and Sc (Salati and Moore, 2010). Aluminum (Al) was used as a reference element to calculate anthropogenic TE enrichments in Marchica lagoon as described by Maanan et al. (2015). EF is calculated as (Salomons and Förstner 1984):

$$EF = (C^i / C^{Al}) / (B^i / B^{Al})$$

where C^i and B^i are the measured concentration and the background value of TE i in the sediment respectively. C^{Al} and B^{Al} are the measured concentration and the background value of Al in the sediment. The EF consists of five classes (Acevedo-Figueroa et al., 2006): no enrichment (EF $\leq$ 1), minor enrichment (1 < EF $\leq$ 3), moderate enrichment (3 < EF $\leq$ 5), moderately severe enrichment (5 < EF $\leq$ 10), severe enrichment (10 < EF $\leq$ 25), very severe enrichment (25 < EF $\leq$ 50) and extremely severe enrichment (EF > 50).

Modified degree of contamination (mCd)

The mCd index, developed by Abrahim (2005), is a quick and efficient method of assessing sediment quality and pollution severity (Vu et al., 2017). It has an advantage over the degree of contamination index (Cd, Hakanson, 1980) because it takes into account the synergistic effects of contaminants at a study site (Brady et al., 2015). The mCd is determinate as:

$$mCd = \frac{\sum_{i=1}^{n} Cf^i}{n} = \frac{\sum_{i=1}^{n} (C^i / B^i)}{n}$$

where Cf^i is the contamination factor of TE i, C^i and B^i are the measured concentrations of the TE i in the sediment sample and its geochemical background values respectively. n is the number of analysed elements. The classification of mCd is (Abrahim and Parker, 2008): unpolluted (mCd < 1.5), low degree of contamination (1.5 $\leq$ mCd < 2), moderate degree of contamination (2 $\leq$ mCd < 4), high degree of contamination (4 $\leq$ mCd < 8), very high degree of contamination (8 $\leq$ mCd < 16), extremely high degree of contamination (16 $\leq$ mCd < 32) and ultra high degree of contamination (mCd $\geq$ 32).

Modified Nemerow pollution index (MNPI)

The NPI (Nemerow, 1991) has been considered a comprehensive tool reflecting effects of TE pollution (Duodu et al., 2016). Due to the use of contamination factor in the calculation of the index that does not take into consideration lithogenic and sedimentary inputs, an improved method for determining the pollution index was proposed by using enrichment factors (Brady et al., 2015). The equation for calculating MNPI is as follows:

$$\mathrm{MNPI} = \sqrt{\frac{\left(\mathrm{EF}_{\mathrm{average}}\right)^2 + \left(\mathrm{EF}_{\mathrm{max}}\right)^2}{2}}$$

where $EF_{average}$ is the arithmetic mean of enrichment factor of all measured TEs, and EF_{max} the maximum enrichment factor among the TEs. The sediment qualification using MNPI index is: unpolluted (MNPI < 1), slightly polluted ($1 \leq$ MNPI < 2), moderately polluted ($2 \leq$ MNPI < 3), moderately heavily polluted ($3 \leq$ MNPI < 5), heavily polluted ($5 \leq$ MNPI < 10), severely polluted (MNPI > 10).

Trace Element Pollution Index (TEPI)

The TEPI index compare global TE pollution levels among the monitored stations (Richir and Gobert, 2014) and is calculated as:

$$\mathrm{TEPI} = (Cf_1 * Cf_2 * \ldots Cf_n)^{1/n}$$

where Cf_n is the concentration of TE n in the sample. The higher the index value is, the more contaminated the monitored station is.

This index is a weighted version of the Metal Pollution Index (MPI, Usero et al., 1996) and allows, contrary to the MPI, the comparison of global TE contamination status between monitoring surveys whatever the list of monitored TEs and bioindicator used. Consequently, this index is computed for *C. nodosa* tissues as well.

2.3.2. Sediment biological and ecological risk assessment

Besides assessing the level of pollution, several sediment quality approaches have been developed to evaluate TE adverse biological and ecological effects according to their toxicity.

Sediment quality guidelines (SQGs)

Sediment Quality Guidelines predict biological effects in polluted sediment by providing setting safe levels for TEs in an aquatic ecosystem (Macdonald et al., 2000; Gao and Chen, 2012).

In this study, threshold effect concentration (TEC), probable effect concentration (PEC), effects range-low (ERL), effects range-median (ERM), lowest effect level (LEL) and severe effect level (SEL) were applied (Persaud et al., 1993; Long et al., 1995; MacDonald et al., 2000). These six guidelines values establish three levels in chemical concentrations, where adverse biological effects are rarely (< TEC, < ERL, < LEL), occasionally (≥ TEC and < PEC, ≥ ERL and < ERM, ≥ LEL and < SEL) and frequently observed (≥ PEC, ≥ ERM, ≥ SEL) (McCready et al., 2006; Zhang et al., 2018).

Based on the fact that TEs always occur in the sediment as complex mixtures that may have additive toxicity effects (Long and MacDonald, 1998), the mean ERM quotient (mERMQ) was utilized according to the following equations (Long et al., 1998a):

$$\mathrm{mERMQ} = \frac{\sum_{i=1}^{n}(C^{i}/\mathrm{ERM}^{i})}{n}$$

where C^i is the concentration of TE i in the sediment, ERM^i (effects range median) is the guideline values reported by Long et al. (1995) for the element i, and n is the number of measured TEs. The four classes of mERMQ are (McCready et al., 2006): low risk level with 9% probability of toxicity (mERMQ ≤ 0.1), medium low risk level with 21% probability of toxicity (0.1 < mERMQ ≤ 0.5), high medium risk level with 49% probability of toxicity (0.5 < mERMQ ≤ 1.5), high risk level with 76% probability of toxicity (mERMQ > 1.5).

Modified potential ecological risk index (MRI)

The initial ecology risk method (RI) introduced by Hakanson (1980) evaluates the combined pollution risk caused by overall levels of contamination and reflects the sensitivity of the biological community through the potential ecological risk coefficient and the toxicity response factor (Pejman et al., 2015; Ke et al., 2017). Nonetheless, RI could not account for the complex sediment behaviour since it uses a simple contamination factor in its determination (Duodu et al., 2017a). Modified potential ecological risk index (MRI) has been developed by Duodu et al.

(2016) to give a better assessment of risk by the utilisation of enrichment factor, which can normalise the impact of terrestrial sedimentary inputs. The calculation of MRI is as:

$$\mathrm{MRI} = \sum_{i=1}^{n} \mathrm{Er^i} = \sum_{i=1}^{n} \mathrm{Tr^i} \times \mathrm{EF^i}$$

where Er^i , Tr^i and EF^i are the potential ecological risk coefficient, the toxicity response factor and the enrichment factor of the TE i respectively. n is the number of TEs. The standardized toxic response factor proposed by Hakanson (1980) for Ni, Cd, Pb, Cu, Cr and Zn are 5, 30, 5, 5, 2 and 1 respectively.

The modified ecological risk of coastal sediments posed by TEs is categorized into four classes: low potential risk ($E^i_r < 40$, MRI < 95), moderate risk ($40 \leq E^i_r < 80$, $95 \leq$ MRI < 190), considerable risk ($80 \leq E^i_r < 160$, $190 \leq$ RI < 380), high risk ($160 \leq E^i_r < 320$), and very high risk ($E^i_r \geq 320$, RI ≥ 380).

2.3.3. Bioconcentration and translocation factors

The bioconcentration factor (BCF; Lewis et al, 2007) is based on the assumption of a linear relationship between seagrasses and sediment TE concentrations. It represents the most common method to model the potentiality of seagrasses for accumulating TEs from sediment (Mountouris et al., 2002). Low BCF values are indicative of low bioaccumulation, whereas high values indicate active uptake. BCF of each element from sediment to *C. nodosa* tissues was calculated as follows:

BCF = mean TE concentration in a seagrass tissues (in $mg.kg^{-1}_{DW}$) / mean TE concentration in sediment (in $mg.kg^{-1}_{DW}$).

The translocation factor (TF) estimates the TE mobility within the different *C. nodosa* tissues. A higher TF values reflects a greater capacity of internal translocation (Deng et al. 2004). TF was calculated as:

$$TF_{leaf/root} = C^i_{leaf}/C^i_{root}$$

$$TF_{rhizome/root} = C^i_{rhizome}/C^i_{root}$$

$$TF_{leaf/rhizome} = C^i_{leaf}/C^i_{rhizome}$$

where C^i_{leaf}, $C^i_{rhizome}$ and C^i_{root} are the mean concentrations of the TE i in leaves, rhizomes, and roots of *C. nodosa* respectively.

2.3.4. Statistical analyses

A one-way Anova test was carried out to determine significant differences between stations mean TE concentrations in sediment and *C. nodosa* tissues followed by a Tukey HSD pairwise comparison test of means. Prior to the analysis, the normality and homogeneity of variance assumptions were checked and, when necessary a log (1 + x) transformation data was applied. A non-parametric analysis of variance (Kruskal-Wallis test) was performed when normality and/or homoscedasticity assumptions were not achieved, followed by a Dunn pairwise comparison test of means. The critical level of significance is set at 0.05 (5%).

A two-way ANOVA was used to analyse the variability in TE concentrations among plant tissues as fixed factor with three levels (leaves, rhizomes, roots) and stations as a random factor with seven levels, orthogonal to tissues factor. Post hoc pairwise comparison of the means (Tukey's honestly significant difference test) was performed for factor tissue.

Spearman's rank correlation analysis was calculated to test TE associations in *C. nodosa* tissues and TEPI values, and correlations between TE concentrations, MNPI, mCd, TEPI, mERMQ and MRI values, OM, sand and mud contents for sediment samples. A third Spearman's correlation coefficient analysis was performed to assess the relationship between TE concentrations and TEPI values in sediment and *C. nodosa* tissues.

Principal component analysis (PCA) and cluster analysis (CA) were performed on matrices of centered and reduced data. For the sediment matrix, sampling stations were the objects and TEs, mCD, MNPI, TEPI, mERMQ and MRI values, OM, sand and mud contents were the variables. For the *C. nodosa* matrix, sampling stations were the objects and TEs and TEPI value in the three tissues were the variables. Samples were clustered using Ward's method (average Euclidean distance between objects as measure of similarity).

All statistical analysis was completed in RStudio version 1.1.383 (R Studio Team, 2018), using R's base function (R Core Team, 2018).

3. Results

3.1. Sediment

Sediments granulometry of the seven stations varied from mud to unsaved gravel (Fig. 2). The reference station (R) with the two stations of the lagoon shorelines near the inlet (N1 and N2) were in the fine sands textural group with a mud fraction less than 5 %. N6 samples were muddy sand sediment while N3-N4-N5 samples were sandy mud sediment. A significant negative correlation (r = -0.96, $p < 0.05$, Table S1) was found between sand and mud sediment contents. Organic matter (OM) values ranged between 2.15 % for N1 and 6.18 % for N3 (Fig. 2). Lower values were observed for the three sandy stations R (2.42 %), N1 (2.15 %) and N2 (2.31 %). A high correlation was found between sediment sand and mud composition and OM content without being statistically significant (r = -0.71 and r = 0.75 respectively, $p > 0.05$).

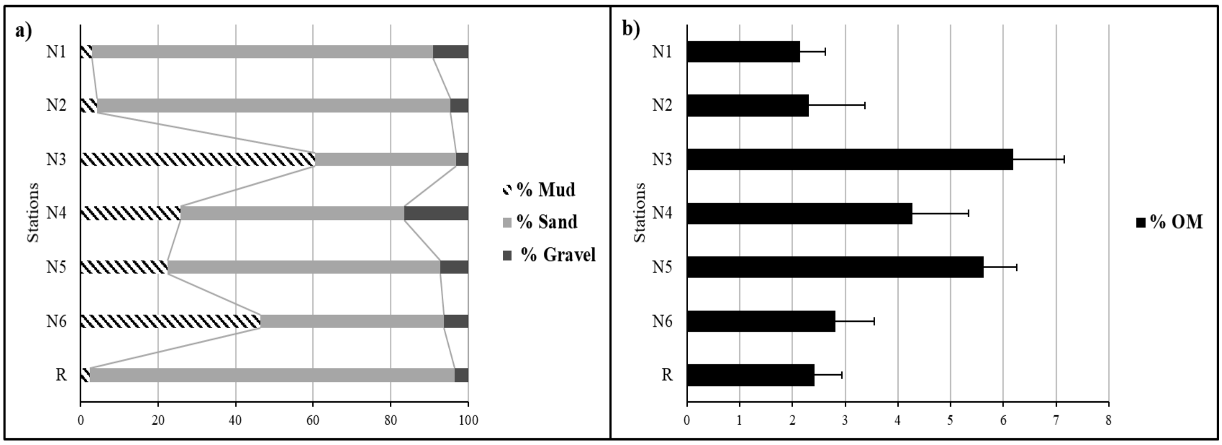

Fig. 2: Sediment characteristics of six stations (N1 to N6) sampled from the Marchica lagoon and one reference station in the open sea (R). a) Relative mean contribution (%) of mud, sand and gravel to the grain size composition (n = 3). b) Percentage of organic matter content (OM, n = 3). Bars represent standard deviation.

The mean concentrations of the seven TEs ($mg.kg^{-1}{}_{DW}$) in the < 0.0625 mm grain size sediment fraction throughout the sampled stations are listed in Table 1. Average concentrations decreased in the order of Al > Zn > Pb > Cu > Cr > Ni > Cd. Maximum contents were found near the urban effluent of Beni Ensar (N3), Nador (N4) and the mouth of the Oued Selouane (N5) stations. Minimum values were observed in reference station (R), near Kariat Arekmane (N6) and the shorelines stations (N1 and N2). Mean concentrations of all TEs in the three former lagoon stations exceeded the local geochemical background (LGB) of non-contaminated sediment (Table S2). Only Cd was above the LGB in the three later lagoon stations.

Table 1: Trace element concentrations (CC, mean ± SD, in mg.kg$^{-1}_{DW}$, n = 3) in sediment and *C. nodosa* tissues (leaves, rhizomes and roots) sampled from six stations in the Marchica lagoon (N1 to N6) and one reference station in the open sea (R). The coefficient of variation in concentrations (CV) between stations for each of the seven TE is calculated. Letters represent significant difference between the concentrations of a given element in the same compartment (sediment or one of the seagrass tissues).

		Trace element						
		Al	Zn	Pb	Cu	Cr	Ni	Cd
Sediment								
	N1	51688 ± 9641^{ab}	70.19 ± 13.0^{ab}	19.8 ± 5.43^{a}	17.8 ± 6.2^{a}	38 ± 18.1^{ab}	24.4 ± 9.43^{a}	0.91 ± 0.38^{ab}
	N2	61706 ± 24372^{abc}	59.48 ± 18.8^{ab}	43.5 ± 11.1^{a}	24.9 ± 5.65^{a}	32.8 ± 10.9^{ab}	16 ± 7.13^{a}	1.08 ± 0.26^{ab}
	N3	130826 ± 29908^{d}	816 ± 204^{c}	229 ± 28.8^{b}	102 ± 51.8^{a}	118 ± 47.6^{bc}	80.6 ± 8.09^{b}	3.02 ± 1.08^{b}
	N4	103820 ± 14598^{cd}	719 ± 84.6^{c}	138 ± 7.94^{c}	126 ± 36.4^{a}	137 ± 48.8^{c}	48.4 ± 9.71^{c}	2.31 ± 1.33^{ab}
	N5	97698 ± 6941^{bcd}	820 ± 383^{c}	207 ± 17.7^{b}	237 ± 82.2^{b}	90.8 ± 39.2^{ab}	53.7 ± 7.17^{c}	2.19 ± 1.06^{ab}
	N6	59074 ± 18800^{abc}	82.2 ± 25.4^{b}	29.7 ± 6.76^{a}	27.8 ± 7.71^{a}	27.2 ± 16.1^{a}	21.8 ± 5.21^{a}	1.23 ± 0.57^{ab}
	R	45024 ± 13662^{a}	33.2 ± 10.6^{a}	30.9 ± 5.56^{a}	20.1 ± 2.22^{a}	24.5 ± 8.71^{a}	20.7 ± 4.86^{a}	0.41 ± 0.39^{a}
	CV%	37	92	84	95	64	61	47
Leaves								
	N1	503 ± 203^{a}	67.3 ± 15.2^{a}	3.45 ± 1.79^{a}	9.08 ± 5.82^{a}	4.84 ± 2.49^{a}	28.7 ± 10.5^{b}	0.72 ± 0.29^{a}
	N2	473 ± 140^{a}	63.8 ± 20.8^{a}	5.89 ± 3.02^{a}	13.2 ± 6.01^{a}	1.62 ± 0.6^{a}	8.48 ± 3.65^{a}	1.12 ± 0.66^{a}
	N3	580 ± 324^{a}	136 ± 69.2^{a}	6.52 ± 1.98^{a}	18.3 ± 4.95^{a}	4.01 ± 1.5^{a}	19.4 ± 7.84^{ab}	1.42 ± 0.23^{a}
	N4	603 ± 205^{a}	109 ± 41.6^{a}	23.2 ± 11.2^{b}	17.3 ± 10.5^{a}	16 ± 9.14^{b}	24.4 ± 5.17^{ab}	1.63 ± 0.55^{a}
	N5	1160 ± 575^{a}	120 ± 79.9^{a}	11.2 ± 4.96^{ab}	20.1 ± 9.14^{a}	3.29 ± 1.41^{a}	21.3 ± 3.58^{ab}	2.27 ± 0.99^{a}
	N6	835 ± 159^{a}	102 ± 59.8^{a}	6.89 ± 3.15^{a}	8.07 ± 2.75^{a}	4.52 ± 2.95^{a}	13.3 ± 2.93^{ab}	1.09 ± 0.18^{a}
	R	385 ± 119^{a}	66.2 ± 31.8^{a}	2.24 ± 1.25^{a}	6.16 ± 1.74^{a}	3.31 ± 2.53^{a}	10.2 ± 5.49^{a}	1.07 ± 0.57^{a}
	CV%	38	29	75	35	90	38	39
Rhizomes								
	N1	123 ± 88.1^{a}	14.7 ± 4.04^{a}	2.56 ± 1.33^{a}	2.34 ± 0.96^{a}	3.13 ± 0.91^{a}	12.7 ± 3.55^{c}	0.18 ± 0.09^{a}
	N2	197 ± 95.3^{a}	24.6 ± 11.8^{ab}	2.49 ± 0.94^{a}	5.19 ± 2.71^{a}	2.82 ± 1.3^{a}	5.45 ± 2.55^{abc}	0.82 ± 0.49^{a}
	N3	464 ± 209^{ab}	35.5 ± 13.5^{ab}	9.35 ± 4.8^{ab}	6.29 ± 2.68^{ab}	2.57 ± 1.1^{a}	2.18 ± 1.02^{a}	0.69 ± 0.5^{a}
	N4	1206 ± 402^{bc}	31.2 ± 16.1^{ab}	16.2 ± 5.97^{b}	8.62 ± 3.93^{ab}	1.63 ± 0.77^{a}	6.23 ± 2.22^{abc}	0.93 ± 0.37^{a}
	N5	1500 ± 517^{c}	48.3 ± 14.7^{b}	8.34 ± 2.14^{ab}	15.3 ± 4.7^{b}	6.35 ± 4.23^{a}	10.6 ± 3.99^{bc}	1.15 ± 0.75^{a}
	N6	854 ± 202^{abc}	26.4 ± 10.2^{ab}	1.67 ± 0.63^{a}	10.8 ± 5.13^{ab}	3.25 ± 1.71^{a}	4.21 ± 1.75^{abc}	0.69 ± 0.38^{a}
	R	280 ± 146^{a}	11.3 ± 5.99^{a}	1.02 ± 0.39^{a}	5.34 ± 2.51^{a}	1.44 ± 0.86^{a}	2.67 ± 1.67^{a}	0.14 ± 0.05^{a}
	CV%	77	38	84	56	49	58	44
Roots								
	N1	864 ± 145^{ab}	32.8 ± 10.9^{ab}	6.12 ± 3.89^{a}	8.48 ± 2.69^{a}	4.26 ± 2.16^{a}	32 ± 13.7^{a}	1.42 ± 0.95^{a}
	N2	365 ± 138^{a}	14.5 ± 5.96^{a}	11.1 ± 5.08^{a}	11 ± 4.32^{a}	3.76 ± 1.4^{a}	15.1 ± 6.22^{a}	1.2 ± 0.12^{a}
	N3	901 ± 126^{ab}	66.2 ± 23.6^{ab}	23.7 ± 11.06^{a}	14.5 ± 6.78^{a}	5.72 ± 3.47^{a}	25.7 ± 11.7^{a}	1.69 ± 0.35^{a}
	N4	1866 ± 864^{bc}	81.3 ± 28.7^{b}	56.4 ± 20.9^{b}	16.3 ± 5.58^{a}	6.48 ± 1.32^{a}	22.3 ± 10.8^{a}	1.89 ± 0.9^{a}
	N5	2514 ± 645^{c}	53.3 ± 22.8^{ab}	20.2 ± 8.61^{a}	19.1 ± 8.24^{a}	7.15 ± 3.24^{a}	19.4 ± 6.02^{a}	1.98 ± 0.49^{a}
	N6	1114 ± 685^{ab}	22.1 ± 12.5^{a}	12 ± 5.52^{a}	17.2 ± 14.8^{a}	5.83 ± 3.05^{a}	21.6 ± 8.1^{a}	1.58 ± 0.34^{a}
	R	635 ± 134^{ab}	26.6 ± 16.3^{a}	8.48 ± 4.53^{a}	7.68 ± 4.11^{a}	4.83 ± 1.59^{a}	6.28 ± 1.85^{a}	0.68 ± 0.37^{a}
	CV%	61	58	84	28	23	25	18

The $1.02 \leq I_{geo} \leq 2.75$ values of Cd (Table S2) in the lagoon stations suggest that their sediment are slightly to strongly polluted by this TE. For the other six TEs, only N3-N4-N5 stations showed a slightly to strongly polluted levels, while the remaining stations would rank as unpolluted. The spatial distribution of EF values (Table S2) showed that all sampling stations had minimum Ni enrichment. No enrichment by Cr and Cu were found at R-N1-N2-N6 stations comparatively to the moderate input at N5 station near the mouth of the Oued Selouane. For Cd, minimum enrichment was found at reference station R, moderate enrichment at stations N1-N2 and moderately severe enrichment at N3-N4-N5-N6 stations. The maximum EF values of Pb and Zn (> 5) were found at N3-N4-N5 stations indicating their moderately severe enrichment.

The assessment of sediment pollution based on multi-TE indices indicates that the TEPI values followed the sequence: N3 (1.45) > N5 (1.44) > N4 (1.41) > N6 (1.19) > N2 (1.18) > N1 (1.17) > R (1.13). Similarly, the elevated mCd values were found in samples from N3-N4-N5 stations detecting high degree of pollution. Intermediate values were found in N6 samples suggesting low pollution level while lower values of N1-N2-R samples pointed out an unpolluted state (Fig. 3). The MNPI index also classified N3-N4-N5 stations as highly polluted. Whereas, N6-N2-N1 stations were ranked as moderately heavily polluted and moderately polluted statue was contributed to R station (Fig. 3). The three pollution assessment indices showed a high correlation level ($0.86 < r < 1.00$, $p < 0.05$). mCd and TEPI exhibited the same pattern with significant correlations with OM, mud, sand and all TEs except Cr (Table S1). Instead, no significant associations have been recorded for MNPI with Al, Pb, mud and sand contents.

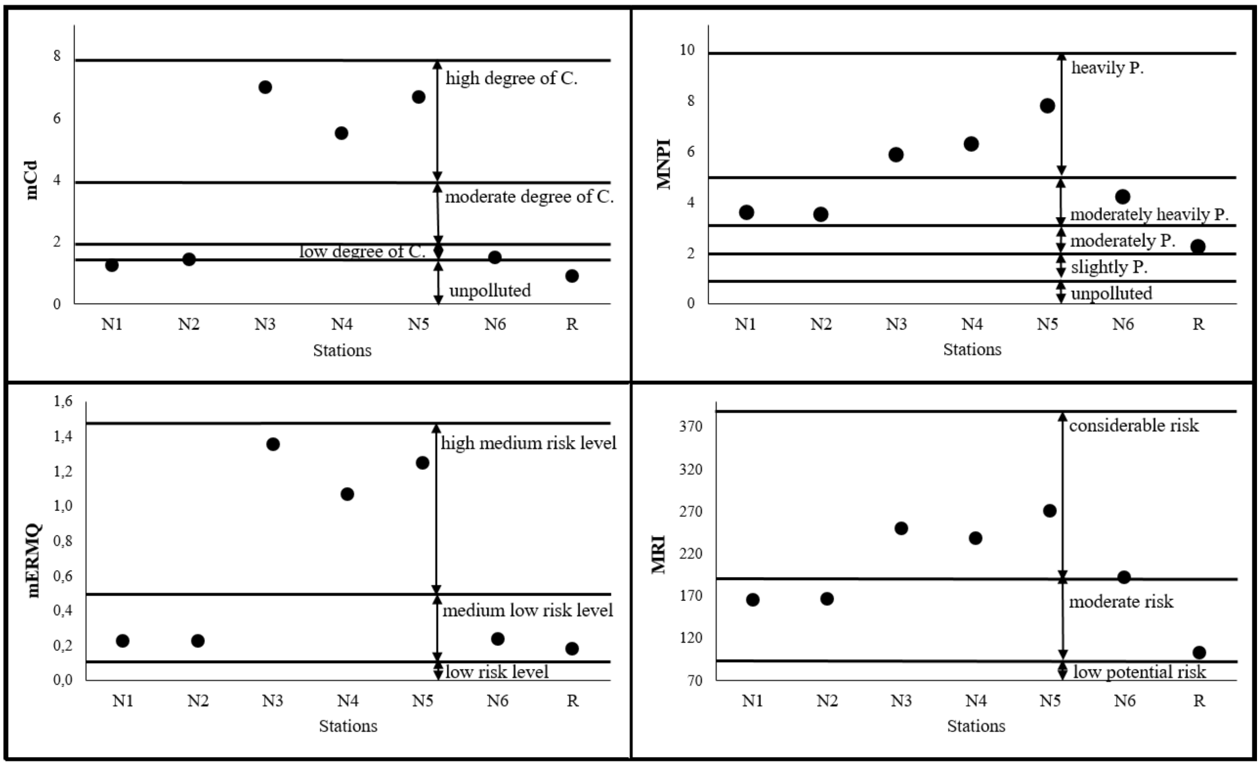

Fig. 3: The spatial distribution of pollution degree posed by the seven investigated TEs using Modified Degree of Contamination (mCd) and Modified Nemerow Pollution Index (MNPI). The ecological adverse risk is evaluated using the Mean ERM Quotient (mERMQ) and Modified Potential Ecological Risk Index (MRI). (N1 to N6): stations sampled in the Marchica lagoon; (R): reference station in the open sea.

The two first principal components PC1 and PC2 resulting from the PCA analysis explained together 94 % of the total variance of the data set with TE concentrations, OM, sand and mud contents, mCD, MNPI, TEPI, mERMQ and MRI indices values (Fig. 4). The dominating features in the PC1 explaining 85 % of the total variance of the data set were mCD, TEPI, mERMQ, Zn, Cd, OM, Al, Pb, Ni, MRI, MNPI, Cr, and Cu (loading values of respectively 0.99, 0.99, 0.99, 0.98, 0.98, 0.97, 0.97, 0.97, 0.94, 0.93, 0.91, 0.91, and 0.82). The PC2 explained 8.9 % of the total variance and was weighted by Sand (0.65) and Mud (-0.71). According to cluster analysis (Fig. 4), the first cluster included N1, N2 and the reference station R. They were characterized by the highest sand content and the lowest levels of the seven investigated TEs, as well as the lowest MRI, MNPI and mud values. The second cluster was formed by N3, N4, and N5 stations that displayed high concentrations of all TEs and high values of TEPI, mERMQ, mCd, MNPI, and OM. Within this group of stations, Ni, mud and OM contents were greater at N3, maximum levels of Cu and sand were found at N5, while both stations displayed highest values of Pb. N4 station showed intermediate values of Cu, sand and mud, lowest values of Pb and OM and highest level of Cr with N3 station. The third cluster was formed by N6 station with intermediate values of all variables comparing to the former clusters.

To place the observed TEs levels into an ecotoxicological context, concentrations are compared with TEC, PEC, ERL, ERM, LEL and SEL values (Table S2). These comparisons suggest a rarely to occasionally Cd biological effect in all the lagoon stations. Cr, Cu, Ni, Pb and Zn are at presumptively toxic levels close to Beni Ensar (N3), Nador (N4) and Oued Selouane (N5). In the reference station, only Cu and Ni concentrations exceeded LEL value. The mERMQ index indicated that N3-N4-N5 stations, near developed anthropogenic activities areas, with mean values of 1.35, 1.07, and 1.25 respectively, present 49% probability of toxicity, while the rest of the stations, present medium-low risk level with 21% probability of toxicity (Fig. 3). According to the potential ecological risk index (E^i_r) of single investigated TE, the severity of pollution decreased in the following sequence: Cd (152) > Pb (21.4) > Cu (9.28) > Ni (8.82) > Zn (4.25) > Cr (2.25). The corresponding potential ecological risk index (MRI) ranged between 103 at R station and 270 at N5 station indicating low, moderate and considerable potential ecological risk at R, N1-N2-N6 and N3-N4-N5 stations respectively (Fig. 3).

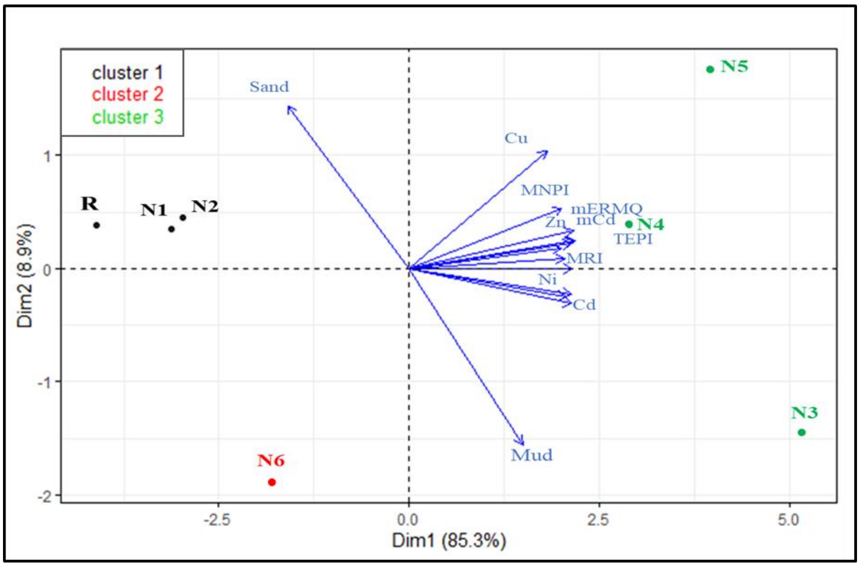

Fig. 4: Factor loading with stations plot for the first two principal components identified in the PCA of trace element concentrations, organic matter (OM), mud and sand contents, Modified Nemerow Pollution Index (MNPI), Modified Degree of Contamination (mCd), Trace Element Pollution Index (TEPI), mean ERM Quotient (mERMQ) and Modified Potential Ecological Risk Index (MRI) values for sediment. The seven stations are color-grouped according to their classification after CA. (N1 to N6): stations sampled in the Marchica lagoon; (R): reference station in the open sea.

3.2. *Cymodocea nodosa*

Trace elements were found in detectable concentrations in the *Cymodocea nodosa* tissues in all study stations. Average TE concentrations (Table 1) decreased in the order:

• Al > Zn > Ni > Cu > Pb > Cr > Cd in leaves;

• Al > Zn > Cu > Ni > Pb > Cr > Cd in rhizomes;

• Al > Zn > Ni > Pb > Cu > Cr > Cd in roots.

The seven TEs analysed showed significant spatial variability and significant variability among tissues ($p < 0.05$) while there was significant interaction only for Pb and Cr (Table S3). Rhizomes displayed the lowest TE values conversely to leaves and roots. Zn accumulated mostly in leaves, while Al and Pb did in roots, and Cu, Cr, Ni and Cd accumulated equally in both tissues (Table S3).

The two first principal components resulting from the PCA explained 60 %, 66 %, and 70 % of the total variance of the data set with TEs and TEPI values for leaves, rhizomes and roots respectively (Fig. 5). According to the cluster analysis performed on leaves data set (Fig. 5 a), the cluster formed by N4 station was characterized by the higher level of Cr, Pb and TEPI values. The second cluster included N3- N5 stations containing high concentrations of Zn, Cu and Cd. Low levels of Cd, Zn, Pb, Cu and TEPI were found in the last cluster linking R-N1N2-N6 stations. For rhizomes data set, the CA (Fig. 5 b) separated the seven stations to three groups with the highest TEPI and five TEs contaminations levels (Al, Zn, Cu, Cd and Cr) were found at N5 station. N3-N4-N6 stations were characterised by high Pb content while the lowest concentrations of the investigated TEs were found at N1-N2-R stations. The CA performed on roots data set (Fig. 5 c) linked N4- N5 stations with highest Al, Cr, Cd and TEPI levels. The second cluster (N3-N6 stations) was characterized by the highest concentrations of Ni. The last cluster (N1-N2-R stations) showed the lowest TEPI and TEs levels.

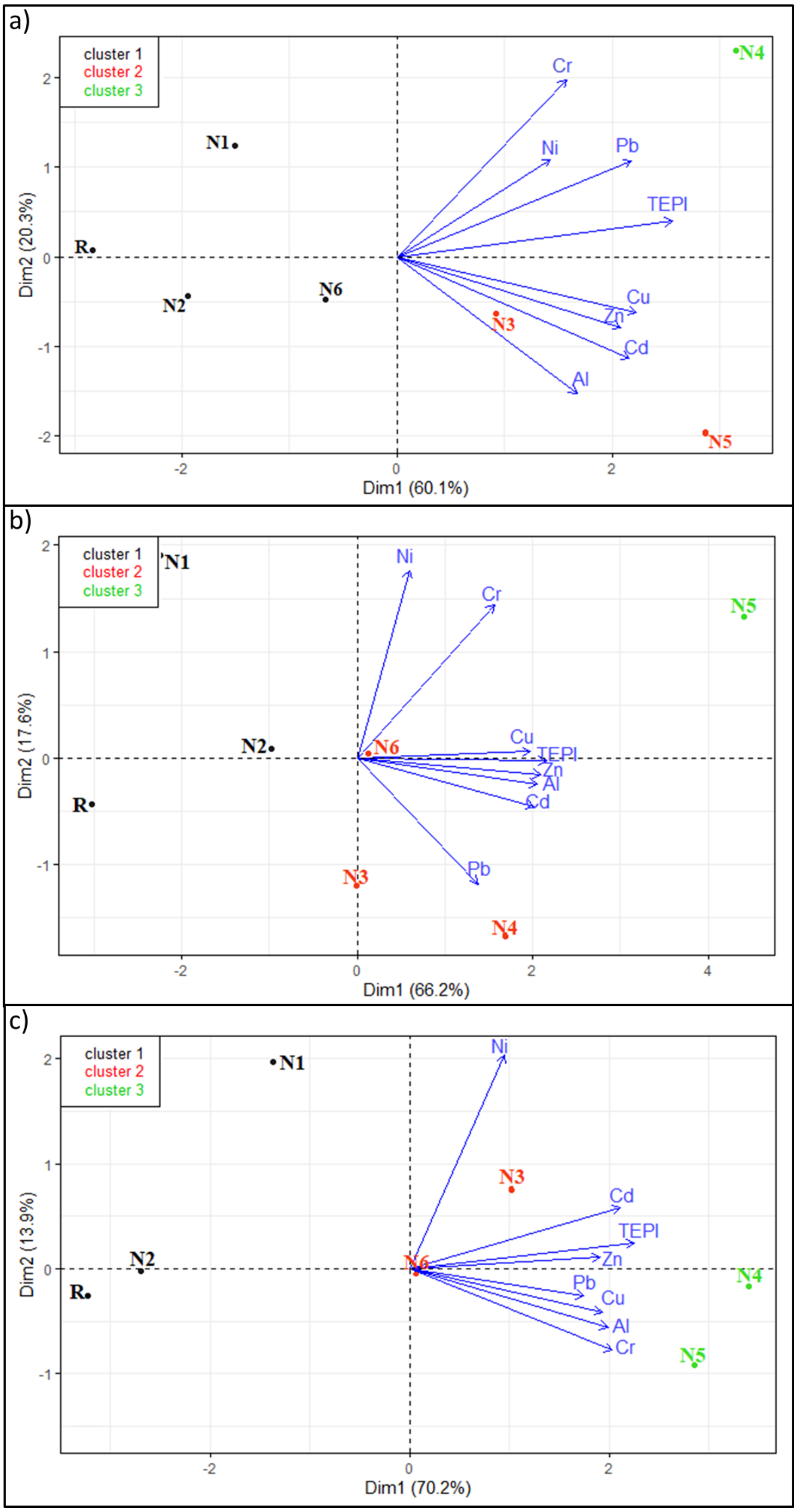

Fig. 5: Factor loading with stations plot for the first two principal components identified in the PCA of trace element concentrations and Trace Element Pollution Index (TEPI) values for *C. nodosa* tissues: a (leaf), b (rhizome) and c (root). The seven stations are color-grouped according to their classification after CA. (N1 to N6): stations sampled in the Marchica lagoon; (R): reference station in the open sea.

The highest mean concentration of Zn, Cu and Cd in leaves were observed at N3-N4-N5 stations (one-way ANOVA, $p < 0.05$). Great Pb and Cr values were found at N4-N5 and N1-N4 stations respectively while Ni was higher at N1-N3-N4-N5 ($p < 0.05$). Considering rhizomes, highest Pb value was found at N4 station while N5 station displayed the highest levels of five TEs: Cu, Zn, Cr, Ni and Cd ($p < 0.05$). For roots, Zn, Pb and Cd were greater at N3-N4-N5 stations ($p < 0.05$). Cu and Cr were also higher at N4-N5 stations while N1-N3-N4 stations displayed the highest level of Ni ($p < 0.05$).

According to TEPI index values, the level of the total TE contamination in *C. nodosa* leaves and roots of the seven stations decreased similarly in the following order: N4 > N5 > N3 > N6 > N1 > N2 > R (Fig. 6). For leaves, TEPI values correlated significantly with Al, Zn, Pb and Cd ($0.79 < r < 0.93$, $p < 0.05$, Table S4). Considering roots, all TEs except Ni correlated with TEPI values ($0.79 < r < 0.96$, $p < 0.05$, Table S4). For *C. nodosa* rhizomes, the level of the total TEs contamination showed this decreasing trend: N5 > N4 > N3 > N6 > N2 > N1 > R (Fig. 6). Positive correlations were observed between TEPI and Al, Zn, Cu and Cd ($0.82 < r < 0.89$, $p < 0.05$, Table S4).

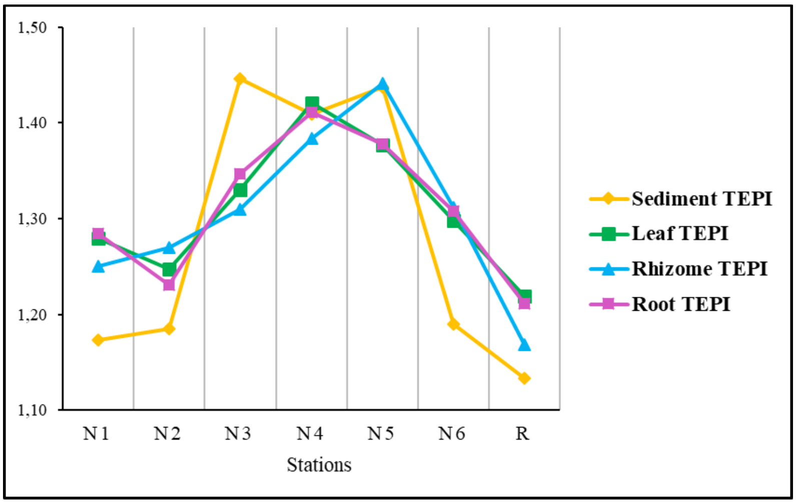

Fig. 6: Trace Element Pollution Index (TEPI) of sediment, *C. nodosa* leaves, rhizomes and roots computed for the seven investigated trace elements. (N1 to N6): stations sampled in the Marchica lagoon; (R): reference station in the open sea.

Trace element translocation differed between the three plant tissues (Table 2). Values in the rhizosphere (rhizome/root) varied from 0.29 for Pb to 0.80 for Zn. Leaf/rhizome translocation was >1 for all elements. Values of leaf/root of the seven TEs were highest for Zn (2.75) and lowest for Pb (0.45).

Table 2: Trace element average translocation factors (TF) in *C. nodosa* tissues from the Marchica lagoon.

	Al	Zn	Pb	Cu	Cr	Ni	Cd
$TF_{leaf/root}$	0.67	2.75	0.45	0.99	0.95	0.95	0.94
$TF_{leaf/rhizome}$	1.62	3.82	1.93	2.08	2.53	3.66	2.44
$TF_{rhizome/root}$	0.52	0.8	0.29	0.55	0.56	0.33	0.44

3.3. Relationships between sediments and *C. nodosa*

Significant positive relationships ($p < 0.05$) were obtained between sediment-leaves, sediment-rhizomes and sediment-roots for Zn-Cu-Cd (0.79 < r < 0.93), Zn-Cu (0.86 < r < 0.96), and Pb-Cu-Cd (0.79 < r < 0.86) respectively (Table 3). High BCF (> 1) were calculated for Cd in leaves and roots with values of 1.08 and 1.13 respectively (Fig. 7). In turn, relatively low BCF values were found in the three tissues for Al (< 0.02), Cr (< 0.12), Pb (< 0.26), Cu (< 0.32) and Ni (< 0.67). Besides, Zn in leaves was the only element with a BCF close to the unit (0.82). For this element in this tissue, high BCF values (> 1) were found at low sediment content stations R-N1-N2-N6 while it reached very low level at N3-N4-N5 stations where sediment Zn concentrations were above 700 $mg.kg^{-1}_{DW}$.

Table 3: Spearman's rank correlation test coefficients between the mean trace element concentrations and Trace Element Pollution Index (TEPI) values for sediment, *C. nodosa* leaves, rhizome and roots. Correlations significant at p < 0.05 are underlined and in bold.

	Al	Zn	Pb	Cu	Cr	Ni	Cd	TEPI
sediment-leaves	0.50	**<u>0.93</u>**	0.5	**<u>0.79</u>**	0.39	0.64	**<u>0.79</u>**	**<u>0.82</u>**
sediment-rhizome	0.54	**<u>0.96</u>**	0.64	**<u>0.86</u>**	0,00	0.04	0.64	**<u>0.79</u>**
sediment-roots	0.50	0.68	**<u>0.79</u>**	**<u>0.86</u>**	0.46	0.61	**<u>0.82</u>**	**<u>0.82</u>**
leaves-rhizomes	**<u>0.82</u>**	**<u>0.86</u>**	0.68	0.43	-0.18	0.64	**<u>0.96</u>**	**<u>0.89</u>**
leaves-roots	**<u>0.93</u>**	**<u>0.79</u>**	**<u>0.86</u>**	0.61	0.25	**<u>0.79</u>**	**<u>0.82</u>**	**<u>1.00</u>**
rhizomes-roots	**<u>0.89</u>**	0.57	0.75	**<u>0.89</u>**	0.29	0.29	0.71	**<u>0.89</u>**

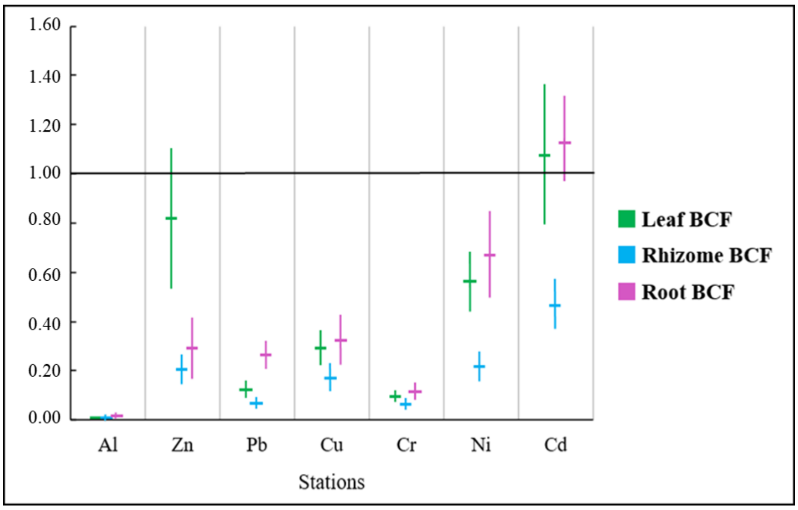

Fig. 7: Mean bioconcentration factor (BCF) from sediment to *C. nodosa* leaves, rhizomes and roots for the seven monitored trace elements int the Marchica lagoon. Bars represent standard deviation. Solid black line corresponds to the BCF value equal to 1.

4. Discussion

4.1. Sediment

The sedimentological study showed a high variability in granulometric composition with a strong affinity of sediment fine particles and organic matter content (OM). Low energy of the tidal surge in the inner stations enable the settlement of organic-rich fine sediment (Grecco et al. 2011). The presence of sand ripple marks near the lagoon inlet is an indicator of intense marine influence (Zourarah et al., 2007; Alaoui et al., 2010) resulting in the development of fine to medium sands poor in OM.

The investigated TEs showed a different distribution pattern throughout the studied stations matching their corresponding sediment characteristics. It is generally accepted that fine-grained particles tend to contain high concentrations of TEs due to additional adsorption sites with higher specific surface area (Fan et al., 2014; Mondal et al, 2018). However, in this study, only Al showed a significant negative correlation with sand ($r = -0.79$, $p < 0.05$), suggesting that fine fraction of sediment play important role in the distribution of this element. In contrast, the levels of TEs in sediment seems to be highly influenced by the OM content (Cd, Cu, Ni and Zn, $0.79 < r < 0.89$, $p < 0.05$) as it was reported by previous findings (Çevik et al., 2008; Guven and Akinci, 2013; Fan et al., 2014; Mujeeb et al. 2020).

The large coefficients of variation (CV > 36 %) within TE concentrations between stations, suggest that their spatial distribution might originate from different sources (Zhang et al. 2016).

The highest mean concentrations were found, near the domestic effluent of Beni Ensar (N3), public treatment station of Nador city (N4), and the mouth of the Oued Selouane (N5) supplied by agricultural drainage water. At these zones, the concentrations of Cd, Cr, Cu, Ni, Pb and Zn were respectively 10, 3, 6, 4, 11 and 12 fold higher than their local preindustrial background, underlining their external loading. The standardization of the TE contents compared to Al given by enrichment factor index (EF) is known to be the best approach identifying TE loads and distinction anthropogenic to natural sources (Hu et al., 2013; El Nemr et al., 2016). The EF values higher than 1 for the seven TEs at N3-N4-N5 stations, suggested that they are more likely to be human-induced (Acevedo-Figueroa et al., 2006). Moreover, the EF values of Cd, Pb, and Zn at N3-N4-N5, and Cu at N5 above 5 pointed out that they are severely enriched in these three locations.

Generally, results of the geo-accumulation index (I_{geo}) were consisted with the EF findings and classified N3-N4-N5 stations as moderately to strongly polluted. Yet, when EF indicated the presence of minimal enrichment, it was considered unpolluted by I_{geo}. Being a logarithmic index implies that I_{geo} is best used to qualify sediment with significant enrichment, which in turn reduces its sensitivity to minor contamination (Brady et al., 2015).

The main limitation of both abovementioned indices is that they are only applicable to a single element, whereas when multiple TEs are present together, they are more likely to have synergistic effects in the environment (Duodu et al., 2016). Therefore, the use of multi-elements indices, e.g. modified degree of contamination (mCd), modified Nemerow pollution index (MNPI), and trace element pollution index (TEPI), provide a more reliable understanding of whole sediment contamination behaviour (Yan et al., 2016). Results of the three multi-elements pollution indices clearly indicated that the sediment at N3-N4-N5 stations are the most impacted by the investigated TEs. However, a sharp contrast in the assessment of pollution is observed for the rest of stations. While mCd classified the sediments at N6 as slightly polluted and unpolluted at N1 and N2, MNPI revealed that their sediments were moderately heavily polluted. This contradiction can be directly related to the employment of the contamination factor and the enrichment factor, respectively, in their calculations. Contamination factor does not take into consideration lithogenic and sedimentary inputs, whereas enrichment factor can detect changes in sedimentary composition due to the use of normalisation element in its computation (Çevik et al., 2008; Brady et al., 2015).

The clustering of the seven stations according to TE concentrations and multi-elements indices values agreed well with the intensity of contamination found at N3-N4-N5 stations. This high degree of contamination was attributed by the seven TEs that displayed a significant high positive correlation between them and with at less one of the three multi-elements indices (mCD, MNPI, and TEPI). This found lead to the conclusion that these TEs are mainly derived from a common origin, i.e. an anthropogenic contribution. Previous study indicated that old iron mine located in the Atalayoun promontory significantly contributed to the excessive enrichment of many contaminants including Zn and Cd (Ruiz et al., 2006; Maanan et al., 2015). Bloundi (2005) and Zerrouqi (2009) reported that illegal urban and industrial discharges of Beni Enser and Nador cities could produce large quantities of Pb, Ni, Cr, Cu and Cd. Pb can also be related to shipping as these TE is commonly present in antifouling paints used on ships and boats (Burton et al., 2004). This might explain the moderate enrichment of the Pb found at the reference station R, which may be related to the presence of many motorized fishing boats. Besides, the extension of modern intensive agriculture in the farmlands around Oued Selouane using excessive pesticides and fertilizers and heavy machinery might result in the increase of Cu, Zn and Cd accumulation in this zone (Ruiz et al., 2006).

To place TE levels observed in the assessed sediment samples into an ecotoxicological context, concentrations comparison with sediment quality guidelines (SQGs) suggest that Cr, Cu, Ni, Pb and Zn at N3-N4-N5 stations are likely to result in a multiple environmental risk with frequent occurrence of adverse effects on living organisms. Although Cd mean concentration was the lowest in the sediment from the Marchica lagoon, high potential ecological risk of it was observed in all stations due to its high toxic-response factor. Similar findings indicating highest potential risk by Cd compared to its low concentrations in sediment have been reported by other studies (Benson et al., 2017; Liu et al., 2018; Zhang et al., 2018).

Based on multi-elements risk indices, spatial distribution of mean ERM quotient (mERMQ) and modified potential ecological risk index (MRI) values showed an obviously similar changing trend with the distribution of mCd, MNPI and TEPI. The sampling stations of N3-N4-N5, which were at the highest pollution level, posed the highest potential ecological risks among the seven sampling stations.

The mean concentrations of TE in the Marchica lagoon sediment were compared with the five Moroccan semi-enclosed coastal ecosystems from the Atlantic side: Moulay Bousselham, Sidi Moussa and Oualidia lagoons, National Park of Khnifiss and Dakhla Bay (Table S4). The comparison shows that Zn, Pb and Al values, and to a lesser extent, Cd, Ni and Cu were higher in Marchica lagoon while Cr level was higher in Sidi Moussa lagoon. In comparison with TE contents measured in others *Cymodocea nodosa* sediment (Table S4), Marchica lagoon showed higher levels than Spanish Mediterranean coast (Sanchiz et al., 2000), Marina Cap Monastir, Bizerte and Ghar El Melh lagoons from Tunisia (Zakhama-Sraieb et al., 2019), Sicily in Italy (Bonanno et al., 2017, Bonanno and Borg, 2018; Bonanno and Raccuia, 2018), Thermaikos Gulf in Greece (Malea and Haritonidis, 1999), and many volcanic seeps around Greece and Italy (Mishra et al., 2020a). In the other hand, Pb-Cd-Zn in Mar Menor lagoon (Spain, Marin-Guirao et al., 2005), Pb-Cd in Antikyra Gulf (Greece, Malea and Haritonidis,1995) and Cr-Ni from Evoikos Gulf (Greece, Nicolaidou and Nott, 1998) exceeded those measured in Marchica lagoon.

4.2. *Cymodocea nodosa* and relationships with sediment

Cymodocea nodosa in the Marchica lagoon accumulated all TEs detected in sediment and reflected their bioavailability for this seagrass. TE concentrations in *C. nodosa* tissues varied significantly among stations ($p < 0.05$). This variability maybe related to the plant phenology and growth dynamics, the element concentration in the external medium, and the environmental variables such as temperature, salinity, pH among others (Yang and Ye, 2009; Malea et al., 2013; Roberts et al., 2013; Birch et al., 2018). Highest TE concentrations for the three tissues were found in the polluted stations N3-N4-N5; this is especially true for Zn, Cu, Pb and Cd displaying positive correlations between seagrass and sediment concentrations. By comparing the global TEs contamination rate measured in sediment and plant tissues given by the TEPI index, similar stations ordination trend has been found in all compartments: N3-N4-N5 > N6 > N1-N2-R (Fig. 6). According to Brix and Lyngby (1982) and Villares et al. (2002), a seagrass is considered as an effective bioindicator when TE contents in it increase proportionally with TE contents in the surrounding environment.

In all stations, rhizomes showed the lowest TE contents. These results are in line with previous studies on other areas that reported the lowest TE concentrations in *C. nodosa* rhizomes (Marin-Guirao et al., 2005; Llagostera and Romero, 2011; Malea and Kevrekidis, 2013; Bonanno and Di Martino, 2016). This could be explained by potentially higher TE absorption capacity of leaves and roots since they are sites of ionic uptake in seagrasses (Romero et al., 2006).

The highest Zn concentration was located on the leaf material, whereas Pb and Al concentrations were higher in the roots while comparable concentrations were found for Cu, Ni, Cr and Cd. This alternation of leaves and roots as main bioaccumulator tissues suggest that *C. nodosa* in Marchica lagoon may adopt a mixed tolerance strategy based on the compartmentalization leading to accumulation of elements in the roots, and on removal strategy by losing temporary tissues (leaves). The compartmentalization in underground tissues is aimed as defensive strategy to protect the species against toxic effects of the photosynthetic tissue (Gratao et al., 2005; Willis et al., 2010; Bonanno and Vymazal, 2017). In turn, the removal strategy rely on active mobilization of toxic elements to the aerial part in order to remove dangerously high concentrations due to the high regeneration rates of leaves and to liberate the roots for more TEs to be absorbed (Malea and Haritonidis, 1999). Beside, rhizomes may act as transit tissues because of the high translocation from underground parts to leaves (Table 2).

Higher Zn concentrations in leaves were also reported in *C. nodosa* in Greece (Nikolaidou and Nott, 1998; Malea et al., 2013), Spain (Sanchiz et al., 2000; Llagostera and Romero, 2011), and Italy (Bonanno and Borg, 2018). As a micronutrient, Zn is involved in protein synthesis (Malea et al., 1995) and is required for seagrass photosynthesis and growth (Kabata-Pendias, 2011; Memon et al., 2001). This may explain its efficient accumulation and retention in *C. nodosa* leaves. Although this essential TE may become toxic if its concentrations exceed certain levels (Babula et al., 2008, Nagajyoti et al., 2010). In this study, high Zn leaves uptake values (BCF > 1) were recorded at lower sediment Zn contents (33 – 82 $mg.kg^{-1}{}_{DW}$) while it decreased to below one when sediment were highly contaminated (> 700 $mg.kg^{-1}{}_{DW}$). Marin-Guirao et al. (2005) mentioned similar observation from Mar Menor lagoon in Spain where *C. nodosa* leaves growing on contaminant-impacted stations accumulated significantly higher concentrations of TEs while BCF levels decreased markedly. However, the significant correlations found between Zn values in leaves and bottom sediment indicated that this tissue reflect sediment pollution gradient.

Al and Pb are both toxic TEs not required by plants growth (Nagajyoti et al., 2010; Prasad, 2013) and were found about two order of magnitude higher in roots than leaves and rhizomes. The investigations of Al concentrations in *C. nodosa* tissues are scarcely reported in literature. In this survey, Al concentrations in the seagrass do not reflect its concentrations in associated sediment as noted previously by Malea (1993) for *C. nodosa* growing at proximity to an aluminium factory without active accumulation for this TE. Concerning Pb, significant positive correlation was found between roots and sediment concentrations ($p < 0.05$) indicating that *C. nodosa* mainly absorb this TE by roots from the sediment. Previous investigations stated different accumulation pattern of Pb in *C. nodosa* either in leaves (Sanchiz et al., 2000; Marin-Guirao et al., 2005), roots (Llagostera and Romero 2011; Bonanno and Di-Martino, 2016) or both (Malea and Haritonidis, 1999). Concentrations of Pb in *C. nodosa* in the present study exceeded the critical toxicity level of this TE permissible in land plants tissues (Krämer, 2010), suggesting the possibility of adverse effects. However, Marin-Guirao et al. (2005) reported no significant sign of decline nor physiological alterations in photosynthetic tissues for *C. nodosa* growing at greater Pb sediment concentrations (11,000 ppm) and accumulating high Pb concentration in leaves (400 ppm).

The remaining investigated TEs, Cu, Cr, Ni and Cd, showed higher concentrations in roots without significant differences to leaves values ($p > 0.05$). The four elements displayed high levels of translocation from belowground tissues to leaves ($0.94 < TF < 3.66$). The translocation occurrence of these TEs is corroborated by the positive correlations between their concentrations and corresponding ones in different tissues. Our findings are consistent with other studies on *C. nodosa* suggesting root uptake with subsequent active mobilization to aboveground tissues for Cu, Cr, Ni and Cd (Malea and Haritonidis 1999, Sanchiz et al. 1999; Malea et al., 2013)**.**

In the case of Cu, concentrations in the three seagrass tissues were positively correlated with sediment concentration ($p < 0.05$). This result suggest that *C. nodosa* reflected faithfully the local availability of Cu in the sediment. Ralph and Burchett (1998) stated that high concentrations of essential micronutrients like Cu may end up being more toxic for plant species than non essential ones due to the presence of high uptake mechanisms for the formers and exclusion mechanisms for the latest. Fortunately, our results remained lower than those measured in *C. nodosa* from highly contaminated site by Cu, but within the range of those from medium levels areas (Table S4).

The Cd uptake for *C. nodosa* showed a mean BCF > 1 in leaves and roots, implying that these tissues tend to accumulate greater Cd concentrations than sediment. Thus, both tissues displayed positive correlations with sediment for this TE. High concentrations of Cd in seagrasses can induce the synthesis of metal-biomolecules such as phytochelatin and metallothionein that can reduce oxidative stress caused by metals (Alvarez-Legorreta et al., 2008; Wang et al., 2010). The mean Cd concentrations recorded in our survey for different *C.*

nodosa tissues were higher than values registered in impacted areas (Marin-Guirao et al., 2005; Serrano et al., 2019).

Regarding Cr and Ni, their mean concentrations in *C. nodosa* tissues showed no relationships to sediment values and consequently do not reflect sediment contents. Despite the higher level of Cr in Marchica lagoon sediment comparing to other areas, tissues concentrations were within the range recorded elsewhere (Table S4). This found is in agreement with previous study reporting low Cr concentrations in *C. nodosa* growing in polluted sediment (Nicolaidou and Nott, 1998). For Ni, results showed high roots BCF values in less contaminated stations while it decreased markedly in contaminated ones. Nevertheless, maximum Ni concentrations in roots recorded in this study were moderately higher than those from highly contaminated sites by this TE (Table S4). These findings are probably a consequence of the exclusion uptake mechanisms activated by the seagrass that cannot tolerate Cr and Ni toxicity.

Understanding the within-plant differences in TE accumulation capacity is of major importance when establishing biomonitoring programs using submerged plants (Llagostera and Romero 2011). Seagrass element accumulation is more element and tissue specific rather than species specific (Bonanno and Orlando-Bonaca, 2017). Roots, as permanent tissues, are more accurate for long-term biomonitoring, whereas leaves, given their periodical regeneration, should be used for short-term periods (Gosselin et al., 2006; Llagostera et al., 2011). Overall, this study support previous evidence on the *C. nodosa* significant TE accumulation capacity. However, higher accumulation can affect the seagrass physiological processes once critical concentrations are reached (Olive et al., 2017). Near submarine volcanic seeps where low pH increase the uptake and availability of TEs, *C. nodosa* enhanced leaf metabolic processes but not the production (Mishra et al., 2020b). Yet, the risk of the combined effects of TEs and toxic gases in these areas is compelling. Unfortunately, TE toxicity effects and tolerance mechanisms on seagrasses remain poorly investigated and generally under laboratory conditions (Ralph et al. 2006; Richir and Gobert, 2014; Goolsby and Mason, 2015; Moawad et al., 2020). Giving that amended sediment do not mimic the conditions of natural one (Van der Ent et al., 2013) and that physiological toxicity responses are species specific (Prange and Dennison, 2000), the phytotoxic thresholds are still unknown (Lin et al., 2016). Additional studies should also be prompted to identify sediment quality guidelines for seagrasses because most of them are based on toxicity levels for macrobenthic infaunal and epifaunal species (Lewis and Devereux, 2009).

5. Conclusion

This study corroborated previous evidences on the accurate use of *Cymodocea nodosa* as sensitive bioindicator of marine and coastal TE pollution. This potential is still not valued in the biomonitoring programs despite that this seagrass species fits different aspects of ideal bioindicator, e.g. wide distribution in the Mediterranean Sea, high accumulation capacity and tolerance to toxicity, sedentary life with availability all year round, and easiness of sampling. Moreover, seagrasses are not only relevant to assess the potential anthropogenic TEs inputs, but also as early sentinels of contamination transfer through the food web with consequent toxic risk at higher trophic level consumers up to humans. Consequently, it is recommended to include *C. nodosa* in TE pollution control and management plans of coastal marine ecosystems.

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Appendix A. Supplementary data

Table S1: Correlation matrix of non-parametric Spearman's rank correlation coefficients between sediment mean trace element concentrations, organic matter (OM), mud and sand contents, Modified Nemerow Pollution Index (MNPI), Modified Degree of Contamination (mCd), Trace Element Pollution Index (TEPI), mean ERM Quotient (mERMQ) and Modified Potential Ecological Risk Index (MRI) values. Correlation coefficients significant at $p < 0.05$ are in bold and underlined. Sediment was sampled from six stations in the Marchica lagoon (N1 to N6) and one reference station in the open sea (R).

Al	1.00														
Zn	**0.79**	1.00													
Pb	**0.86**	0.68	1.00												
Cu	**0.79**	**0.86**	**0.79**	1.00											
Cr	**0.86**	0.75	0.64	0.64	1.00										
Ni	0.71	**0.89**	0.64	0.64	**0.79**	1.00									
Cd	**0.96**	**0.86**	**0.79**	**0.82**	**0.82**	**0.79**	1.00								
OM	**0.79**	**0.82**	**0.86**	**0.86**	0.57	**0.79**	**0.86**	1.00							
Mud	**0.79**	0.71	0.54	0.64	0.54	0.61	0.89	0.75	1.00						
Sand	-0.71	-0.75	-0.43	-0.57	-0.57	-0.71	-0.86	-0.71	**-0.96**	1.00					
MNPI	0.75	**0.96**	0.61	**0.89**	**0.79**	**0.82**	**0.82**	0.75	0.64	-0.68	1.00				
mCd	**0.93**	**0.93**	**0.82**	**0.86**	0.75	**0.82**	**0.96**	**0.89**	**0.86**	**-0.82**	**0.86**	1.00			
TEPI	**0.93**	**0.93**	**0.82**	**0.86**	0.75	**0.82**	**0.96**	**0.89**	**0.86**	**-0.82**	**0.86**	**1.00**	1.00		
mERMQ	**0.93**	**0.93**	**0.82**	**0.86**	0.75	**0.82**	**0.96**	**0.89**	**0.86**	**-0.82**	**0.86**	**1.00**	**1.00**	1.00	
MRI	**0.86**	**0.96**	**0.79**	**0.93**	0.71	**0.79**	**0.89**	**0.86**	0.75	-0.71	**0.93**	**0.96**	**0.96**	**0.96**	1.00
	Al	**Zn**	**Pb**	**Cu**	**Cr**	**Ni**	**Cd**	**OM**	**Mud**	**Sand**	**MNPI**	**mCd**	**TEPI**	**mERMQ**	**MRI**

Table S2: Geo-accumulation Index (I_{geo}) and Enrichment Factor Index (EF) computed for sediment sampled from six stations in the Marchica lagoon (N1 to N6) and one reference station in the open sea (R). Local geochemical background (LGB) and sediment quality guidelines (SQGs) are also given. TEC: threshold effect concentration, PEC: probable effect concentration, ERL: effects range-low, ERM: effects range-median, LEL: lowest effect level, SEL: severe effect level.

N1	Igeo	--	-0.58	-0.6	-1.66	-1.17	-0.35	1.02
	EF	--	1.55	1.53	0.73	1.07	1.83	4.71
N2	Igeo	--	-0.82	0.54	-1.18	-1.33	-0.96	1.27
	EF	--	1.1	2.82	0.86	0.77	1,00	4.68
N3	Igeo	--	2.98	2.93	0.86	0.51	1.38	2.75
	EF	--	7.13	6.99	1.67	1.31	2.38	6.15
N4	Igeo	--	2.78	2.2	1.17	0.73	0.64	2.36
	EF	--	7.92	5.32	2.59	1.92	1.8	5.92
N5	Igeo	--	2.97	2.79	2.07	0.14	0.79	2.28
	EF	--	9.59	8.49	5.18	1.35	2.13	5.97
N6	Igeo	--	-0.35	-0.01	-1.02	-1.6	-0.51	1.46
	EF	--	1.59	2.01	1,00	0.67	1.42	5.57
R	Igeo	--	-1.66	0.04	-1.49	-1.75	-0.58	-0.13
	EF	--	0.84	2.75	0.95	0.79	1.78	2.44
LGB[1]		8 (%)	70.0	20.0	37.5	55.0	20.7	0.30
SQG-	TEC[2]	--	120	36	32	43	23	1
	PEC[2]	--	460	130	150	110	49	5
	ERL[3]	--	120	35	70	80	30	5
	ERM[3]	--	270	110	390	145	50	9
	LEL[4]	--	120	31	16	26	16	0.6
	SEL[4]	--	820	250	110	110	75	10

[1] Maanan et al., 2015
[2] MacDonald et al., 2000
[3] Long et al., 1995
[4] Persaud et al., 1993

Table S3: Results of two-way analysis of variance testing the effects of study stations and the three *Cymodocea nodosa* tissues (leaves, rhizomes and roots) on trace element (TE) concentrations, with Tuckey's honestly significant difference analysis for factor tissue. Bold number indicates significant differences settled at 0.05. Lv: leaves, Rz: rhizomes, Rt: roots.

TE	Source of variation	df	SS	MS	F	p-Value	Post hoc
Zn	station	6	22053	3676	35760	**0.006**	Lv > Rt = Rz
	tissue	2	52992	26496	257784	**0.00005**	
	station x tissue	12	7497	625	0.6079	0.823	
	Residuals	42	43169	1028			
Pb	station	6	5259	876	190240	**0.0000001**	Rt > Lv = Rz (For N1 station: Rt = Lv = Rz)
	tissue	2	2255	1128	244736	**0.00009**	
	station x tissue	12	1640	137	29677	**0.004**	
	Residuals	42	1935	46.07			
Cu	station	6	953	158837	42160	**0.002**	Rt = Lv > Rz
	tissue	2	444	222248	58992	**0.006**	
	station x tissue	12	281	23400	0.6211	0.812	
	Residuals	42	1582	37674			
Cr	station	6	169	28179	33245	**0.009**	Rt = Lv = Rz (For N4 station: Rt = Lv > Rz)
	tissue	2	79	39485	46584	**0.015**	
	station x tissue	12	320	26686	31484	**0.003**	
	Residuals	42	356	8476			
Ni	station	6	1869	311	73042	**0.022**	Rt = Lv > Rz
	tissue	2	2376	1188	278689	**0.00002**	
	station x tissue	12	626	52.21	12244	0.299	
	Residuals	42	1791	42.64			
Cd	station	6	87051	14508	51522	**0.0005**	Rt = Lv > Rz
	tissue	2	82541	41271	146559	**0.015**	
	station x tissue	12	19392	0.1616	0.5739	0.85	
	Residuals	42	118270	0.2816			
Al	station	6	13537330	2256222	167123	**0.000001**	Rt > Lv = Rz
	tissue	2	3864796	1932398	143136	**0.018**	
	station x tissue	12	3098020	258168	19123	0.061	
	Residuals	42	5670163	135004			

Table S4: Correlation matrix of non-parametric Spearman's rank correlation coefficients between mean trace element concentrations and Trace Element Pollution Index (TEPI) values for *Cymodocea nodosa* tissues. a) leaves correlation matrix. b) Rhizomes correlation matrix. c) Roots correlation matrix. Correlations significant at $p < 0.05$ are underlined and in bold.

a)

	Al	Zn	Pb	Cu	Cr	Ni	Cd	TEPI
Al	1.00							
Zn	0.71	1.00						
Pb	**<u>0.86</u>**	0.64	1.00					
Cu	0.57	0.71	0.64	1.00				
Cr	0.21	0.29	0.29	-0.14	1.00			
Ni	0.43	0.50	0.36	0.36	0.71	1.00		
Cd	0.64	0.64	**<u>0.82</u>**	**<u>0.86</u>**	-0.18	0.11	1.00	
TEPI	**<u>0.82</u>**	**<u>0.82</u>**	**<u>0.93</u>**	0.75	0.43	0.61	**<u>0.79</u>**	1.00

b)

	Al	Zn	Pb	Cu	Cr	Ni	Cd	TEPI
Al	1.00							
Zn	**<u>0.79</u>**	1.00						
Pb	0.43	0.71	1.00					
Cu	**<u>0.96</u>**	0.75	0.25	1.00				
Cr	0.25	0.43	0.00	0.39	1.00			
Ni	0.00	0.04	0.25	-0.07	0.50	1.00		
Cd	0.75	**<u>0.79</u>**	0.57	0.64	0.21	0.14	1.00	
TEPI	**<u>0.89</u>**	**<u>0.89</u>**	0.61	**<u>0.86</u>**	0.50	0.25	**<u>0.82</u>**	1.00

c)

	Al	Zn	Pb	Cu	Cr	Ni	Cd	TEPI
Al	1.00							
Zn	0.64	1.00						
Pb	0.68	0.68	1.00					
Cu	**<u>0.86</u>**	0.29	0.68	1.00				
Cr	**<u>0.96</u>**	0.61	0.71	**<u>0.82</u>**	1.00			
Ni	0.29	0.54	0.18	0.11	0.07	1.00		
Cd	**<u>0.93</u>**	0.71	**<u>0.79</u>**	**<u>0.86</u>**	**<u>0.86</u>**	0.39	1.00	
TEPI	**<u>0.89</u>**	**<u>0.79</u>**	**<u>0.86</u>**	**<u>0.79</u>**	**<u>0.82</u>**	0.46	**<u>0.96</u>**	1.00

Table S5: Trace element concentrations (mg.kg$^{-1}_{DW}$) reported in sediment (Sed) and *Cymodocea nodosa* tissues (Lv: leaves; Rz: rhizomes; Rt: roots) from various geographical locations. Values are either ranges of concentrations (R), mean concentrations (M) or ranges of mean concentrations (RM). ~: values estimated from figures, LOD: limit of detection, C: contaminated sites, I: uncontaminated sites.

Location	Compartment	Data type	Trace element						Reference
			Zn	Pb	Cu	Cr	Ni	Cd	
Nador lagoon, Morocco	Sed	M-R	428(59.5-820)	111(19.8-229)	90(17.8-237)	73.9(27.2-137)	40.8(16-80.6)	1.79(0.91-3.02)	Present study
	Lv	M-R	100(63.8-136)	9.5(3.45-23.2)	14.3(8.07-20)	5.7(1.6-16)	19.3(8.48-28.7)	1.38(0.72-2.27)	
	Rz	M-R	30.1(14.7-48.3)	6.8(1.675-16.2)	8.07(2.34-15.3)	3.3(1.6-6.4)	6.9(2.18-12.7)	0.74(0.18(1.15)	
	Rt	M-R	45(14.5-81.3)	21.6(6.12-56.4)	14.4(8.48-19.1)	5.5(3.8-7.2)	22.7(15.1-32)	1.63(1.2-1.98)	
Merja Zega, Sidi Moussa, oualida, Khnifiss lagoons and Dakhla bay, Morocco	Sed	RM	32.5-90.4	4.75-16.1	34.5-75.9	28.4-127	9.50-30.8	0.11-1.32	Boutahar et al., 2019
Mediterranean coast, Spain	Sed	RM	8-130	0.8-90	----	----	----	0.04-0.58	Sanchiz et al., 2000
	Lv	RM	11-75	2-126	----	----	----	0.16-1	
	Rz	RM	10-35	1.6-10	----	----	----	0.23-0.43	
	Rt	RM	5-30	2-37	----	----	----	0.17-0.97	
Mar Menor lagoon, Spain	Sed	~ R	C(800-7200)I(250-100)	C(375-11500)I(80-200)	----	----	----	C(1.25-7.5)I(0.3-0.45)	Marin-Guirao et al., 2005
	Lv	~ R	C(180-320)I(50-60)	C(200-300)I(10-5)	----	----	----	C(0.4-0.3)I(0.08-0.06)	
	Rz	~ R	C(90-160)I(20-40)	C(30-250)I(5-10)	----	----	----	C(0.11-0.125)I(0.01-0.005)	
	Rt	~ R	C(130-520)I(20-30)	C(100-850)I(0-5)	----	----	----	C(0.3-0.5)I(0.02-0.04)	
Mar Menor lagoon, Spain	Sed	RM	C(1030-279)I(323-44)	C(8700-2530) I(26-27)	C(43-12)I(20-8)	C(30-23)I(3-3.5)	C(29-22) I(LOD)	C(19-9)I(LOD)	Serrano et al., 2019
	whole plante	RM	C(8300-3700)I(16-9)	C(4350-810) I(264-8)	C(155-25)I(7-5)	LOD	C(7-LOD) I(4-LOD)	LOD	
Alfacs and Fangar bays, Spain	Lv	~ R	10-50	0.5-3.8	3.5-7.5	----	1-3.2	0.3-1	Llagostera and Romero, 2011
	Rz	~ R	10-25	0.2-0.8	1.5-3.5	----	0.2-1	0.18-0.4	
	Rt	~ R	05-30	1-3.2	2.5-6.5	----	0.8-3.2	0.10-0.6	
Marina Cap Monastir, Bizerte and Ghar El Melh lagoons, Tunisia	Sed	RM	32.2-74.5	15.1-19.8	19.2-39.5	----	6.02-6.7	0.28-0.52	Zakhama-Sraieb et al., 2019
	Lv	RM	15.9-74.7	0.85-4.76	2.37-6.77	----	1.53-2.21	0.23-0.58	
	Rz	RM	18-22.1	0.39-1.3	1.04-4.35	----	0.73-0.99	0.14-0.28	

Sicily, Italy	Sed	RM	3.32-38.6	1.05-17.2	0.3-34.6	3.88-76.3	3.32-45.4	0.12-0.85	Bonanno and Di-Martino, 2016, Bonanno et al., 2017, Bonanno and Borg, 2018, Bonannoa and Raccuia, 2018
	Lv	RM	42.6-69.6	1.4-6.99	3.61-28.8	3.16-6.34	3.1-6.65	0.1-0.6	
	Rz	RM	18.6-38.9	0.21-1.87	2.06-24.5	1.05-2.67	0.88-5.34	0.04-0.22	
	Rt	RM	34.5-64.3	1.67-6.99	2.85-25.8	4.35-6.9	3.32-8.8	0.15-0.55	
Kopfer Bay, Slovenia	Sed	RM	86-194	19.5-262	22-76.3	25.3-64.3	26.5-127	0.07-1.2	Faganeli et al., 1997
	whole plante	RM	49.1-787	5.59-29.1	11.8-13.6	0.8-2.15	4.58-8.1	1.21-2.38	
Antikyra Gulf, Greece	Sed	M-R	28.5(12.3-69.8)	173(22.5-633)	108(5.2-397)	----	----	15.9(0.3-87)	Malea and Haritonidis, 1995
	whole plant	M-R	31.8(17.3-50.5)	50.9(14.7-297)	16.1(2.1-98.3)	----	----	18.8(0.88-83)	
Evoikos Gulf, Greece	Sed	RM	44.3-152.5	----	16.6-13.2	105-677.6	60.5-889.4	0.1-0.1	Nicolaidou and Nott, 1998
	Lv	RM	57.5-147	----	9.6-23.5	2-4.8	7.6-77.3	1.2-2.3	
	Rt	RM	22.92-62.4	----	12.8-22.4	5.1-10.5	5.2-26.4	2.1-2.6	
Thermaikos Gulf, Greece	Sed	M-R	42.7(30.8-100)	----	14.8(11.7-18)	30.9(21-39.7)	23.9(16.2-34.9)	0.15(0.01-0.25)	Malea and Haritonidis, 1999
	Lv	M-R	175(29.3-627)	156(60-210)	12.7(6.6-20.9)	4.65(0.01-14.7)	2.99(1.29-4.66)	0.58(0.32-0.79)	
	Rt	M-R	48.1(31-77.7)	157(70-220)	12.2(4.2-24.2)	19.9(0.13-66.9)	1.84(0.62-3.26)	0.56(0.32-0.44)	
Thermaikos Gulf, Greece	Sed		40.5(15.2-120)	11.8(6.1-19.8)	10.7(5.34-17)	----	----	0.032(0.002-0.05)	Malea et al., 2013
	Blades of Lv	M-R	185(59.2-343)	7.76(1.17-18.24)	18.7(11-45)	----	----	0.45(0.16-0.86)	
	Rz	M	131	2.76	12.6	----	----	0.19	
	Rt	M-R	163(61.2-305)	7.14(1.51-11.52)	21.2(10.4-31.7)	----	----	0.43(0.18-0.54)	
Vulcano, Adamas and Peleochori seeps, Italy and Greece	Sed	RM	14.7-35.8	2.27-3.97	2.3-44.8	----	8--48	0.14-0.97	Mishra et al., 2020
	Lv	RM	11.9-15.3	0.09-3.80	2.77-14.7	----	2.33-10.71	0.14-1.74	
	Rz	RM	14.6-28.5	0.53-3.26	2.03-26.9	----	2.11-3.71	0.12-0.67	
	Rt	RM	8.9-82.8	0.81-9.81	6.76-258	----	1.64-48	0.14-1.14	

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Appendix B.

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Trace element bioaccumulation in the seagrass *Cymodocea nodosa* from a polluted coastal lagoon: Biomonitoring implications

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ABSTRACT

This is the first investigation of the potential for using *Cymodocea nodosa* to biomonitor trace element (TE) contamination in Marchica lagoon (Morocco), a Mediterranean pollution hotspot. We measured concentrations of seven TEs in seagrass tissues (leaf-rhizome-root) and sediments. Single and multi-element indices confirmed that sediments near illegal discharges were heavily polluted and we predicted risks of frequent adverse biological effects in these areas. Four of the TEs increased concentrations in *C. nodosa* leaf and root along sediment pollution gradient. Leaves and roots were both good indicators of Cu and Cd contamination in sediment, whereas leaves were the best indicator of Zn and roots for Pb. This seagrass was not a bioindicator of Al, Cr and Ni contamination. These results show the bioaccumulation patterns of TEs in *C. nodosa*, and can be used to design biomonitoring programs.

1. Introduction

Pollution of coastal ecosystems by trace elements (TEs) has becoming a major environmental problem due to their toxicity, persistence, and bioaccumulation into the food chain which lead to potential threat to aquatic biota and human health (Zhuang and Gao, 2014; Suresh et al., 2015; Abreu et al., 2016; Ihedioha et al., 2017; Ali and Khan, 2018).

The entry of TEs within the aquatic environments arises from natural processes (i.e. geological weathering and soil erosion) and anthropogenic activities (i.e. urban sewage discharge, industrial wastewaters, fertilizer leaching from adjacent agricultural lands and riverine fluxes) (Vikas and Dwarakish, 2015; Duodu et al., 2017; Boutahar et al., 2019). Then, they are accumulated in bottom sediment to levels significantly higher compared with the concentration of the overlying water (Benson et al., 2016; Simpson and Spadaro, 2016; Matache et al., 2018).

Various sediment quality indices have been widely applied to quantify the degree of TE contamination and evaluate their biological adverse risk in aquatic ecosystems (Duodu et al., 2016; Birch, 2018). However, high TE concentration in the sediment does not indicate their high bioavailability to the living organisms (Ralph et al., 2006). Accordingly, when determining the ecotoxicological relevance of TEs, it is critical to monitor their bioavailable form (Brady et al., 2016; Duodu et al., 2017). The worldwide adopted procedures to extract the active fraction of TEs in sediment use single step extraction by dilute acids and chelating agents (Sahuquillo et al., 2003; Hu et al., 2011) or multiple-sequential extraction methods (Tessier et al., 1979; Rauret, 1998; Cuong and Obbard, 2006). Although these approaches have brought significant knowledge on the interactions between TEs and sediment components, their ability to provide a robust evidence on the fraction interacting with organisms is limited (Mourier et al., 2011; Brady et al., 2016; Liu et al., 2018). Because of this deficiency, biological indicators have been identified as the useful tool to assess the availability of TEs through the trophic levels (Farias et al., 2018).

Worldwide, seagrasses have been recommended as efficient biomonitor and bioindicator species to assess the contamination of the marine environment by TEs (Bonanno and Raccuia, 2018; Boutahar et al., 2019; Boutahar et al., 2020; Gopi et al., 2020; Jeong et al., 2021). As primary producers, seagrasses may be used as early detectors of

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CHAPTER 4 - SEAGRASS *CYMODOCEA NODOSA* AT AL HOCEIMA NATIONAL PARK: CURRENT POPULATION DYNAMICS STATUS AND MANAGEMENT MEASURES.

Abstract

Seagrasses habitats rank amongst the most valuable ecosystems in the biosphere. They support fisheries production, climate change mitigation, water quality improvement and coastal protection. Faced with the current global crisis of accelerating losses of this key component of coastal environments, strong efforts are expended within the conservation movement to flatten their decline curve. Although understanding of the functioning of seagrass ecosystems and the way they respond to stressors has improved over the last years, major gaps exist for West Africa including basic ecological and distributional knowledge. This study aimed to investigate, for the first time, two *Cymodocea nodosa* meadows structural development in Al Hoceima National Park (Mediterranean coast of Morocco) using the reconstruction techniques as an indirect measurement of seagrass growth. *C. nodosa* leaves were remarkably invaded by epiphytes while leaf production, shoot density, above and belowground biomass, vertical and horizontal rhizomes extend were in the last rang of values recorded elsewhere. Age structure showed that since the second year class, the survival of shoots decreases. With regard to the severe slow recruitment rates, the population net growth was declining. Besides this fragile status, the seagrass is facing numerous pressures mainly fishing by rawling, and alien species invasion. The primary conservation challenge for *C. nodosa* of Al Hoceima National Park is that this marine protected area become operationally implemented and actively managed. However, the seagrass habitat-forming species is not safeguarded until then.

Key-words:

Cymodocea nodosa; Al Hoceima National Park; reconstruction techniques; population dynamics; effective management and conservation.

1. Introduction

Seagrasses are flowering marine plants that provide extensive valuable ecosystem goods and services to human livelihoods and well-being. Human disturbances such as coastal development, eutrophication, pollution and physical destruction by dredging and trawling are causing seagrasses increasingly worldwide decline; along with the services they supply (De los Santos et al., 2019; Salinas et al., 2020). In addition, climate change is a growing concern as rising sea levels and increasing ocean temperature may cause future seagrass losses (Fortes et al., 2018). It has been estimated that 30% of the known seagrass areal extent disappeared since seagrass areas were initially recorded in 1879 (Waycott et al., 2009). Yet, there is major gaps in global data sets for seagrass extent and distribution, and so many losses remained unknown (Keulen et al., 2018). The growing evidence of seagrass vast storage ability and sequestration of carbon, derived the Paris Climate Agreement to require placing an increased global focus on the spatial distribution, loss and restoration of seagrass meadows (McKenzie et al., 2020). Critical need of a deep scientific knowledge based on the exhaustive characterization of natural populations in response to local and global changes is therefore strongly needed (York et al., 2017; De los Santos et al., 2019).

In order to update all the management plans of the marine protected area of Al Hoceima National Park (Mediterranean coast of Morocco), the mapping inventory of key marine habitats of conservation interest, performed inf the framework of the MedKeyhabitats 2 Project, identified the presence of *Cymodocea nodosa* (Ucria) Ascherson meadows in the park. This seagrass species is common in the Mediterranean Sea, the North Atlantic coasts of Africa, South Atlantic coast of Europe and around Madeira and the Canary Islands (Cunha and Duarte, 2007). Even though it is considered to be resilient to natural and anthropogenic stresses and shows a high environmental plasticity to colonise lagoons, bays, estuaries and open coastal waters (Canals and Ballesteros, 1997; Papathanasiou et al., 2015), it is classified as an endangered species under Annex II of the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean of the Barcelona Convention (PNUE-PAM-CAR/ASP, 2013). Along the Mediterranean coast, the extension decline of *C. nodosa* meadows was recorded in many areas: Mar Menor lagoon, Spain (Perez-Ruzafa et al., 2012), Urbinu lagoon, France (Fernandez et al., 2006), Gulf of Tigullio, Italy (Barsanti et al., 2007), and Ghar El Melh lagoon, Tunisia (Shili et al., 2002).

Given the underrepresented knowledge of seagrasses in West Africa (McKenzie et al., 2020), and the marked regression of this species through-out its range distribution, this study aimed to closely monitor the development of *C. nodosa* meadows in Al Hoceima National Park. It provides the first baseline quantitative and qualitative database to fill the knowledge gaps on these ecosystems and promote their conservation. We investigated *C. nodosa* structural development using the reconstruction technique, an indirect measurement of seagrass growth, which has been proposed to overcome the lack of long-term data. This dating method allows a fast evaluation of leaf production and rhizome growth rates and their changes over time and, from this, derive shoot demography and population dynamics (Duarte et al., 1994). The balance between shoot recruitment and mortality rates are thus used to forecast the expansion, the steady state or the regression of meadows (Duarte and Sand-Jensen, 1990).

In addition, the management status of the park is discussed and measures to promote the conservation of seagrass habitats are proposed.

2. Materials and Methods

2.1. Study site

Al Hoceima National Park (AHNP) is located on the Mediterranean coast of Morocco (35°10′N, 4°07′O) at approximately 150 km east of the Strait of Gibraltar, west of the city of Al Hoceima. The surface area of its land part is 28860 ha, while the marine part covers 19600 ha covering a coastline of 40 km (PNUE-PAM-CAR/ASP, 2020). AHNP is the unique official marine protected area of the Mediterranean Moroccan coast under the Specially Protected Areas and Biological Diversity Protocol of the Barcelona Convention. Moreover, it was classified in 2009 by the United Nations as a specially protected area of Mediterranean importance (SPAMI) (PNUE-PAM-CAR/ASP, 2009).

The dominant features of the park coast are its very high rocky cliffs reaching more than 500 m that fall into the sea with steep slopes, constituting a grandiose natural landscape. These cliffs are locally interrupted by creeks of sand and gravel that often correspond to the wadis' outlets. Some of these streams only function after abundant and continuous rainfall. Hence, the irregularity of the water regimes of these wadis (IUCN, 2012). The beaches are relatively rare, small in extent and appear only in the protected zones of the bays. The marine area of the Park, located in the Alboran Sea, is under the influence of the Western Anticyclonic Gyre (WAG) formed by the opposite circulatory movements of the Mediterranean Sea and the Atlantic Ocean waters entering through the Strait of Gibraltar (Garcia-Lafuente et al., 2017). The exchange of these two water masses with different properties result in high primary productivity in this region (Abdellaoui et al., 2017), and remarkably enhance the diversity of benthic habitats and species communities listed in many international conventions and protection agreements (PNUE-PAM-CAR/ASP, 2020).

2.2. Sample collection and laboratory analyses

During the marine survey of July 2019, two *Cymodocea nodosa* meadows were identified near of Boumehdi Beach (35°14’N-04°00'W) and East of the Cala Iris islet (35°9′N-04°2′W) at -15 and -9 m depth respectively (Fig. 1).

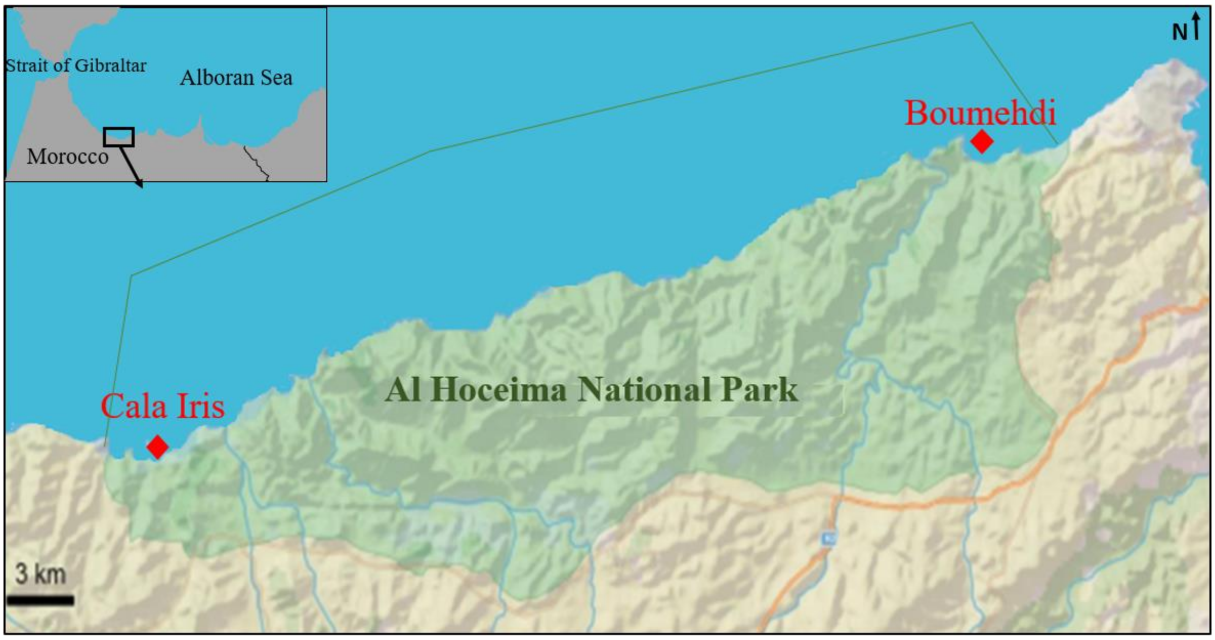

Fig. 1: Map showing the localisation of the two studied *Cymodocea nodosa* meadows in Boumehdi beach and Cala Iris bay, in the marine part of the Al Hoceima National Park, along the Mediterranean coast of Morocco.

At the center dense part of each meadow, five randomly thrown quadrat of 1 x 1 m^2 were photographed to estimate the meadows cover. 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; Boutahar et al., 2020).

Shoot density was estimated *in situ* as the number of shoots within 20 x 20 cm quadrats (10 replicates at each station). For biometry and biomass determination, five cylindrical cores of 0.15 m in diameter and 0.12 m long were extracted. Around 200 shoots in connection to their horizontal rhizomes were handily harvested for plant growth history and population dynamics.

All samples were then rinsed free of sediment carefully to avoid shoot breakage, transferred into plastic bags and frozen until further analysis.

In the laboratory, cores' material was sorted into aboveground (leaves), and belowground (rhizomes and roots) *C. nodosa* tissues. Foliar epiphytes were scraped off using a glass slide (Dauby and Poulicek, 1995). Twenty shoots were randomly selected from the five aboveground cores samples and dissected for number of total leaves per shoot, number, length and width of leaf by category (differentiated (with sheath), and not differentiated (without sheath)). Leaf area index (LAI) was determined by multiplying mean surface area of shoots (only one face) by meadow shoot density. Plant tissues and epiphytes where then oven dried until constant weight (minimum 48 h at 60 °C) to determine dry weight per shoot ($g_{DW}.shoot^{-1}$) and per meadow surface unit ($g_{DW}.m^{-2}$).

The age of vertical rhizomes (144 at Boumehdi and 130 at Cala Iris) was estimated by counting the number of scars and standing leaves and dividing the resultant number by the annual mean number of leaves produced by the plant (Duarte et al., 1994; Fourqurean et al., 2003). Age estimation is generated in plastochrone interval unit (PI, the time elapsed between the formation of two consecutive leaves) which was then converted into an absolute time unit. Average leaf turnover was determined as the product of the inverse of the rate of production of new leaves and the average number of standing leaves per shoot. The annual vertical rhizome elongation rate was calculated as the slope of linear regression between the length of vertical rhizomes and the shoot age. The production rate of horizontal rhizome internodes was estimated as the linear regression slope of the number of horizontal internodes between consecutive shoots against their age difference (Duarte et al. 1994). The product of this slope and the average length of the rhizome internodes estimated the average annual horizontal rhizome elongation rate (Duarte et al. 1994). The population dynamics of the two meadows was characterised by the shoot mortality, and recruitment through clonal growth as described by Duarte et al. (1994) and Fourqurean et al. (2003). The annual gross shoot recruitment rate (R_{gross}) was calculated as the difference between the total number of shoots and the number of shoots older than one year. Shoot mortality rate (M), assumed constant over shoot age and years, was obtained from the exponential decay regression of the shoot age frequency distribution, where it is:

$N_t = N_0.e^{-M.t}$,

where N_t is the number of shoots in age class t, N_0 is the number of shoots recruited into the population, and M is the mortality rate. The net rate of shoot population growth ($R_{net} = R_{gross} - M$) was then used to forecast the future development of the meadows if growth conditions were maintained. Net recruitment positive values indicate expanding seagrass meadows; negative values indicate the regression of meadows.

2.3. Statistical analyses

Differences in measured parameters among both meadows were analyzed using a t-Student test. Raw or log-transformed data were tested for normality and homogeneity of variance to meet the assumptions for parametric statistics. Statistical analyses were performed using the Statistica Software. Statistical analyses were not performed for the horizontal rhizome elongation, mortality and recruitment rates, because just one value was obtained for each population.

3. Results and Discussion

In Boumehdi and Cala Iris, *C. nodosa* grows in dense monospecific bed covering 18.4 ha and 4.63 ha respectively (PNUE-PAM-CAR/ASP, 2020).

Descriptive morphological, structural and growth parameters of both meadows are reported in tables 1 and 2. Shoot density, cover and leaf biomass were higher in Boumehdi without statistical differences ($p > 0.05$). Despite the higher leaves length of Cala Iris meadow ($p < 0.05$), the leaf surface exposed to light (m^2 of leaves per m^2 of sediment) was lower, as a direct consequence of the lower density value. Moreover, leaves of Cala Iris meadow were remarkably more invaded by epiphytes that exceed leaf biomass per shoot and per m^2. Epiphytes load is a result of the balance between nutrient supply, light penetration and grazing pressure (Castejon-Silvo and Terrados, 2012). Lower depth development of Cala Iris meadow (9 m vs 15 m at Boumehdi) under combination of human induced stressors (fishing, tourism and nearby port activities) may promoted the epiphytic overgrowth. In addition, the stronger currents in this area may eliminated grazers allowing for more epiphytes to grow (Mabrouk et al., 2014).

Table 1: *Cymodocea nodosa* shoot density, cover, biomass, leaf morphometric measurements, rhizome growth and leaf formation at Boumehdi and Cala Iris of Al Hoceima National Park. Significant differences between meadows ($p < 0.05$) are presented in bold. Standard deviation of the mean (within parentheses) is provided when possible. Dw: dry weight.

Meadows / Parameters	Boumehdi	Cala Iris
Shoot density (shoots.m^{-2}, n = 10)	493 (118)	450 (184)
Shoot cover (%, n = 5)	98 (4)	94 (5.1)
Total number of leaves per shoot (leaves.$shoot^{-1}$, n = 20)	**4.7** (1.2)	3.1 (0.7)
Number of differenciated leaves per shoot (leaves.$shoot^{-1}$, n = 20)	**2.7** (0.7)	1.8 (0.4)
Number of undifferentiated leaves per shoot (leaves.$shoot^{-1}$, n = 20)	**2.0** (0.8)	1.4 (0.5)
Leaf length (mm, n = 20)	148 (82)	**185** (89)
Leaf width (mm, n = 20)	2.7 (0.8)	2.5 (0.6)
Leaf Area Index ($m^2.m^{-2}$, n = 20)	**0.97** (0.5)	0.7 (0.2)
Shoot weight (g_{Dw}, n = 20)	0.05 (0.02)	0.04 (0.02)
Epiphyte load ($g_{Dw}.g^{-1}_{Dw}.shoot^{-1}$, n = 20)	0.02 (0.01)	**0.09** (0.07)
Leaf biomass ($g_{Dw}.m^{-2}$, n = 5)	41 (5)	30 (12)
Epiphyte biomass ($g_{Dw}.m^{-2}$, n = 5)	16 (3)	**51** (28)
Rhizome biomass ($g_{Dw}.m^{-2}$, n = 5)	**50** (12)	17 (2)
Root biomass ($g_{Dw}.m^{-2}$, n = 5)	**26** (8.2)	8.4 (3.6)
Belowground biomass ($g_{Dw}.m^{-2}$, n = 5)	**76** (15)	25 (4)
Total seagrass biomass ($g_{Dw}.m^{-2}$, n = 5)	**117** (10)	55 (13)
Vertical rhizome elongation (cm.$year^{-1}$)	1.51	0.94
Horizontal rhizome elongation (cm.$year^{-1}$)	7.2	4.8
Leaf production (leaves.$shoot^{-1}.year^{-1}$)	15	11
Leaf apparence (leaves.$shoot^{-1}.day^{-1}$)	0.04	0.03
Leaf turnover time (day)	116	106

High epiphyte concentrations can severely reduce the availability of light to seagrass leaves (Tuya et al., 2013), one of the most limiting factor for their vegetative development (Leoni et al., 2008). Low number and longer leaves in Cala Iris meadow is probably an adaptive coupled mechanism to maximize light capture and avoid self-shading within the canopy (Ralph et al., 2007). This trend has also been observed in seagrasses from impacted sites with low light levels (Longstaff and Dennison, 1999; Marin-Guirao et al., 2005; Orfanidis et al., 2010; Papathanasiou et al., 2015).

Leaf formation rate was lower at Cala Iris meadow with 11 leaves.shoot^{-1}.year^{-1} compared to 15 leaves.shoot^{-1}.year^{-1} in Boumehdi with turnover rate estimated to 103 days and 116 days respectively. Accordingly, leaves disappeared faster than they appeared at Cala Iris meadow indicating less growth conditions. It is well documented that continuous light deprivation by excessive epiphyte overgrowth leads to significant declines in leaf production, shoot density and standing biomass (Leoni et al., 2008; Bryars et al., 2011; Kelaher et al., 2013). Our founding is consistent with previous research that reported high *C. nodosa* leaf turnover rate in localities under higher anthropogenic disturbance (Perez et al., 1994).

Table 2: Shoot demography and population dynamics of *Cymodocea nodosa* at Boumehdi and Cala Iris of Al Hoceima National Park. Standard deviation of the mean (within parentheses) is presented for the shoot age. Number of shoots used for the analysis were 144 and 130 respectively.

Meadows / Parameters	Boumehdi	Cala Iris
Shoot mean age (year)	1.7 (1.1)	1.3 (0.7)
Maximum shoot age (year)	9.50	3.40
Gross shoot recruitment rate (year^{-1})	0.37	0.37
Shoot mortality rate (year^{-1})	0.58	0.81
Net shoot population growth (year^{-1})	-0.20	-0.44
State of the meadow	regression	regression

The relative contribution of leaf canopy to the total biomass accounting only for 35% in Boumehdi, testified the well-developed layer of rhizomes and roots in this meadow. The average length of vertical rhizomes was 2.19 ± 1.80 cm, where the longest length was 14.5 cm. Their annual elongation rate was estimated to 1.51 cm.year^{-1}. Vertical rhizomes of Cala Iris were much shorter with the longest length up to 5.40 cm with an annual elongation rate of 0.94 cm.year^{-1}. Horizontal rhizome growth was very low in both meadows as reflected by the slow elongation rates (4.8 - 7.2 cm.year^{-1}). These values are smaller when compared to the rhizome growth of *C. nodosa* in other localities at smaller depths (Table 3). In contrast to our results, Terrados et al. (2006) examined the vegetative development of *C. nodosa* along depth gradients and found that horizontal rhizome elongation of meadows growing at depths (8 - 11 m), comparable to those of Al Hoceima (9 - 15 m), were three times greater than at shallow waters.

Examination of age structure showed that since the survival of shoots decreases since the second year class, reflecting the high mortality rates especially in Cala Iris (Fig. 2).

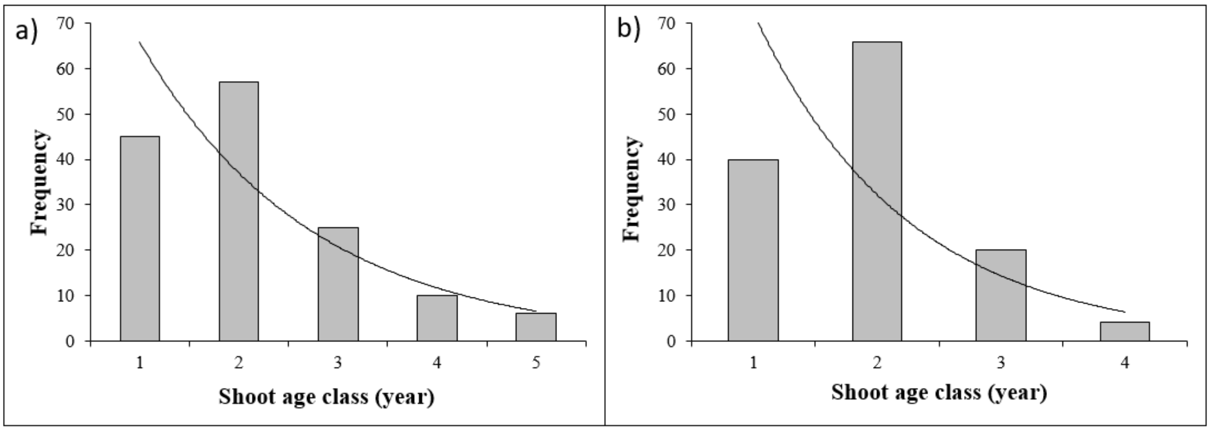

Fig. 2: Age frequency distribution of *Cymodocea nodosa* populations at Boumehdi (a) and Cala Iris (b) meadows of Al Hoceima National Park. Solid line shows the fitted exponential decay line used to obtain the mortality rate (M).

Similar founding with higher *C. nodosa* shoots mortality in stands with greater quantity of leaf-biofilm was registered in Mar Menor lagoon (Spain) by Marin-Guirao et al., 2005. However, mortality rate values estimated in Al Hoceima were smaller than elsewhere (0.58 – 0.81 year^{-1} comparing to 0.22 – 12.4 year^{-1}, Table 3), while the recruitment rates showed severely lower values (0.37 year^{-1} in both meadows). The reduced recruitment of the shoots was consistent with the low extend of rhizomes as described previously by several researchers (Cunha and Duarte, 2005; Cabaço et al., 2010; Tupan and Uneputty, 2017). Rhizome growth regulates the seagrass shoot development, which is the basis of meadows resilience and dispersion (Duarte and Sand-Jensen, 1990; Marba and Duarte, 1994). The negative net growth calculated with reference to the recruitment and mortality rates concluded that *C. nodosa* populations at Al Hoceima were declining as a direct result of epiphytic overload and limited rhizomes growth.

Table 3: Literature data on annual range of *Cymodocea nodosa* density, morphological, biomass, growth rates and population dynamics.

Parameter	Value	Location	Reference
Shoot density (shoot.m^{-2})	450-493	Al Hoceima National Pak	Present study
	849-1594	Alfacs Bay, Spain	Perez et al., 1994
	597-1140	Canary Island, Alicante and Mallorca, Spain	Manez-Crespo, 2020
	573-822	Ria Formosa, Portugal	Cabaço et al., 2010
	633-704	Monastir Bay, Tunisia	Sghaier et al., 2017
	204-814	Gabes Gulf, Tunisa	Zakhama Sraieb et al., 2010
	978-1658	Grado Lagoon, Italy	Guidetti, 2002
	757-1520	Urbinu Lagoon, Corsica	Agostini et al., 2003
Leaf number (leaves.shoot^{-1})	3.1-4.7	Al Hoceima National Pak	Present study
	3-4	Alfacs Bay, Spain	Perez et al., 1994
	3.1-3.3	Ria Formosa, Portugal	Cabaço et al., 2010
	4-5	Monastir Bay, Tunisia	Sghaier et al., 2017
	3-5	Gabes Gulf, Tunisa	Zakhama Sraieb et al., 2010
	1-4	Venice Lagoon, Italy	Rismondo et al., 1997
Leaf length (cm)	148-185	Al Hoceima National Pak	Present study
	20-43	Alfacs Bay, Spain	Perez et al., 1994
	24-38	Ria Formosa, Portugal	Cabaço et al., 2010
	5-20	Monastir Bay, Tunisia	Sghaier et al., 2017
	11-44	Gabes Gul, Tunisa	Zakhama Sraieb et al., 2010
	12-48	Venice Lagoon, Italy	Rismondo et al., 1997
Aboveground Biomass (g_{DW}.m^{-2})	30-41	Al Hoceima National Pak	Present study
	20-130	Alfacs Bay, Spain	Olesen et al., 2002
	62-104	Ghar El Melh lagoon, Tunisia	Sghaier et al., 2011; 2012
	44-810	Venice Lagoon, Italy	Rismondo et al., 1997
	30-160	Grado, Italy	Guidetti, 2002
	48-98	Vulcano, Adamas and Peleochori seeps, Italy and Greece	Mishra et al., 2021
	200-400	Urbinu Lagoon, Corsica	Agostini et al., 2003
Beloweground Biomass (g_{DW}.m^{-2})	25-76	Al Hoceima National Pak	Present study
	123-192	Blanes Bay, Spain	Marba and Duarte, 2001
	186-116	Sant Pol beach, Spain	Terrados et al., 2006
	10-539	Canary Island, Alicante and Mallorca, Spain	Manez-Crespo, 2020
	188-539	Ghar El Melh lagoon, Tunisia	Sghaier et al., 2011; 2012
	108-358	Vulcano, Adamas and Peleochori seeps, Italy and Greece	Mishra et al., 2021
	300-650	Urbinu Lagoon, Corsica	Agostini et al., 2003
Plastochrone interval (days)	25-34	Al Hoceima National Pak	Present study
	28-45	Alfacs Bay, Spain	Olesen et al., 2002
	38-40	Sant Pol beach, Spain	Terrados et al., 2006
	12-43	Ghar El Melh lagoon, Tunisia	Sghaier et al., 2011; 2012
	23	Ischia Island, Italy	Cancemi et al., 2002
Leaf production (leaves.shoot^{-1}.year^{-1})	11-15	Al Hoceima National Pak	Present study
	13-20	Alfacs Bay, Spain	Perez et al., 1994
	16	Ghar El Melh lagoon, Tunisia	Sghaier et al., 2011; 2012

	12	Monastir Bay, Tunisia	Sghaier et al., 2017
	16	Ischia Island, Italy	Cancemi et al., 2002
Vertical rhizome elongation (cm.year^{-1})	0.94-1.51	Al Hoceima National Pak	Present study
	1.50-3.19	Ria Formosa, Portugal	Cunha and Duarte, 2005
	2.44-5.79	Ria Formosa, Portugal	Cabaço et al., 2010
	0.68-1.85	Vulcano, Adamas and Peleochori seeps, Italy and Greece	Mishra et al., 2021
Horizontal rhizome elongation (cm.year^{-1})	4.8-7.2	Al Hoceima National Pak	Present study
	15-35	Alfacs Bay, Spain	Olesen et al., 2002
	14-31	Ria Formosa, Portugal	Cunha and Duarte, 2005
	7-18	Ghar El Melh lagoon, Tunisia	Sghaier et al., 2011; 2012
	4.6-25	Monastir Bay, Tunisia	Sghaier et al., 2017
	3.7-22	Venice Lagoon, Italy	Rismondo et al., 1997
Mean age (year)	1.3-1.7	Al Hoceima National Pak	Present study
	0.80-0.94	Alfacs Bay, Spain	Perez et al., 1994
	2.5-3.2	Sant Pol beach, Spain	Terrados et al., 2006
	0.43-0.9	Ria Formosa, Portugal	Cabaço et al., 2010
Recuitment (year^{-1})	0.37	Al Hoceima National Pak	Present study
	0.6-2.3	Alfacs Bay, Spain	Olesen et al., 2002
	0.82-2.36	Ria Formosa, Portugal	Cabaço et al., 2010
	0.36-0.77	Vulcano, Adamas and Peleochori seeps, Italy and Greece	Mishra et al., 2021
Mortality (year^{-1})	0.58-0.81	Al Hoceima National Pak	Present study
	0.87-1.62	Alfacs Bay, Spain	Perez et al., 1994
	0.7-1.2	Alfacs Bay, Spain	Olesen et al., 2002
	3.15-12.4	Ria Formosa, Portugal	Cabaço et al., 2010
	0.22-0.99	Vulcano, Adamas and Peleochori seeps, Italy and Greece	Mishra et al., 2021
Net growth rate (year^{-1})	(-0.2)-(-0.44)	Al Hoceima National Pak	Present study
	(-0.1)-1.1	Alfacs Bay, Spain	Olesen et al., 2002
	0.4-0.68	Mar Menor Lagoon, Spain	Marın-Guirao et al., 2005
	(-2.35)-0.29	Ria Formosa, Portugal	Cunha and Duarte, 2005

High epiphytic production may be related to the nutrient-rich Atlantic Jet via the Strait of Gibraltar that result in the accumulation of nutrients in the Alboran Sea and thus induce a permanent fertilisation of this area (Lorente et al., 2019). Fresh water runoff conducted from watershed during the rainy months may also enhance nutrients enrichment. Concerning clonal growth regulation, previous studies underlined that it exhibit intraspecific variability in response to meadow genetic diversity (Manez-Crespo et al., 2020) and local scale environmental conditions (hydrodynamism, sediment dynamics, light, salinity, temperature, nutrient, heavy metals among others; Cunha and Duarte, 2005; Ambo-Rappe 2011; Sghaier et al., 2017; Tuya et al., 2019; Manez-Crespo et al., 2020). Azizi et al. (2020) recorded an average monthly salinity of the park water column oscillating between 36 and 40. The optimum *C. nodosa* meadows productivity is at around oceanic salinity (33-37), while extreme or suboptimal values negatively affect their photosynthesis, metabolism and growth, determining their biomass, productivity and survival (Vermaat et al., 2000; Pagès et al., 2010; Fernandez-Torquemada and Sánchez-Lizaso, 2011; Sghaier et al., 2017). The illegal fishing activities are other problems that can threaten the survival of Al Hoceima meadows. Many trawls crossing scars have been observed in the park too close to the coast although the law prohibits trawling activity at depths less than 80 m. This physical disturbance is one of the major direct damage to seagrasses injuring roots and rhizomes, reducing shoot density, leading to fragmentation and permanent loss of habitat (Short and Wyllie-Echeverria, 1996; Ardizzone et al., 2000; Neckles et al., 2005). Fishing using highly toxic chemicals such as copper sulphate is also very common in the Park, which causes metal pollution. Overall, the risk of degradation of the AHNP seagrass meadows by fishing activities, evaluated by combining the sensitivity of this habitat and the amplitude of the pressures, was qualified as strong (PNUE-PAM-CAR/ASP, 2020). Other potential threats are related to port activities, urban discharges and/or terrigenous inputs after heavy rainfall that washes away all the dumped waste. The presence of eight invasive macroalgal species in the park is a supplementary biological stress to *C. nodosa* meadows (PNUE-PAM-CAR/ASP, 2020). Seven of the recorded species are included in the list of the worst invasive phytobenthos in the Mediterranean Sea (Streftaris and Zenetos, 2006; Verlaque et al., 2015) while *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim is actually showing an overflowing expansion capacity in detriment of native species around the Strait of Gibraltar (Garcia-Gomez et al., 2021).

Cymodocea nodosa meadows of Al Hoceima National Park are one of the fragile ecosystems that are sensitives to the different encountered pressures, which is incompatible with the primary aim of SPAMIs to maintain marine ecosystem functionality and health. The site suffers from a lack of coastal planning and effective governance system. A management and development plan have been proposed in 1993, resumed in 2002 and revised in 2011, but has never been completed. There is also a major lack of legislations and regulations to ensure compliance with the good environmental practices. According to Soriani et al. (2015), most of the problems and conflicts are related to non-coordination of sectoral actions, rigidity of procedures, absence of prospective vision and poor law enforcement. They also reported that the inclusion of research outcomes in coastal policy decisions rarely exceeds a statement of interest.

The park is therefore facing a great challenge to become operationally implemented and actively managed. However, the various habitat-forming species of the site are not safeguarded until then. To meet the international commitments to the United Nations Convention on Biological Diversity, the United Nations 2030 Agenda for Sustainable Development and Paris Climate Agreement, effective conservation strategies are urgently needed to address the current *C. nodosa* decline and boost its recovery. This can be ensured by:

- Establishment of habitats protection zones using the detailed mapping of biological communities inside the park;
- Enforcement of legislations along with strong control to respect regulations especially against illegal fishing activities;
- Uncontrolled urban solid and liquid waste disposal to the sea and tourism projects not in line with sustainable development must be reviewed to improve water quality;
- Costal constructions (e.g., ports, marinas and touristic establishments) should be located far from seagrass meadows to minimize their impacts;
- Address the current gaps in physicochemical datasets and environmental conditions affecting meadows growth. The launch of a marine observatory at Al-Hoceima National Park under the EU funded ODYSSEA project, a multiplatform network of observing and forecasting systems across the Mediterranean basin, will certainly contribute to water column data acquisition, however, the sediment should also be involved in the regular monitoring programs;
- Development of Citizen Science monitoring approach involving local scuba divers for a rapid underwater benthic seascape changes detection;
- The park management body should insure long-term seagrass monitoring along with seaweed invasion by providing the financial support to scientific researches;
- Improve communication on the benefits provided by seagrass meadows to increasing society’s awareness of the importance of this ecosystem.

4. Conclusion

This study is the first report on the structure and dynamic of *Cymodocea nodosa* populations in Al Hoceima National Park. Our results provide support to the increasingly worldwide decline of seagrasses meadows; along with the services they supply. For conservation purpose, build on and coordinate efforts to identify local scale factors driving the development pattern of these meadows and address the surrounding anthropogenic stressors are urgently required to implement effective management actions to promote their recovery and restoration.

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CHAPTER 5 – HOW CUMULATIVE EFFECTS IMPACT THE ECOSYSTEM FUNCTIONING AND ORGANIZATION: TOWARDS AN ECOSPACE APPROACH COMBINING MULTIPLE DRIVERS

A part of this chapter was treated as an article published in the journal 'Aquatic Botany' indexed in 'Scopus' and 'Web of Science' databases:

Boutahar, L., Espinosa, F., Richir, J., Lepoint, G., Gobert, S., Maanan, M., Bazairi, H., 2020. Deep-water *Zostera marina* meadows in the Mediterranean. Aquat. Bot. 166, 103269. https://doi.org/10.1016/j.aquabot.2020.103269.

Abstract

Zostera marina Linnaeus is one of the world's most widespread seagrass of the Northern Hemisphere. In the Mediterranean, although relatively rare, it is present in coastal lagoons and the innermost part of very sheltered bays. Along Mediterranean coasts of Morocco, the species has disappeared from many localities where it was historically cited. Recently, in 2014, in the framework of the MedKeyHabitats I project, marine surveys focused on the Mediterranean key habitats of the marine part of the site ‘Jbel Moussa’ (southern coasts of the Strait of Gibraltar), two *Z. marina* meadows (lower limit at -17 m depth with patches extending up to -20 m) were identified in Belyounech and Oued El Mersa bays. Data collected during 2014 and 2015 surveys showed good physiological, morphological status with low contamination level by trace elements and no inputs of land-derived organic matter or nutrients defining an overall good health status of *Z. marina* meadows of Jbel Moussa. These Moroccan meadows, localized within the warm temperate-southern limit of the species, were well developed compared to many places worldwide. During the following four years, the meadows experienced high summer temperatures, rapid expansion of the invasive seaweed *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim linked to the global climate change, and persistent trawling activities until a complete destruction. This habitat change induced a significant extinction of soft bottom amphipods communities up to 70% of the total variation.

Keywords: *Zostera marina*, cumulative stressors, global change.

1. Introduction

As required by Barcelona Convention's Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol), the executive agency Regional Activity Center for Specially Protected Areas (RAC/SPA) developed the MedKeyHabitats I project with a financial support of the MAVA foundation. The project aimed at mapping of key marine habitats in the Mediterranean and promoting their conservation through the establishment of Specially Protected Areas of Mediterranean Importance network (SPAMI).

In this context, in 2014, marine surveys of the marine part of the site ‘Jbel Moussa’ (southern coasts of the Strait of Gibraltar) identified two *Zostera marina* Linnaeus (eelgrass) meadows (lower limit at 17 m depth with patches extending up to 20 m) in Belyounech and Oued El Mersa bays (PNUE-PAM-CAR/ASP 2016).

This seagrass is the most widely distributed species through temperate marine environments of the Atlantic and Pacific Oceans to the Arctic Circle (Short et al., 2010). In the Mediterranean, *Z. marina* remains rare and mainly occurs in intertidal and shallow subtidal lagoons and very sheltered bays (Pergent et al., 2014). Because of its global distribution close to major human disturbances, *Z. marina* has experienced alarming loss rates in many locations, including in northern Europe (Frederiksen et al., 2004), southern Europe and the Mediterranean (Cunha et al., 2011; Pergent et al., 2014), the northwestern Atlantic (Beem and Short, 2009; Costello and Kenworthy, 2011; Lefcheck et al., 2017), and the western coast of the USA (Short and Wyllie-Echeverria, 1996). Their alarming loss rates are primarily because of human disturbances such as the destruction of physical habitats, sediment runoff, intensive algal blooms, increased nutrient enrichment, pollution and water temperature rising (Cunha et al., 2011; Moore and Jarvis, 2008; Short et al., 2011; McGlathery et al., 2013; Zhou et al., 2015; Lefcheck et al., 2017; Hammer et al., 2018), as well as disease by the pathogen *Labyrinthula zosterae* (Hughes et al., 2018).

Along Mediterranean coasts of Morocco, the species has disappeared from many localities where it was historically cited (Bitar, 1987; Bazairi, 2015). To the best of our knowledge, Jbel Moussa meadows represent the only remaining ones along Mediterranean coasts of Morocco, or even of North Africa, and the deepest ones in the entire Mediterranean.

The worldwide decline in the distribution and abundance of *Z. marina* meadows, with the singularity of those of Jbel Moussa, require sound monitoring and management following the

Integrated Monitoring and Assessment Programme (IMAP) adopted by the Contracting Parties to the Barcelona Convention. IMAP, in line with the Ecosystem Approach Process, is a key achievement of a quantitative, integrated analysis of the state of the marine and coastal environment, covering pollution, biodiversity, and hydrography, based on common regional indicators (UNEP, 2017). The ultimate goal of this road map is to assess the status of the Mediterranean Sea and support the integration process at the national level (review and update of the national monitoring programs).

The Moroccan national monitoring program for the biodiversity component was developed and discussed during a national validation workshop as part of the EcApMEDII project activities (Rabat, Morocco, July 4th, 2017). The participants adopted a list of species, habitats, and non-indigenous species (NIS) to be monitor, including *Z. marina* meadows of Jbel Moussa. This PhD thesis directed in cotutelle between Mohammed V University of Rabat (Morocco) and the University of Seville (Spain) is a part of the implementation of the 'National Integrated Monitoring and Assessment Program' at the Jbel Moussa site.

To this end, a series of ecological and biochemical descriptors were investigated in the present work for monitoring of *Z. marina* habitats. First, seagrass extent metrics (area extent, depth limit), seagrass density metrics (cover, shoot density, total and above-ground biomass), seagrass shoot morphometry and biomass, and associated amphipods crustaceans species in response to the biodiversity common indicators 1 (habitat distributional range) and 2 (condition of the habitat's typical species and communities) of the IMAP program. Second, the presence of alien species as the common indicator 6 (trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species). 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 (Ikem and Egiebor, 2005; Ralph et al., 2006; Jackson et al., 2013). By integrating such measurements, greater information about the environmental status of these sensitive seagrass meadows can be provided.

2. Materials and methods

2.1. Study area

The site Jbel Moussa is a Site of Biological and Ecological Interest (SIBE) of priority 1 in the Master Plan of Protected Areas in Morocco (PDAPM, 1996) and was listed as a RAMSAR site in 2019. Its boundaries delimit an area of 5,000 ha (land area: 35.55 km^2, marine area: 14.45 km^2). 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 4,500 live in the middle of the SIBE in the municipality of Belyounech. The area of Jbel Moussa is characterized by a variety of natural resource exploitation methods. The main activities are agriculture and livestock, artisanal fishing and tourism. Agriculture remains a food-producing activity that is not very developed when compared to livestock farming practiced by a large part of the population. 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, 2019). Tourism and leisure activities remain limited in Jbel Moussa.

The SIBE Marine Area encompasses the marine fringe between Cap Ciress and Belyounech, including the islet of Leila/Perejil. The SIBE coastal zone is characterized by the dominance of rocky shores and cliffs, alternating with caves, creeks and small sandy beaches.

The Jbel Moussa area is located in the southern part of the Strait of Gibraltar. Oceanic circulation in the region is characterised by exchanges of different water masses, consisting of the denser Mediterranean waters outflew at depth, and the less salty Atlantic waters inflow occupying the upper layer (García-Lafuente, 2017). However, the thickness and depth of each water profile varies along the Strait under the influence of physical mechanisms that operate on different time scales, such as atmospheric pressure variations, winds, tidal currents, and bathymetric circulation processes (García-Lafuente, 2007). Within this context, the entrance of Atlantic waters oscillates between two main circulation patterns on a seasonal scale with stronger flows northeastward during the first half of the year and a weaker flow southward towards the end of the year (Vargas-Yáñez et al., 2002). The zonal wind has been reported as the main driving force for surface circulation (Macías et al., 2016). Following an annual cycle, the westerly winds during winter enhance the surface inflow while the strong summer easterly winds induce the extreme collapses (Dorman et al., 1995). Furthermore, the entrance of Atlantic flood tidal currents via Gibraltar, leads to the periodic generation of large-amplitude internal waves propagating eastward and forming turbulence, especially in shallow areas where wave overtopping occurs (Sanchez-Garrido et al., 2011). Under extreme weather conditions, waves can reach over 8 m high (Mazas and Hamm, 2011). In the bottom layer, occupied by the Mediterranean waters, the speed of the currents gradually increases until it reaches its maximum at about 290 m below the surface. This maximum is about 130 cm/s. Below this depth, the velocity decreases (Sanchez-Roman et al. 2008). 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 a part of the global scale ocean warming, an increasing temperature trend is observed in the Strait of Gibraltar since 1995 (Sanz-Fernández et al., 2019). In the northern part of the Strait, the temperature of the sea surface increased over the period 2000-2015, with a minimum monthly average of 14 °C and peaking at 23 °C in July 2015 (Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, 2019).

2.2. Sampling design and laboratory analyses

The mapping of marine key habitats of Jbel Moussa between Punta Ciress and Belyounech using a Remotely Operated Vehicle (ROV: VideoRay PRO) has identified two *Z. marina* meadows in the bays of Oued El Mersa and Belyounech spreading over 29.19 and 9.54 ha respectively (PNUE-PAM-CAR/ASP 2016; Fig. 1).

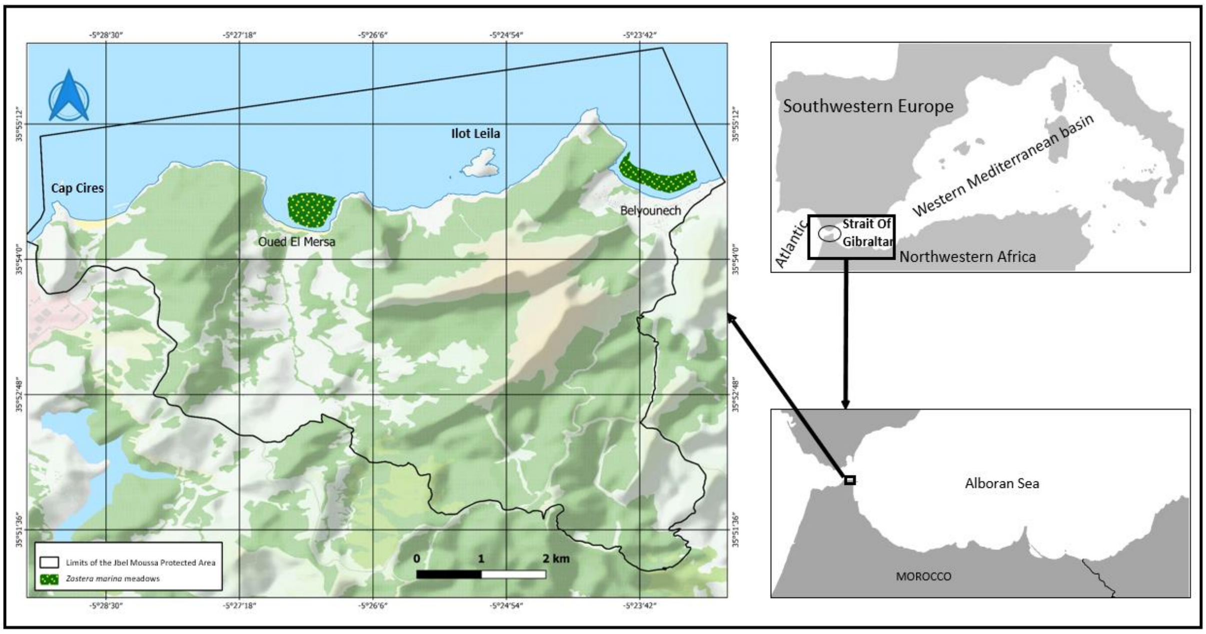

Fig. 1: Map showing the georaphical position of the study meadows Belyounech and Oued El Mersa bays in the marine part of the site Jbel Moussa, along the Mediterranean coast of Morocco, southern coasts of the Strait of Gibraltar (From PNUE-PAM-CAR/ASP 2016 modified).

2.2.1. *Zostera marina* abundance and biomass

The first sampling was carried out in mid-September 2014 in the central dense part of both meadows. Seasonal monitoring survey was performed in September 2017, April, July, September and December 2018 from 1x1 m permanent quadrats at the center and the edge of each meadow, (more details in 2.2 section).

Z. marina inside a 20x20 cm randomly thrown quadrat were counted to estimate shoot density (n = 10). Mature shoots were collected individually (n = 20) to measure leaf biomass and foliar biometric parameters: length and width by leaf category, *i.e.*, differentiated (with sheath) and not differentiated (without sheath) and the number of leaves by shoot.

To estimate the aboveground and belowground biomass per unit of surface, five cores were randomly collected using a cylindrical PVC corer of 0.15 m in diameter. They were sieved through a 0.5 mm mesh size sieve and rinsed with seawater. Plant material was separated into aboveground (leaves) and belowground tissues (rhizomes and roots), transferred into plastic bags and frozen. In the laboratory above and belowground parts of *Z. marina* were oven dried 48 h at 60 °C to constant weight to determine tissue dry weight (g_{DW}).

2.2.2. *Zostera marina* and *Caulerpa cylindracea* cover

In September 2015, at the center and the edge of each meadow, permanent quadrats of 1x1 m (n = 5) were set up and fixed in the sediment with 50 cm long metal stakes to monitor the seagrass cover seasonally. Cover seasonal monitoring was then performed in September 2017, April, July, September and December 2018. Each quadrat was photographed, and images digitally analysed using Adobe Photoshop 6.0©. A digital network of 64 squares was superimposed onto the photographs and adjusted using the distortion tool. The coverage of each species was then measured (Espinosa et al., 2014).

2.2.3. Sediment characterization and chemical analyses

In September 2015, at the center of Belyounech bay, seagrass with sediment cores (n = 3) were randomly collected in the vegetated seabed using a cylindrical PVC corer of 0.15 m in diameter. Leaves, rhizomes and roots were separated in the field and all samples were transferred into plastic bags and stored frozen until analysis. In the laboratory, we reported very low occurrence of epiphytic algae, so if present, they were removed by scraping leave with a glass slide (Dauby and Poulicek, 1995). Seagrass compartments and sediment were oven dried 48 h at 60 °C to constant weight. Plant material was then ground with the support of a Mixer Mill (MM200, Retsch GmbH) (30 Hertz, 3 min). Each sediment core was sifted through 0.0625 mm nylon mesh sieve for trace elements (TEs) and elementary content analysis. The mud fraction (< 0.0625 mm) was selected because contaminants tend to associate with sediment fine particles (Jickells and Knap, 1984). Chemical elements, P, C and N contents and the stable isotope ratio values of C and N were then measured.

In 2017 and 2018 sampling times, sediment samples (n = 3) were taken from the center and the edge of each meadow and subdivided into two homogeneous subsamples for organic matter content and grain size partitioning.

Organic matter content was determined by the loss on ignition method (Heiri et al., 2001) in the first subsample of each sediment core. Subsamples were dried at 105 °C up to a constant weight (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 determination of the different fractions' ratios (gravel, sand and mud; Wentworth, 1922) using Afnor sieves (63 to 2000 µm).

For major and TEs analysis, grinded seagrass samples 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 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.). Analyses included P, 4 major elements : Na, Mg, K, 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. Concentrations of Hg were determined 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 PACS-2 (Marine sediments) for sediments and BCR 60 (*Lagarosiphon major*), BCR 61 (*Platihypnidium riparioides*), GBW 07603 (brush 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). Vegetal CRM mean recovery was 103 % (there was no certified value for Sn, Ti and U). Chemical element concentrations are expressed in $mg.kg^{-1}{}_{DW}$ of sediment mud or seagrass compartments.

The analyses of C and N content and their stable isotopes ratio values ($\delta^{13}C$ and $\delta^{15}N$, ‰) were conducted via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS) at the University of Liège (ULiège, Belgium) 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; mean ± SD: $\delta^{13}C = -10.8 \pm 0.5$ ‰) and ammonium sulfate (IAEA-N_2; $\delta^{15}N = 20.3 \pm 0.2$ ‰) were used as certified reference materials (CRM). Both CRMs are calibrated against international isotopic references, *i.e.* the Vienna Pee Dee Belemnite (VPBD) for carbon and Atmospheric Air for nitrogen. 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 $\delta^{13}C$ and $\delta^{15}N$ respectively. Elemental data are express in $\%_{DW}$ after which atomic C:N and N:P values were calculated.

2.2.4. Amphipod collection and data analyses

To determinate the composition of the amphipod community and evaluate its temporal dynamics at the seasonal scale, both zones (center and edge) of *Z. marina* meadows (Oued El Mersa and Belyounech) were prospected. The samples collection was carried out in September 2017, April, July, September and December 2018, using a hand-held core of 0.02 m^2. Five replicate core samples were taken at each position. Samples were washed through a 0.5 mm mesh sieve with seawater and fixed with ethanol. In the laboratory, each sample was examined using binocular microscopes. All amphipod specimens were identified to species level where possible and counted. Abundance (N: ind. m^{-2}), number of species (S), Shannon-Wiener diversity index (H′, $\log_2$) and Pielou's evenness index (J′) were calculated.

The spatiotemporal variations in these parameters were explored using an Analysis of Variance (ANOVA) with the following factors: time (fixed factor with 5 levels: one of each sampling times), meadow (fixed with 2 levels, either Oued El Mersa or Belyounech), and zone (fixed with 2 levels, the center and the edge). Prior to ANOVA analyses, the homogeneity of variances was tested using Cochran test. When ANOVA indicated a significant difference for a given factor, the source of difference was identified using the *posthoc* test.

Following the same three-factor design, a distance based permutational multivariate analysis of variance (PERMANOVA) was carried out to test differences in the amphipod's composition. The statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. Data were fourth root transformed to reduce the importance of extreme values, and a similarity matrix was generated using the Bray-Curtis similarity index. Terms found to be significant were examined using pairwise a posteriori comparison with the t statistic. A permutational analysis of multivariate dispersions (PERMDISP) was applied to test whether the amphipods composition differences were due to different dispersion of samples or to the location of centroids.

Hierarchical agglomerative cluster analysis (CA) was conducted using the mean values per station to assess the relationship between stations in different sampling times. The group-average linking was used and interpreted with the similarity profile test (SIMPROF). The similarity percentages procedure (SIMPER) was performed to identify the species that contributed most to similarity and dissimilarity within and between the identified assemblages. A cut-off criterion was applied to allow identification of a subset of species whose cumulative percentage contribution higher than 75% of the similarity values. A nonmetric multidimensional scaling (nMDS) was applied as an ordination method to visualize patterns in data.

3. Statistical analyses

Differences in measured parameters among meadows (Belyounech and Oued El Mersa bays), zones (center and edge of meadows), sampling times and benthic community structure indexes were analyzed using three-way ANOVA in STATISTICA software package (StatSoft Inc., version 6). Macrofaunal multivariate analyses were carried out with the PRIMER v.6 + PERMANOVA package (Clarke and Gorley, 2006; Anderson et al., 2008).

3. Results

3.1. *Zostera marina* meadows characterization

In 2015, Belyounech *Z. marina* meadow cover was continuous while that of Oued El Mersa where trawling scar has been observed (Fig. 2) was highly fragmented.

Fig. 2: Trawl crossing scars observed in the Ouad El Mersa near the *Zostera marina* meadows.

The analysis of pictures of the permanent quadrats deployed in the two seagrass beds showed a *Z. marina* cover of about 100% in the center and of more than 80% at the edges (Fig. 3). During sampling, the invasive algae *Caulerpa cylindracea* Sonder was observed in both meadows. It was more abundant at the edge of Oued El Mersa meadow than Belyounech meadow, and almost absent in the center of meadows except a few in Belyounech (Fig. 4). Differences in *C. cylindracea* cover were significant ($p < 0.05$) between zones only.

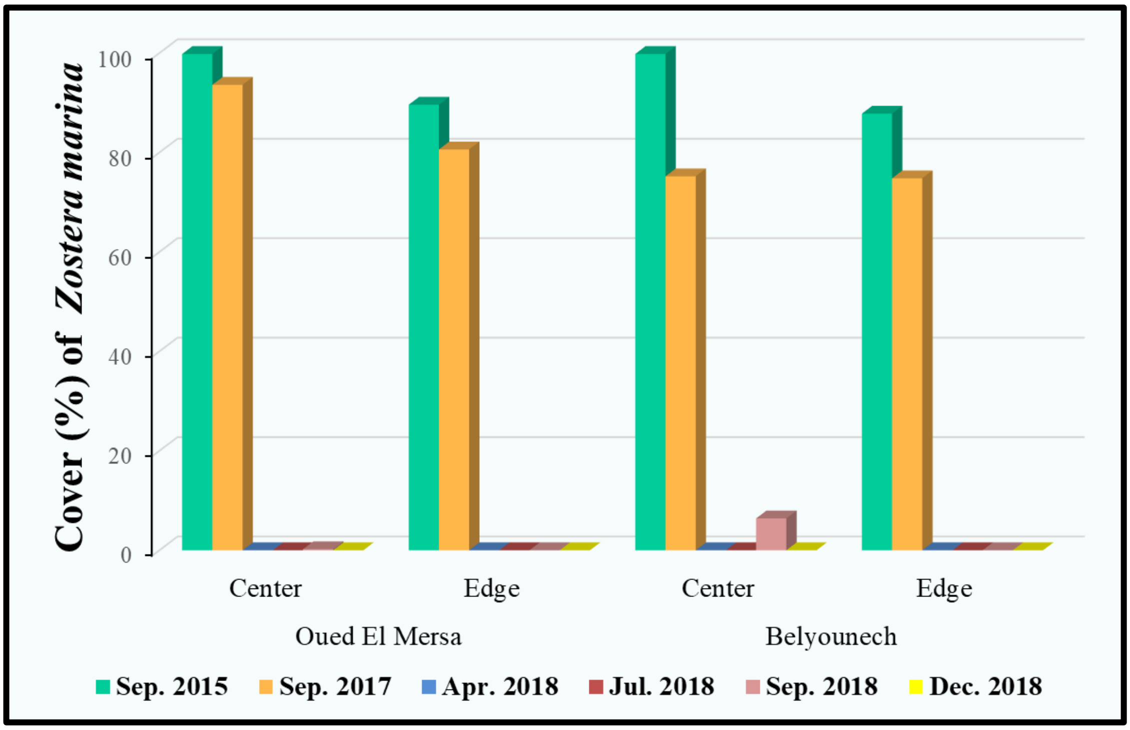

Fig. 3: Evolution between 2015, 2017 and 2018 of the *Zostera marina* percentage cover (n = 5) by meadow (Oued El Mersa vs Belyounech) and by position in the meadows (Center vs Edge).

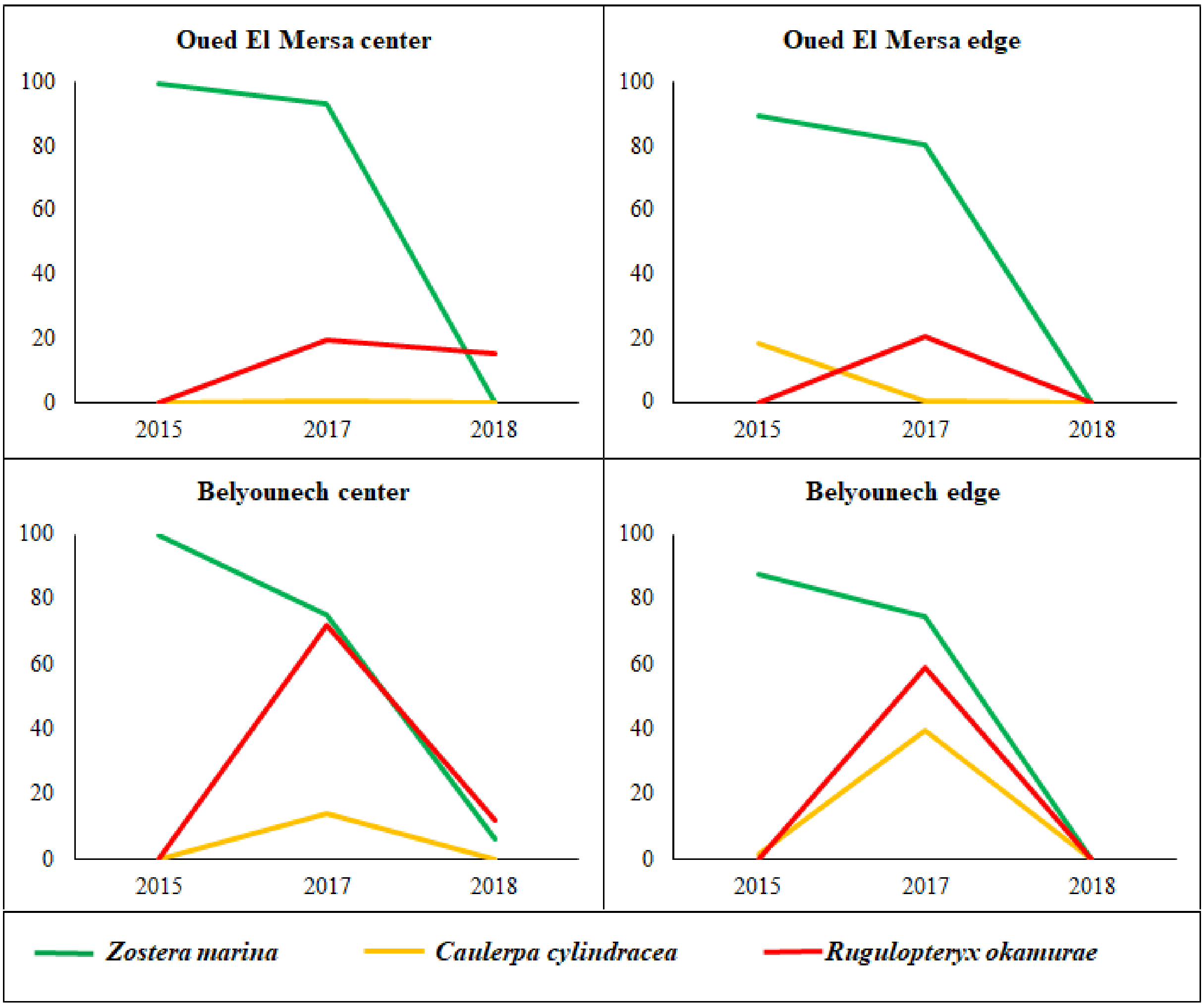

Fig. 4: *Zostera marina*, *Caulerpa cylindracea* and *Rugulopteryx okamurae* percentage covers (n = 5) in the center and on the edge of Oued El Mersa and Belyounech meadows.

From 2015 to 2018, a significant decrease in *Z. marina* percentage cover (%) was recorded in both meadows with a total disappear of the seagrass in April, July and December 2018 (Fig. 3; Fig. 5).

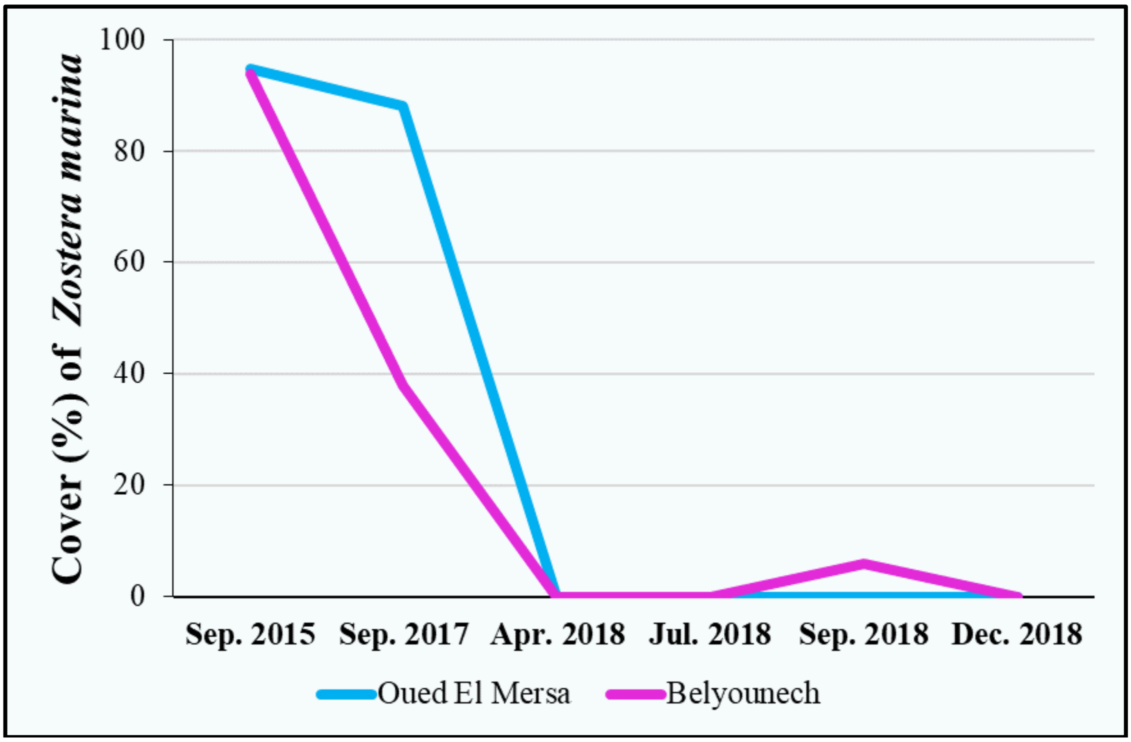

Fig. 5: Evolution between 2015, 2017 and 2018 of the *Zostera marina* percentage cover (n = 5) by meadow (Oued EL Mersa vs. Belyounech).

Since 2017, a significant proliferation of the invasive alga *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim has been recorded in the marine part of Jbel Moussa. In *Z. marina* meadows, it accounted for 94% and 98% of the total alga biomass measured in Oued El Mersa and Belyounech respectively. It was more abundant in Belyounech with 62% and 65% of the total vegetated biomass of the center and the edge zones respectively (Fig. 6).

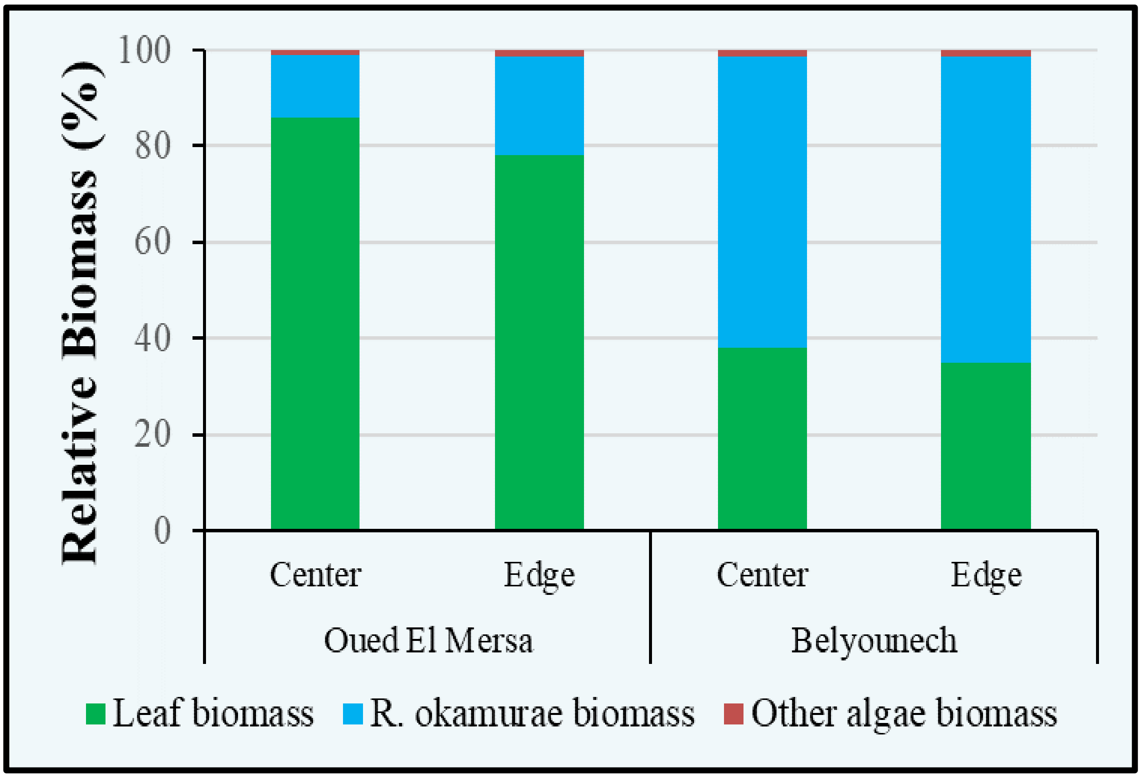

Fig. 6: Relative mean contribution (%) of *Zostera marina* leaf, *Rugulopteryx okamurae* and other algae weights to the total vegetative biomass (n = 5) in September 2017.

The mean number of total leaves per shoot was similar in the central zone of both meadows with an enhance from 5 leaves shoot^{-1} in 2014 to 6 leaves shoot^{-1} in 2017. This is due to the increase of differentiated leaves numbers (Table 1). In 2014, Oued El Mersa leaves were significantly longer (146 mm) comparing to Belyounech meadow (109 mm length, $p < 0.05$) while a contrary was found in 2017 with 133 mm length for the former and 153 mm for the later. However, the mean shoot weight remained higher in Oued El Mersa comparing to Belyounech meadow in both years. From 2014 to 2017, a clear decrease in the above- and below-ground biomass of *Z. marina* has been recorded in both meadows. This was remarkably observed in Belyounech bay where leaf biomass decreased from 60% of the total biomass in 2014 to only 37% in 2017 (Table 1). Regarding the zone effect explored in 2017, Belyounech edge, the lower limit of Jbel Moussa *Z. marina* meadows (17 m depth), showed the lowest total number of leaves per shoot and the longest ones. Accordingly, no differences were found for shoots weight comparing to the central zone.

Table 1: *Zostera marina* biomass (n = 5) and morphometric measurements (mean ± standard deviation, SD, n = 20) of Belyounech and Oued El Mersa seagrass meadows in 2014 and 2017.

Time	September 2014				September 2017							
Meadow	O.E.Mersa		Belyounech		O.E.Mersa		Belyounech		O.E.Mersa		Belyounech	
Zone	Center				Center				Edge			
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Number of total leaves per shoot (leaves.shoot^{-1}, n=20)	5.2	1.2	5.2	0.9	6.1	1.4	6.0	1.5	6.0	0.9	5.2	1.2
Number of differenciated leaves per shoot (leaves.shoot^{-1}. n=20)	2.3	0.9	2.5	0.5	3.2	0.7	3.2	0.8	3.2	0.6	2.7	0.6
Number of undifferentiated leaves per shoot (leaves.shoot^{-1}. n=20)	2.9	0.6	2.7	0.8	2.9	1.1	2.8	1.1	2.8	1.0	2.5	0.8
Mean leaves length (mm. n=20)	146	96	109	64	133	73	153	76	121	46	172	98
Mean leaves width (mm. n=20)	3.5	0.9	3.3	0.6	3.7	0.9	3.6	1.5	3.5	1.6	3.3	0.8
Shoot weight (mg. n=20)	125	92	64	34	122	40	110	48	82	13	106	29
Leaf biomass ($g_{Dw}.m^{-2}$. n=5)	260	26	273	40	159	21	72	28	116	34	60	5.8
Rhizomes biomass ($g_{Dw}.m^{-2}$. n=5)	54	20	60	21	96	72	50	4.3	86	26	41	6.9
Roots biomass ($g_{Dw}.m^{-2}$. n=5)	250	105	121	63	134	37	75	7.8	76	31	59	25
Belowground biomass ($g_{Dw}.m^{-2}$. n=5)	303	111	181	82	230	88	124	11	162	30	99	21
Total seagrass biomass ($g_{Dw}.m^{-2}$. n=5)	563	113	454	104	390	103	196	21	277	36	159	23

3.2. Sediment description

The mean organic matter content of sediment from both seagrass meadows was low and ranged between 1.7% and 3.8%. The sediment grain size varied from mud to very coarse sand. The sand fraction dominated and accounted for at less 95% in the total samples (Table 2).

Table 2: Granulometric characteristics (mean. in %) of sediment (n = 3) sampled between 2017 and 2018 in the center and the edge of the *Zostera marina* meadow.

		September 2017			April 2018			July 2018			September 2018			December 2018		
		Sand	Mud	OM	Sand	Mud	OM	Sand	Mud	OM	Sand	Mud	OM	Sand	Mud	OM
Oued El Mersa	Center	99	1.5	2.1	98	2.1	1.9	98	1.9	2.5	99	1.4	2.4	98	2.1	1.7
	Edge	99	1.5	2.9	98	2.5	3.2	98	2.5	3.8	98	2.3	2.7	98	2.3	2.8
Belyounech	Center	95	5.4	3.1	95	4.7	3.8	97	3.0	2.1	97	3.3	3.4	97	3.2	3.1
	Edge	96	4.5	2.2	95	5.0	2.4	97	3.2	2.8	97	3.1	2.9	97	3.1	2.8

3.3. Chemical analyses

Concentrations of major and TEs in the < 0.0625 mm sediment grain size fraction and *Z. marina* compartments (leaves. roots and rhizomes) sampled in the center of Belyounech bay meadow with the concentration ratios between the seagrass compartments and with the sediment are given in Table 3.

Most elements except a few were more concentrated in sediment than in the seagrass compartments. Indeed. the plant compartment over sediment concentration ratio 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 different compartments of the seagrass compared to the sediment. Ni, As, Sn, Sb and U were highly accumulated in roots only compared to sediment. Overall, chemical element concentrations decreased in *Z. marina* compartments in the following order: root > leaf > rhizome. Element distribution differed between the three compartments of the plant (Table 3). Values in the rhizosphere (rhizome/root) ranged between 0.008 for Ni and 3.11 for K. Values of chemical elements from rhizome to leaf varied appreciably from 0.73 for U to 6.91 for Ni. Values of leaf/root of different chemical elements were highest for K (3.52) and lowest for Ni (0.05).

Table 3: Chemical element concentrations ($mg.kg^{-1}_{DW}$) and concentration ratios in the < 0.0625 mm sediment grain size fraction (n = 1) and *Zostera marina* compartments (leaves. rhizomes. roots; n = 1) sampled in Belyounech bay meadow.

		Na	Mg	Al	K	Ca	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Sr	Li	As	Mo	Ag	Cd	Sn	Sb	Ba	Tl	Pb	Bi	U	Hg
Sediments		15400	23100	18400	4200	81600	39.6	21.0	23100	207	7.0	11.2	13.7	46.0	171	20.8	7.25	1.53	0.02	0.08	0.26	0.07	22	0.32	16.8	0.04	1.06	0.01
Z. marina compartments	leaves	68100	10400	498	33000	8230	2.50	1.32	492	37.0	0.49	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
	rhizomes	61100	8090	171	29100	6410	1.10	0.32	285	6.00	0.12	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
	roots	47100	12800	6190	9370	29700	21.0	7.86	6980	92.0	6.27	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
Ratios	leaves/sediments	4.42	0.45	0.03	7.86	0.10	0.06	0.06	0.02	0.18	0.07	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
	rhizomes/sediments	3.97	0.35	0.01	6.93	0.08	0.03	0.02	0.01	0.03	0.02	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
	roots/sediments	3.06	0.55	0.34	2.23	0.36	0.53	0.37	0.30	0.44	0.89	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
	leaves / rhizomes	1.11	1.29	2.91	1.13	1.28	2.27	4.13	1.73	6.17	4.08	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
	leaves / roots	1.45	0.81	0.08	3.52	0.28	0.12	0.17	0.07	0.40	0.08	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
	rhizomes / roots	1.30	0.63	0.03	3.11	0.22	0.05	0.04	0.04	0.07	0.02	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 content, *Z. marina* compartments from Belyounech bay meadow showed higher N contents than sediment. Conversely, sediment was more enriched in P and $\delta^{15}N$ than *Z. marina* compartments. The organic C and $\delta^{13}C$ were respectively higher and more negative in leaves comparing to belowground parts. The high C and low P and N in rhizomes led to the higher C:N and lower N:P ratios than leaves and roots (Table 4).

Table 4: Mean C and N contents (in %). ratio and their stable isotope compositions in the < 0.0625 mm sediment grain size fraction (n = 1) and *Zostera marina* compartments (leaves. rhizomes. roots; n = 1) sampled in the center of Belyounech bay seagrass meadow.

		$\%C_{DW}$	$\%N_{DW}$	$\%P_{DW}$	C:N (at:at)	C:P (at:at)	N:P (at:at)	δ13C (‰)	δ15N (‰)
Sediments		-	0.05	0.48	-	-	0.23	-	4.72
Z. marina compartments	leaves	32.8	2.17	0.19	17.6	446	25.3	-12.3	2.3
	rhizomes	30.9	0.67	0.14	53.8	570	10.6	-11.7	1.88
	roots	18.2	0.81	0.16	26.2	294	11.2	-11.4	2.84

3.4. Amphipods composition

A total of 52 amphipod species (6 caprellids and 46 gammarids) were recorded from the four study positions during the five sampling times (Table 5). The ANOVA results showed that maximum value for species richness, density and diversity were registered in September 2017 comprising 40 species and 53% of the total specimens (Table 6). The center zones showed significantly higher densities than the edges with the great number recorded in September 2017 at Oued El Mersa center. In turn, amphipods of Belyounech meadow were more equitable (Table 6).

Ten species were exclusively found in Oued El Mersa meadow. Since 2017, 3 disappeared (*Amphilochus* sp., *Caprela danilevskii*, *Gammmaropsis maculata*), 6 appeared (*Echinogammarus sp., Hyale sp., Megaluropus sp., Peltocoxa marioni, Pleonexes heller, Nototropis swammerdamei*) and one persisted (*Stenothoe monoculoides*). In Belyounech meadow, from 9 exclusive species, 5 disappeared after 2017 (*Harpinia crenulata, Iphimedia sp., Leptocheirus pectinatus, Lysianassa sp., Microdentopus versiculatus*), and 4 were newly recorded (*Leptocheirus sp., Microprotopus longimanus, Parvipalpus major, Tryphosites longipes*). Two more species appeared after 2017 in both meadows: *Harpinia ala* and *Monoculodes griseus*.

Table 5: List of amphipod species recorded at Jbel Moussa and mean densities (ind/m^2) at each sampling station and sampling time. M: Oued El Mersa, B: Belyounech, C: center, E: edge.

	September 2017				**April 2018**				**July 2018**				**September 2018**				**December 2018**			
Species \ Stations	**MC**	**ME**	**BC**	**BE**	**MC**	**ME**	**BC**	**BE**	**MC**	**ME**	**BC**	**BE**	**MC**	**ME**	**BC**	**BE**	**MC**	**ME**	**BC**	**BE**
Abludomelita gladiosa																				
Ampelisca sp.																				
Amphilochus sp.																				
Ampithoe ramondi																				
Aora gracilis																				
Aoridae sp.																				
Apherusa sp.																				
Bathyporea cf. elegans																				
Caprela acanthifera																				
Caprela danilevskii																				
Cerpopsis cf. longipes																				
Corophium sp.																				
Dexamine spiniventris																				
Dexamine spinosa																				
Echinogammarus sp.																				
Ericthonius argenteus																				
Gammarella fucicola																				
Gammmaropsis maculata																				
Gammmaropsis palmata																				
Harpinia ala																				
Harpinia crenulata																				
Harpinia pectinata																				
Hippomedon massiliensis																				

Hippomedon sp.																				
Hyale sp.																				
Iphimedia sp.																				
Leptocheirus pectinatus																				
Leptocheirus sp.																				
Leucothoe incisa																				
Lysianassa sp.																				
Megaluropus sp.																				
Metaphoxus sp.																				
Microdentopus versiculatus																				
Microdentopus stationis																				
Microprotopus longimanus																				
Microprotopus maculatus																				
Monoculodes griseus																				
Nototropis guttatus																				
Nototropis massiliensis																				
Nototropis swammerdamei																				
Pariambus typicus																				
Parvipalpus major																				
Peltocoxa marioni																				
Pentocrates arenarius																				
Pereionotus testudo																				
Perioculodes longimanus																				
Phtisica marina																				
Pleonexes helleri																				
Pseudoprotella phasma																				

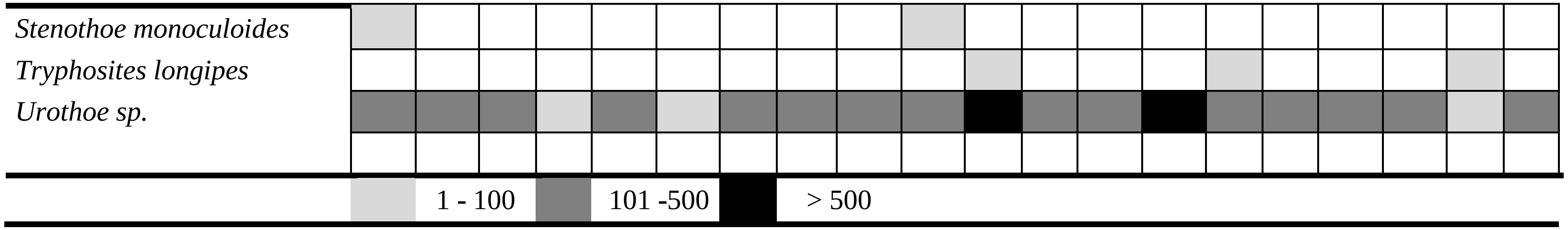

Table 6: Three-way ANOVA and post hoc for the species richness (S), density (N), Shannon diversity index (H′) and Pielou evenness index (J′) of the amphipod communities at the scales of time (5 levels, fixed), meadows (2 levels, fixed), and zone (2 levels, fixed) levels. M: Oued El Mersa, B: Belyounech, C: center, E: edge, Sep: September, Dec: December, Apr: April, Jul: July. *$p<0.05$; **$p<0.01$; ***$p<0.001$*

Source of deviation	df	S				N				H'				J'			
		MS	F	*p*	post hoc	MS	F	*p*	post hoc	MS	F	*p*	post hoc	MS	F	*p*	post hoc
Time	4	252	33.9	***		54550626	27.6	*		6.46	25.9	***		0.078	10.4	***	Sep 2017≠Apr 2018-Jul 2018≠Sep 2018-Dec 2018
Meadow	1	16	2.15	0.146		5041269	2.55	0.114		3.26	13.1	***		0.109	14.8	***	B≠M
Zone	1	23	3.10	0.082		11938939	6.05	*		0.009	0.035	0.851		0.025	3.31	0.072	
Time×Meadow	4	7.15	0.96	0.433		12109475	6.13	***	Sep 2017 M≠Sep 2017 B≠Jul 2018 B-Jul 2018 M-Sep 2018 M-Sep 2018 B≠Dec 2018 M-Dec 2018 B-Apr 2018 B≠Apr 2018 M	0.12	0.49	0.737		0.015	2.01	0.102	
Time×Zone	4	22	2.97	*	Sep 2017 C≠Sep 2017 E≠Jul 2018 C-Jul 2018 E≠Sep 2018 C-Sep 2018 E-Dec 2018 C≠Dec 2018 E-Apr 2018 C-April 2018 E	12288605	6.22	***	Sep 2017 C≠Sep 2017 E≠Jul 2018 C-Jul 2018 E-Sep 2018 C-Sep 2018 E-Dec 2018 C≠Dec 2018 E-Apr 2018 C-Apr 2018 E	0.296	1.19	0.323		0.008	1.03	0.397	
Meadow×Zone	1	154	20.7	***	BC-ME≠MC-BE	6760640	3.42	0.068		5.23	20.9	***	BC≠ME-BE≠MC	0.019	2.64	0.108	
Time×Meadow×Zone	4	7.01	0.94	0.443		836618	0.42	0.791		0.94	3.77	**		0.008	1.11	0.359	
Residuals	80	7.43				1974670				0.25				0.007			

PERMANOVA results (Table 7) revealed statistically significant differences in amphipods composition between meadows, times and also for all the interactions. This indicated that differences among meadows were not constant in sampling times and nor were differences among times constant in space.

Table 7: Results of the multivariate permutational analysis (PERMANOVA) for amphipod assemblages Time (5 levels, Fixed), Meadow (2 levels, fixed), and Zone (2 levels, fixed) factors. Analyses based on Bray–Curtis similarity matrixes from fourth root transformed data. *$^{**}p<0.01$; $^{***}p<0.001$*

Source of variation	df	SS	MS	Pseudo-F	Unique perms	*P*(perm)
Time	4	55824	13956	10.214	9895	***
Meadow	1	11113	11113	8.1332	9952	***
Zone	1	2546.2	2546.2	1.8635	9957	0.0771
Time× Meadow	4	19814	4953.5	3.6253	9890	***
Time × Zone	4	10690	2672.4	1.9559	9906	**
Meadow × Zone	1	7226.1	7226.1	5.2886	9955	***
Time × Meadow × Zone	4	13011	3252.6	2.3805	9886	***
Residual	80	109310	1366.4			
Total	99	229530				

Pairwise comparisons (Table 8) showed that within Oued El Mersa meadow, edge assemblages differed significantly over all sampling times. In the center, September 2017 differed significantly from other periods. Between both zones, differences occurred for April and July assemblages. In Belyounech meadow, more similarity between time assemblages were found within both zone positions. However, September 2017 differed significantly from other sampling times. By comparing zones of both meadows, similarity occurred in April between the centers (t = 1.61, p(perm) = 0.065) and in September 2018 between the edges (t = 1.35, p(perm) = 0.14). Between both meadows, amphipod assemblages were significantly different in all times (p(perm) < 0.05).

Table 8: Multivariate pair-wise comparison of amphipod assemblages for the term a) Time × Meadow at the type-of-Meadow level, for the term Time × Meadow × Zone b) at the type-of-Zone level, c) at the type-of-Meadow level, d) at the type-of-Time level. The significant values are indicated in bold. M: Oued El Mersa, B: Belyounech, C: Center, E: Edge, Sep: September, Dec: December, Apr: April, Jul: July. **p<0.05; **p<0.01; ***p<0.001*

Time Meadow/Zone		Sep. 2017	Apr. 2018	Jul. 2018	Sep. 2018	Dec. 2018
a) M vs B	*t*	2.5206	1.8027	1.7275	1.7052	2.8536
	p(perm)	***	**	*	*	***
b) MC vs ME	*t*	1.2197	1.7234	2.5503	1.3985	1.2629
	p(perm)	0.1398	*	**	0.0505	0.2216
BC vs BE	*t*	1.7069	1.2024	1.0527	1.1993	1.5031
	p(perm)	**	0.2331	0.3909	0.2613	*
c) MC vs BC	*t*	2.2004	1.6103	1.6265	1.6622	2.0219
	p(perm)	**	0.0652	**	*	**
ME vs BE	*t*	1.9835	2.0278	2.2884	1.3524	2.4135
	p(perm)	**	**	**	0.1363	**

d) Station	MC		ME		BC		BE	
Time	*t*	*p(perm)*	*t*	*p(perm)*	*t*	*p(perm)*	*t*	*p(perm)*
Sep. 2017, Apr. 2018	2.9993	**	2.165	**	2.1935	**	1.616	*
Sep. 2017, Jul. 2018	3.6444	**	2.3617	**	1.7805	**	1.5512	*
Sep. 2017, Sep. 2018	3.0549	**	4.1638	**	2.3795	**	1.5847	*
Sep. 2017, Dec. 2018	4.1824	*	4.1166	**	2.3203	**	2.1242	**
Apr. 2018, Jul. 2018	1.7721	*	1.9626	**	1.3381	0.1357	1.7262	**
Apr. 2018, Sep. 2018	1.4808	0.1233	2.987	**	1.94	**	1.2789	0.1782
Apr. 2018, Dec. 2018	1.7989	*	2.8193	**	1.4639	0.0587	1.5035	0.074
Jul. 2018, Sep. 2018	0.83827	0.6737	2.9171	**	1.2749	0.1123	0.86817	0.6012
Jul. 2018, Dec. 2018	1.6172	*	3.0838	**	1.5976	**	1.8917	**
Sep. 2018, Dec. 2018	1.2609	0.2192	2.4766	**	1.389	0.1268	1.6576	*

The lack of significant differences on the PERMDISP procedure indicated that differences detected by PERMANOVA were due to the location effect of centroids rather than different multivariate samples dispersion between meadows (F = 3.63, *p*(perm) = 0.08) or zones (F = 0.49, *p*(perm) = 0.52).

The cluster analyses with SIMPROF test identified five distinct assemblages (Table 9). The first and second groups gathered Belyounech zones (G1) and Oued El Mersa zones (G2) of September 2017 respectively. The third group included April of Oued El Mersa zones (G3), and G4 included all the remaining sampling times (G4) except the isolated Oued El Mersa edge station of July 2018 (G5).

SIMPER test highlighted that 19 species were responsible for within group similarity (76 to 98% of contribution) and between group dissimilarity (48 to 98% of contribution) (Table 9). These species dominated the group assemblages with 81, 93, 91, 93 and 71% in G1, G2, G3, G4 and the isolated Oued El Mersa edge station of July 2018 respectively. Moreover, they comprised from 71 to 100% of amphipod composition of the four sampled positions during the five sampling times.

The resulting MDS plot suggests the same PERMANOVA patterns where labels are used to denote different levels of the meadow and zone factors and symbols are used to denote different levels of the time factor (Fig. 7). The plot revealed the absence of clear and constant effects of the main factors considered on amphipods composition. Distinction was relevant between September 2017 and 2018 times. Nevertheless, amphipod structure seems to be also influenced by meadow (Oued El Mersa and Benyounech) because samples of each one appears mostly segregated in the MDS plot.

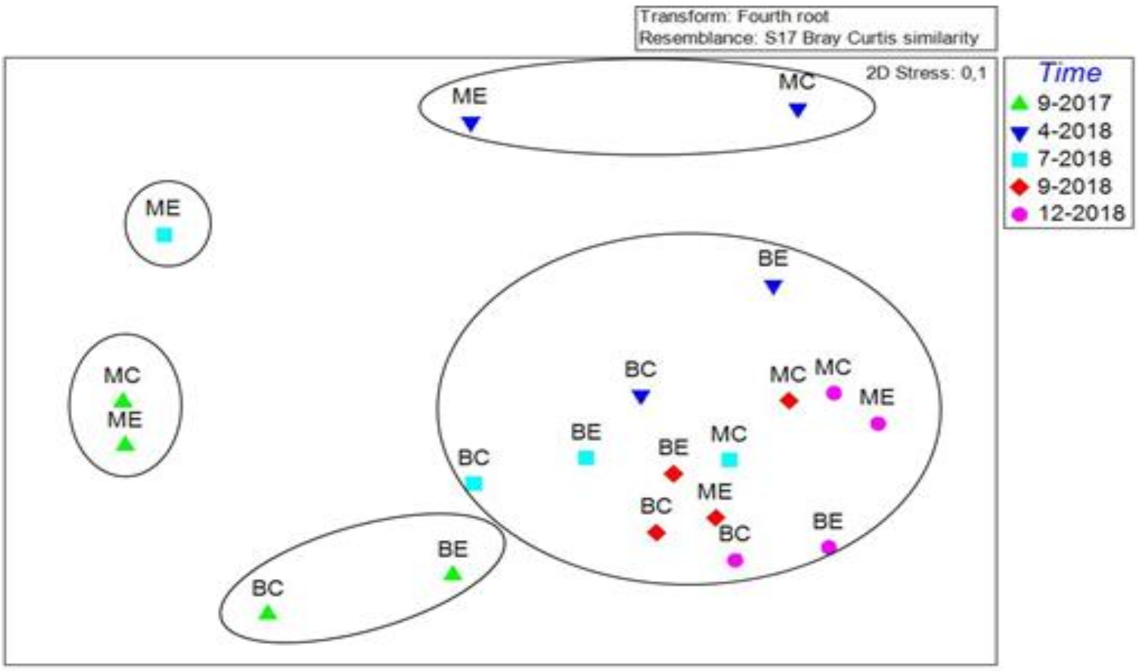

Fig. 7: Two-dimensional non-parametric multidimensional scaling (nMDS) plot for amphipods species composition. Black circles represent the five groups obtained after SIMPROF analysis. M: Oued El Mersa, B: Belyounech, C: center, E: edge.

Table 9: Contribution of important amphipods species to the average Bray-Curtis similarity and dissimilarity within and between groups of stations according to the SIMPER analysis.

Species	Group 1	Group 2	Group 3	Group 4	Group 5	Between Groups
	% similarity: 38	% similarity: 60	% similarity: 35	% similarity: 44	% similarity: 45	% dissimilarity: 64-79
	% contribution					
Ampelisca sp.	1.6			9.5		1.6 - 8.5
Ampithoe ramondi	0.3	4.5				1.0 - 5.2
Aora gracilis		6.0		0.3	2.6	0.9 - 5.6
Aoridae	10	14	24	11	27	4.3 - 9.9
Apherusa sp.	0.7	0.14			4.7	0.1 - 5.3
Bathyporea cf. elegans	0.4			4.6		1.0 - 5.7
Cerpopsis cf. longipes	2.1			4.7		2.2 - 6.0
Ericthonius argenteus	3.5	20	3			2.2 - 17
Gammarella fucicola	5.4	4.7				1.6 - 5.6
Gammmaropsis palmata		7.1				0.6 - 6.5
Harpinia pectinata	15	4.9	2	16	16	3.3 - 11
Hippomedon massiliensis	0.9			4.4		1.5 - 5.6
Leucothoe incisa	3.3			4.8		2.2 - 5.5
Metaphoxus sp.	0.9	5.4	3	2.2		1.5 - 5.7
Microdentopus stationis	1.7	4.8		0.0	2.3	1.5 - 5.1
Nototropis swammerdamei				0.1	2.8	0.3 - 5.6
Pariambus typicus	11	4		6.7		3.3 - 7.8
Phtisica marina	8.7	10	4	0.2	6.4	2.9 - 6.9
Urothoe sp.	17	4.5	60	34	14	3.1 - 9.0
Total contribution (%)	83	90	96	98	76	45 - 85

4. Discussion

In the Mediterranean, *Z. marina* is often restricted to shallow lagoons (Buia and Marzocchi, 1995). The only deep subtidal beds that have been reported are in open bays of southern Spain in the Alboran Sea at depths of 5–14 m (Rueda et al., 2008). The observation of deep *Z. marina* meadows at Jbel Moussa extending from 3 to 17 m deep with patches extending up to 20 m make them colonizing deeper waters than in many other places worldwide where the species is present (Table 10).

During 2014 survey, the Moroccan meadows were well developed comparing to the other Mediterranean areas. High *Z. marina* densities were reported in the Italian lagoons for depths less than 1 m. However, leaf and belowground biomass of Oued El Mersa bay remained slightly higher. Moreover, shoot density in shallow waters of the Thau lagoon (France) was much lower than in Jbel Moussa meadows (Table 10 and references therein). Densities, leaves numbers, above and belowground biomasses of Jbel Moussa meadows were higher than in Southern Spain meadows of Cañuelo Bay at a quite similar depth (Rueda et al., 2008). Comparing to the Atlantic side, densities recorded in Arcachon bay (France, Auby et al., 2018) and along coasts of British Isles (UK, Jones and Unsworth, 2016) were much lower, but with longer and wider lives. Röhr et al. (2018) studied *Z. marina* densities and biomasses of 54 sites in 13 countries worldwide. Only Eastern Atlantic meadows showed slightly higher densities than in Jbel Moussa meadows, while above and belowground biomass were much higher in the Moroccan site.

The great hydrodynamic pressure could have prevented the development of the seagrass in the shallowest depths rather than in the deepest zone sheltered from the strong wave action as some authors have pointed out (Krause Jensen et al., 2003). Besides, the development of healthy seagrasses in very deep areas indicates that they receive enough light for their photosynthesis which would predict a good water clarity according with Krause Jensen et al. (2005)

Table 10: Mean biometric parameter values of *Zostera marina* meadows at different locations, measured in September for each referenced study. If September value not available, annual range or the maximum value (Max) of the annual range is given. ~: values estimated from figures; L: leaf; LAI: leaf area index; Ab: aboveground; Bel: belowground; B: biomass.

Site (location)		Year	Depth range (m)	Shoot density (shoots.m^{-2})	Shoot height (mm)	L. number (leaves.shoot^{-1})	L. width (mm)	LAI (m^{2}.m^{-2})	Ab. B. (g$_{DW}$.m^{-2})	Bel. B. (g$_{DW}$.m^{-2})	Total B. (g$_{DW}$.m^{-2})	Reference
Mediterranean Sea	Belyounech bay, Morocco	2014	3 to 17	745	179	5.2	3.3	1.4	273	181	454	Present study
	Oued El Mersa bay, Morocco			680	227	5.2	3.5	1.9	260	303	563	
	Cañuelo Bay, Spain	2004-2005	5 to 14	402	338	4.7	~2.8	1.4	~90	~98	188	Rueda et al., 2008
	Thau lagoon, France	1994-1996	0.5 to 2.6	296	726 (Max)	5.7	-	-	287	-	300	Laugier et al., 1999
	Bouzigues, France	2015	2.1	223	-	-	-	-	73	144	-	Röhr et al., 2018
	Venice lagoon, Itlaly	1994-1995	0.5 to 1.5	~900	321	~3	-	-	-	-	-	Sfriso and Marcomini, 1997
	Grado laggon, Italy	1997	0.5 to 1	774 (Max)	~490	~5.3 (Max)		~5.2 (Max)	~220	~270	~490 (Max)	Guidetti et al., 2002
Black Sea	Ropotamo, Bulgaria	2015	2.5	697	-	-	-	-	176	103	-	Röhr et al., 2018
Eastern Atlantic	Portugal, Culatra	2015	1.18	348	-	-	-	-	76	660	-	Röhr et al., 2018
	Arcachon bay, France	2015	-	111	633	3.6	8.2	3.04	79	55	134	Auby et al., 2018
	British Isles, UK	2013	0 to 3	193	346	4.6	5.5	-	-	-	-	Jones and Unsworth, 2016
	Rövika, Nrway	2015	1	1019	-	-	-	-	105	174	-	Röhr et al., 2018
Western Atlantic	Dorothy Cove, USA	2015	2	372	-	-	-	-	307	237	-	Röhr et al., 2018
	St-Ludger bay, Canada	2015	1.75	1215	-	-	-	-	163	142	-	Röhr et al., 2019
Eastern Pacific	San Quintin Bay, Mexico	2015	1.15	488	-	-	-	-	285	129	-	Röhr et al., 2018
	Prince Rupert, Canada	2015	1.04	211	-	-	-	-	139	27	-	Röhr et al., 2019
Western Pacific	Greenland, Denmark	2009-2012	2 to 3.8	871-2044	180-560	3.6-5.1	-	-	~93-271	~100-300	193-571	Olesen et al., 2015
	Swan Lake, China	2009-2010	0.3 to 1.2	334-645	138-1782	-	-	-	-	-	67-1705	Zhou et al., 2014
	Hokkaido, Japan	2002-2003	0.5 to 2.5	68-1334	-	-	-	-	~25-242	~15-98	~40-340	Hasegawa et al., 2008

Zostera marina cover at Jbel Moussa reached 100% cover in the center and more than 80% cover at the edges. Seagrass meadows with a coverage exceeding 75% are considered as continuous and homogeneous, with a high foliar coverage (De-Jong et al., 2004). In Oued El Mersa bay, the meadow was fragmented by trawling scars. In addition to trawling physical stresses, along Jbel Moussa meadows' edges with less dense *Z. marina* cover, the presence of *Caulerpa cylindracea* is a supplementary threat, especially in Oued El Mersa bay where it covers up to 19%. This alien seaweed is as an important threat to the biodiversity in the Mediterranean Sea (Ceccherelli and Campo, 2002; Ruitton et al., 2005), and stress reaction of seagrasses when in contact with *C. cylindracea* has been demonstrated (Klein and Velarque, 2008). However, no macro-effect of the green seaweed interaction to *Z. marina* in Jbel Moussa meadows has been observed.

Jbel Moussa sediment mainly consisted in sand, the maximum value of mud fraction only represented 4.4% of total sediment. As for the OM content, it did not exceed 2.3%. Silt-clay content in the sediment of healthy *Z. marina* meadows should range between 2.30 and 56.3 % (Koch, 2001). OM content thresholds of 5% to 13% above which *Z. marina* growth may be limited (Koch, 2001; Krause-Jensen et al., 2011). The low OM content in Jbel Moussa sediment and the transparency of its coastal waters could contribute to the ability of *Z. marina* of Jbel Moussa to expand into deeper water depths.

Major and TE contents in Belyounech bay sediment (mud fraction) were generally lower of those determined in other studies (Table 11). Comparing to the Nador lagoon in the Moroccan Mediterranean side, concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the present study were similar to levels in uncontaminated stations (Boutahar et al., 2021). TEs concentrations were comparable to the values considered as little or not enriched for semi-enclosed water bodies along the Atlantic coast (Boutahar et al., 2019).

Table 11: Trace element concentrations (mg.kg$^{-1}_{DW}$) reported in sediment (Sed) and *Zostera marina* compartments (Lv: leaves; Rz: rhizomes; Rt: roots) from various geographical locations. Values are either single concentrations (SC, n = 1 pooled sample), ranges of concentrations (R), mean concentrations (M) or ranges of mean concentrations (RM). BD: below detection.

	Location	Compartment	Data type	Trace element							Reference
				Cd	Cu	Cr	Ni	Pb	Zn	As	
Mediterranean Sea	Belyounech Bay. Morocco	Sed	SC	0.08	13.7	21.0	11.2	16.8	46.0	7.25	Present study
		Lv		0.64	6.61	1.32	2.42	2.64	29.5	1.82	
		Rz		0.35	1.69	0.32	0.35	0.45	15.2	0.99	
		Rt		0.79	9.12	7.86	46.1	5.89	39.2	7.94	
	Thau lagoon. France	Sed	M	-	19	21	9	13	36	-	De Casabianca et al.,2004
		Lv		-	10	0.3	0.6	1	83	-	
		Rt		-	9	2	1	2	44	-	
	Nador lagoon. Morocco	Sed	R	0.12-1.6	10-151	22-72	20-45	16-135	55-555	-	Maanan et al., 2014
Eastern Atlantic	Merja Zega, Sidi Moussa, Oualida, Khnifiss lagoons and Dakhla bay. Morocco	Sed	RM	0.11-1.32	34.5-75.9	28.4-127	9.50-30.8	4.75-16.1	32.5-90.4	3.26-13.4	Boutahar et al., 2019
Eastern Pacific	Puget Sound. Washington. USA	Lv of impacted site	M	2	16	1	45	0	102	3	Ferrat et al., 2012
		Lv of not impacted site		2	10	1	23	0	110	3	
		Rt and Rz of impacted site		11	43	6	63	6	169	10	
		Rt and Rz of not impacted site		3	10	1	64	0	96	1	
	Yaquina Bay. Oregon. USA	Lv	M	1.7	10	7.2	20	BD	29	4.6	Kaldy. 2006a
		Rz		0.8	8.3	7.4	25	BD	18	4.8	
		Rt		6.4	47	38	40	BD	52	12	
	Baja California. Mexico	Whole plant	R	0.01–66	0.94–15	-	-	2.6–46	0.76–221	-	Marcías-Zamora et al., 2008
Black sea	Bosphorus Strait. Turkey	Sed	RM	2.06-3.01	25.0-55.2	25.8-58.9	18.0-28.8	43.7-272	86.3-119	-	Guven et al., 1993
		Whole plant		1.92-2.33	23.4-39.8	8.34-13.6	12.9-17.5	26.1-32.1	48.7-91.3	-	
Western Pacific	Pos'et Bay.Russia	Whole plant	RM	1.6-2.5	2.1-5.3	1.00-4.2	2.1-3.5	5.3-11.4	-	-	Chervnova et al., 2002

The accumulation of the studied chemical elements by *Z. marina* tissues was mostly made by roots (except, Mo, Ag, Cd, Na and K), followed by leaves then rhizomes. *Z. marina* roots are therefore the main tissue for chemical elements uptake from the environment. Additionally, elements transition from roots to rhizomes and leaves was low. The compartmentalization strategy in underground seagrass tissues is aimed as defensive mechanism to protect the species against toxic effects of the photosynthetic tissue (Gratao et al., 2005; Willis et al., 2010). These results are in line with previous studies on other seagrasses species reporting that roots store generally the highest concentrations of toxic elements (Malea and Kevrekidis, 2013; Bonanno et al., 2017; Boutahar et al., 2021). Scholars that have investigated the level of TEs in *Z. marina* in the Mediterranean Sea are relatively scarce, but several ones elsewhere in the world considered this specie as powerful accumulator of TEs (Lewis and Devereux, 2009). A comparison of TE concentrations in *Z. marina* from Jbel Moussa with different geographical areas is summarized in Table 11. In Thau lagoon, Fe and Cr were also accumulated in the 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 sediments than in the macrophytes, and higher in *Z. marina* than in algae (Guven et al., 1993). Ferrat et al., (2012) repported that *Z. marina* accumulated high levels of TEs when growing on contaminant-impacted sites. Consistently with our results, they found that belowground tissue accumulated higher quantity of TEs. Similar observation was also found in Yaquina Bay, Oregon, USA (Kaldy 2006a). The variation found in TE accumulation by *Z. marina* over geographic scales and tissue types has been linked to the characteristics of sampling environment and local availability (De Casabianca et al., 2004), metabolic processes and age of the plant (Marcías-Zamora et al., 2008), seasonal variation in TE concentrations (lyngby and Brix 1982) and different procuderes of analysis (Brix et al., 1983).

Concentrations of nutrient (N and P) in *Z. marina* from Jbel Moussa was more pronounced in leaves than belowground tissues accordingly with what was reported for this species by several authors (Pedersen and Borum 1993, 1995; Rigolet et al., 1998; Kaldy 2006b). Nutrient accumulation in *Z. marina* tissues depends primarily on their availability in the aquatic environment (Fourqurean et al., 1992; Erftemeijer et al., 1994). According to Gerloff (1975), P can be expected to be limiting if leaf concentration is below 0.07 $\%_{DW}$. In our case, P content above 0.10 $\%_{DW}$ (Table 12), was not limiting. Duarte (1990) indicated that seagrass growth is N-limited when average N concentrations in leaves decrease below 1.80$\%_{DW}$. In the present study, N concentration was above that threshold. The N:P ratio, reflecting the overall nutrient availability to the plant, reached 25 in leaves and therefore N and P were in sufficient amount 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 belowground tissues from Belyounech meadows (0.74 %) are low compared to other areas (Table 12).

Z. marina δ^{15}N is an effective time integrated indicator of anthropogenic N inputs (Costanzo et al., 2001, Schubert et al., 2012). Indeed, such anthropogenic effect is often associated to elevated δ^{15}N values in seagrass tissues (Lassauque et al., 2010). Leaf δ^{15}N values in Belyounech bay was lower than values along Algerian coast in the seagrass *P. oceanica* (Belbachir et al., 2019).

The high *Z. marina* abundance and biomass obsedved in Jbel Moussa meadow could therefore explain the nutrients depletion in sediment. Thus, the low mud fractions in Belyounech bay does not promote high nutrient concentrations in porewater (Kenworthy et al., 1982; Huettel and Rusch, 2000). This low nutrient availability resulted in the absence of the epiphytic microalgae occurrence on seagrass leaves (Burkholder et al., 2007).

Considering organic C, similar levels between *Z. marina* leaves and rhizomes were measured. C fixed through photosynthesis can be stored in rhizome as soluble carbohydrate or starch, which are used to support plant growth (Durako and Moffler 1985; Dawes and Guiry 1992). The C content and δ^{13}C values of *Z. marina* compartments from Belyounech bay show little differences when compared to oceans and seas from other places woldwide (Table 4). In these studies, seagrass samples were collected from sheltered depositional environments with relatively high accumulation of autochthonous organic and inorganic material. The deep meadow of Belyounech bay growth is relatively exposed to hydrodynamism that do not promote extensive carbon sequestration and export the organic matter produced in the meadow to further adjacent locations (Serrano et al., 2016; Kindeberg et al., 2018). Previous studies found the lowest C stocks for *Z. marina* at sandy, exposed sites in the Baltic Sea (Röhr et al., 2016). A second contributor to the low C stocks may be sediment type. Fine grained particles, such as mud, tend to promote more carbon adsorption (Kennedy et al., 2010). The Jbel Moussa sediment contained a large proportion of sand.

Table 12: C, N and P levels (in $\%_{DW}$), their ratios (atom:atom),- and $\delta^{13}C$ and $\delta^{15}N$ values (in ‰) in *Zostera marina* compartments (Lv: leaves; Rz: rhizomes; Rt: roots) and sediment (< 0.0625 mm; Sed) from Belyounech bay meadow and other geographical locations. Values are either single concentrations (SC, n = 1 pooled sample), mean concentrations (M) or ranges of mean concentrations (RM). ~: value estimated from figures; at: atom.

	Location	Data type	Matrix	C	N	P	C:N	C:P	N:P	$\delta^{13}C$	$\delta^{15}N$	Reference
Mediterranean Sea	Belyounech bay, Morocco	SC	Sed	-	0.05	0.48	-	-	0.23	-	4.72	Present study
			Lv	32.8	2.17	0.19	17.6	446	25.3	-12.3	2.30	
			Rz	30.9	0.67	0.14	53.8	570	10.6	-11.7	1.88	
			Rt	18.2	0.81	0.16	26.2	294	11.2	-11.4	2.84	
	Thau lagoon, France	M	Lv	-	1.72	0.24	-	-	16	-	-	De casabianca et al., 2004
			Rz- Rt		1.12	0.17			16			Rigollet et al., 1998
	Venice lagoon, Italy	M	Lv	-	2.34	0.23	-	-	24	-	-	Rigollet et al., 1998
			Rz-Rt		1.23	0.10			28			
Black Sea	Ropotamo, Bulgaria	M	Lv	32.8	-	-	-	-	-	-17.8	10	Röhr et al., 2018
			Rz							-15.5	-	
Eastern Atlantic	Heiligenhafen Bay, Germany	RM	Lv	35.6-36.6	0.86-2.51	-	23-27	-	-	-	(-0.54)-13.5	Schubert et al., 2012
	British Isles, UK	M	Lv	47.7	3.58	0.21	16.5	646	39	-	-	Jones and Unsworth, 2016
	Greenland, Denmark	RM	Lv	36-40	1.35-2.33	0.25-0.42	20-31	246-372	11.9-12.3	-	-	Olesen et al., 2015
			Rz-Rt	28-36	0.99-1.3	0.12-0.73	32-33	603-127	3.9-18			
Western Atlantic	USA and Canada	M	Lv	27.5	-	-	-	-	-	-9.2	7.20	Röhr et al., 2018
			Rz							-10.1	-	
Eastern Pacific	Yaquina Bay, Oregon, USA	RM	Sed	0.21-0.62	0.03-0.07	-	8.88-10.2	-	-	-	-	
			Lv	-	-		15-20	155-440	13-32			Kaldy 2006a
			Rz	-	-		20-60	130-784	7.00-26			Kaldy 2006b
			Rt	-	-		20-30	399-739	19-31			
	Tomales Bay, California, USA	RM	Lv	29-41	1.13-3.79	0.11-0.90	11.5-38	106-455	5.8-28.5	(-15)-(-7.50)	6.30-12.5	Fourqurean et al., 1997
Western Pacific	Jindong Bay, Korea	RM(~)	Lv	32.5-38	1.5-4.5	-	09-25	-	-	-	-	Kim et al., 2012
			Rz	29-38	0.5-5.5		08-55					
	Hokkaido, Japan	RM	Sed	0.94-1.22	0.08-0.11	-	9.4-14	-	-	-	-	Hasegawa et al., 2008
			Lv	33.7-36.9	1.1-3.1		12-30					Hasegawa et al., 2007
Various		M	Lv	36	2.5	0.39	16.8	238	14.2	-	-	Duarte (1990)

Overall, data collected in 2014 for Jbel Moussa *Z. marina* meadows details their good health status with low contamination level by TEs and no enrichment in organic matter or nutrient. This low organic and nutrient content results in good water clarity and light availability, and eventually increasing depth limits (Nielsen et al., 2002a, 2002b).

The 2017 investigation was concomitant with rapid expansion of the invasive species *Rugulopteryx okamurae* in the Strait of Gibraltar (Altamirano et al., 2017; El Aamri et al., 2018; García-Gomez et al., 2018). This brown seaweed became the dominant alga species in *Z. marina* meadows of Oued El Mersa and Belyounech bays and accounted for 94 and 98% of the total alga biomass respectively. Although *C. cylindracea* have been established in the area earlier, no macro-effects of its presence on *Z. marina* have been observed. The outcompeting capacity of *R. okamurae* displaced *C. cylindracea* and reduced the coverage of the native seagrass. This was mainly recorded in Belyounech meadow where *R. okamurae* represented 64% of total vegetated biomass. Contrarily to the presence of a non-indigenous species (NIS), which may be present in recipient ecosystem for long time without significantly affecting it, the fast growth of invasive exotics generates strong ecological impacts on resident biota (Sanchez et al., 2005; Thomsen et al., 2017).

R. okamurae, originated from the northwestern Pacific Ocean (Hwang et al., 2009), was first detected in the Mediterranean in the coastal lagoon of Thau (France) in 2002 and probably introduced via oyster aquaculture (Verlaque et al., 2009). The species has been reported on the south coasts of the Strait of Gibraltar since 2015 with huge bloom in the city of Ceuta (North Africa) where more than 5,000 tons of wracks were extracted from its beaches (Altamirano-Jeschke et al., 2016). The Strait of Gibraltar supports an intense maritime traffic (Endrina et al., 2018) making it subject to marine bioinvasions since ballast water of large merchant ships are considered as NIS primary vectors (Ribera-Siguan, 2003). Since then, *R. okamurae* has spread its spatial establishment through all the Strait of Gibraltar coastal areas with westward and eastward propagule (García-Gómez et al., 2020).

Habitat changes generated by *R. okamurae* induced an efficient loss of resident species. In the coralligenous rocky bottoms of Jbel Moussa, Sempere-Valverde et al. (2020) registered a shift in the community structure and the regression of bioindicator species. Large change was also reported in community composition one year after the first recording of this species on the Island of Tarifa, Spain (García-Gómez et al., 2020).

In addition to this biological invasion stress, high summer temperatures linked to the global climate change was a supplementary threat to *Z. marina* meadows of Jbel Moussa. These Moroccan meadows are localized within the warm temperate-southern limit of the species, where the average sea surface thermal amplitude ranges between 14 °C and 24 °C (Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible, 2019). Previous studies have reported that high water temperatures above 25 °C markedly affect *Z. marina* meadows by decreasing its growth and increasing shoot mortality (Moore and Jarvis, 2008; Moore et al., 2014; Lefcheck et al., 2017). Unusually warm temperatures are also associated with the wasting disease that decimated eelgrass across the northern hemisphere in the 1930s (Hughes et al., 2018). The heatwave recorded in July 2015 of 24 °C probably facilitated *R. okamurae* shift from exotic to invasive in short time. Wernberg et al. (2016) assigned that climate driven regime shifts increase the settlement success of invasive species and thereby set the biogeographical boundary of native ones.

From September 2017 to April 2018 survey, *Z. marina* meadows of Jbel Moussa were completely destroyed with significant injury to roots and rhizomes (Fig. 8, Fig. 9). Metal stakes fixed in the sediment for permanent monitoring quadrats were not found or inclined probably because of illegal trawling activities (Fig. 8).

Fig. 8: Destroyed *Zostera marina* meadow in Oued El Mersa Edge with inclined metal stake of permanent monitoring quadrat. Photo taken in April 2018 survey.

Fig. 9: Abraded *Zostera marina* shoots and *Rugulopteryx okamurae* wracks in the center of Belyounech degraded meadow. Photo taken in April 2018 survey.

Bottom trawling for finfish is one of the major direct damage to seagrasses injuring roots and rhizomes that is lethal to seagrass shoots (Ardizzone et al., 2000). Seagrass structure is rarely homogenous and variation (e.g., shoot density, cover, canopy height) can occur naturally within meadows due to fluctuations in the system (Guidetti and Bussotti, 2000). However, physical disturbance intensifies habitat change, lead to fragmentation, a reduction in shoot density and coverage, and permanent loss of habitat (Short and Wyllie-Echeverria, 1996). Neckles et al (2005) found that one year after the last trawl, *Z. marina* shoot density and total biomass were reduced 2- 3 % and < 1 % respectively in comparison to the reference sites. The authors estimated that an average of 11 years was required for *Z. marina* shoot density to match pre-trawling reference values.

Hereby, interactions among global change stressors, namely heatwave linked to the global climate change, biological invasion, i.e., *R. okamurae* and mechanical threat i.e., trawling, all together could induced the withdrawal of Jbel Moussa *Z. marina*, the deepest meadows reported for the Mediterranean (lower limit at 17 m in depth with patches extending up to 20 m).

In September 2018 survey, sparse new *Z. marina* shoots were observed establishing in the area (Fig. 10).

Fig. 10: Appearance of a new shoot of *Zostera marina*. *Rugulopteryx okamurae* can be seen in the upper right corner.

After disturbance, seagrass recolonization process has often been reported to increases via sexual reproduction through seedbank, followed by the expansion of seagrass meadows via clonal growth through lateral shoot production (Jarvis et al., 2012). However, areas affected by intense seagrass diebacks events where seedlings initiate the seagrass recovery show slower recolonization due to low germination rates and high seedling mortality (Orth et al., 2003; Lee et al., 2007; Boese et al., 2009). For instance, less than 5% of *Z. marina* seedlings from germinated seeds developed to adult shoots in the southern coast of Korea (Kim et al., 2014). In Denmark, *Z. marina* seedlings exposed to physical disturbance encountered a threefold higher mortality rate than seedlings in unthatched zone (Valdemarsen et al., 2010). The injury caused to roots and rhizomes of Jbel Moussa seagrass could prevent the vegetative growth through rhizome elongation, and the small new shoots may easily be uprooted due to the persistence of illegal trawling, while the injury caused by this fishing activity within the sea bottom would prevent the renewal of seedbank and, subsequently, would limit the sexual reproduction.

Z. marina is an ecological autogenic and allogenic engineer so the loss of Jbel Moussa meadows will certainly lead to a decline in the functioning and stability of the whole ecosystem and may trigger significant extinction cascades. As expected, the study of amphipods communities inhabiting Oued El Mersa and Belyounech soft bottom reviled a significant decrease in richness, diversity and abundance after *Z. marina* decline. Although communities remained dominated mostly by the same taxa comparing the presence time of *Z. marina* and its disappearance, a high total variation of 70% was detected. The presence of seagrasses has traditionally been considered a primary determinant of faunal communities (Thomsen et al., 2018). This has been linked to the complexity of the structural microhabitats formed by leaf canopies (Leopardas et al., 2014) and root-rhizomes systems providing additional hide-spaces for prey, foraging grounds and food resources to invertebrate assemblages (Romero et al., 2014). Moreover, with live *Z. marina* shoots, denser meadows of Ouad El Mersa hosted a higher number of individuals comprising 67% of the total sampled species comparing to Belyounech meadows with greater *R. okamurae* biomass and low cover. McCloskey and Unsworth (2015) also observed significantly higher faunal diversity and species richness in high versus low cover seagrass. Hirst and Attrill (2008) found that the size of *Zostera* metrics made no impact on diversity, however, the presence or absence of seagrass made a significant difference. Subsequently, the primary mechanism that may underlie the observed amphipods community shift is the loss of habitat complexity

Another plausible mechanism contributing to the ecological stress of amphipods community may be the chemical substances produced by *R. okamurae* that could exhibited phytotoxic properties against *Z. marina* and its associated fauna. Previous studies on the effects of *C. cylindracea* over Mediterranean seagrasses elucidated that the toxic secondary metabolite (caulerpenyne) caused sedimentary sulphides (Holmer et al., 2009), a factor that has been coupled with higher seagrasses mortalities (Frederiksen et al., 2007). In turn, *R. okamurae* collected from the invaded area (Strait of Gibraltar) contain six bioactive compounds of the diterpenoid class with markedly higher concentration of dilkamural (Casal-Porras et al., 2021) compared to specimens in their native range (Ninomiya et al., 1999). Casal-Porras et al. (2021) reported that the major metabolite of *R. okamurae* (i.e. dilkamural) has a deterrent herbivory effect and lethal capacity over native consumers witch could hence the success invasion by this species in the Strait of Gibraltar.

Beyond the direct effects of seagrass meadows loss on the ecosystem itself, there are also potential effects on adjacent ecosystems. *Z. marina* of Jbel Moussa was in close proximity to important populations of coralligenous habitat and their benefits from the eelgrass services may be affected. It is well known that the connectivity of ecosystems across the seascape suggests a direct corals and other calcified organisms benefit from seagrasses ability to buffer dissolved inorganic carbon during photosynthesis (Koweek et al., 2018) which contribute to enhance the resilience of these vulnerable species to ocean acidification (Wahl et al., 2017). Corals also benefit from seagrasses ability to reduce the incidence of pathogenic marine bacteria causing their disease (Lamb et al., 2017) and, therefore, coralligenous assemblages found in the area (Sempere-Valverde et al., 2020) could be endangered by losing seagrass habitats.

Moreover, the loss of native benthic communities with the explosive expansion of *R. okamurae* in Jbel Moussa entailed significant detrimental economic impacts. Recreational and social services linked to touristic activities in this touristic hot spot suffered from the algae accumulation on the beaches which caused decomposition odours and very disturbing view (Fig. 11)

Fig.11: Stranding of *Rugulopteryx okamurae* in M’diq beach near of Jbel Moussa (From El Aamri et al., 2018)

Fishermen also complained from cleaning fishing nets and materials from massive quantities of trapped seaweed. Despite fisheries were not investigated, fishes captures close to the coastal border are expected to be affected since seagrass complex structure provide suitable habitats for a variety of permanent and temporary fish residents, including vulnerable life stages such as juveniles seeking to avoid predation (Nordlund et al., 2018).

5. Conclusion

Marine ecosystems are influenced by multiple global and local anthropogenic factors acting jointly (Lubchenco and Grorud-Colvert, 2015; Halpern et al., 2019). *Z. marina* meadows of Jbel Moussa abundance has decreased dramatically over 4 years and the combined effect of warming, invasion and trawling that extirpated the totality of the eelgrass population in unprecedented way. New shoots recruits were not able to grow under the persistence of stressors which in consequence resulted on the significant decrease of associated amphipods community. Since climate change is a global crisis, implementation of mitigation efforts against transportation of non-indigenous invasive species (regional conservation), and physical disturbances (local conservation) are urgently required. The entry of invaders is primary associated with ballast waters, it is therefore necessary to make effective the International Convention for the control and management of ballast water and sediments of ships to prevent new introductions. It is also highly recommended to establish zones where seagrass meadows remain preserved against habitat fragmentation caused by physical disturbance. Furthermore, fisherman must be involved to connect between fisheries management and biodiversity conservation concern.

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Appendix A.

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Deep-water *Zostera marina* meadows in the Mediterranean

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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 ± 183 shoots.m^{-2} and 273 ± 40 g_{DW}. m^{-2} 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.

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^{-1}. year^{-1} for enhancing fish biomass (Blandon and Zu-Ermgassen, 2014) and up to $13.7 billion. year^{-1} 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

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CONCLUSIONS AND RECOMMENDATIONS

Seagrass meadows are considered among the most productive and valuable marine habitat types, for ecological goods and benefits services they provide. However, this marine heritage is declining worldwide at an incising rate. Increasing human stressors are putting their future viability in doubt. Only including seagrass ecosystems in effective management strategies could help reverse their loss.

The objective of this research was to provide knowledge on seagrass meadows status and ecosystem services in the Moroccan coasts, and provide steps for their conservation measures.

Biomonitoring environmental status in semi-enclosed coastal ecosystems using *Zostera noltei* and *Cymodocea nodosa* meadows

Along the full latitudinal climatic gradient of the Atlantic coast of Morocco (Mediterranean, semi-arid and arid climate), we investigated for the first time the suitability of *Zostera noltei* leaves as a bioindicator of trace element contamination in five semi-enclosed coastal ecosystems (Moulay Bousselham lagoon, Sidi Moussa lagoon, Oualidia lagoon, Khnifiss lagoon and Dakhla bay) submitted to different anthropogenic pressures (urbanism, agriculture, industries and mining). Moreover, it is the first worldwide study of the ability of *Z. noltei* leaves to concentrate 27 chemical element (Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U, Bi, Hg, Na, Mg, K and Ca) from its surrounding environment. Results pointed out that chemical element concentrations for both sediments and *Z. noltei* leaves showed inter-site and intra-site (downstream/upstream) variability. Thus, similar contamination occurrence was detected in both environmental matrix and its bioavailability to seagrass leaves for Al, Fe, Mn, Zn, Ag, Cd, Ba and Hg while Cd, Mo, Sb, Ag, Zn and U were efficiently uptaked by *Z. noltei* from sediments. Ultimately, we concluded that *Z. noltei* leaves are powerful bioindicator of Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination monitoring purpose.

From the Mediterranean coast**,** the contamination level and ecological risk status of six trace elements (Ni, Cd, Pb, Cu, Cr and Zn) in sediments of Marchica lagoon were investigated using single and multi-element indices: contamination factor, enrichment factor, index of geo-accumulation (I-geo), (modified) degree of contamination (Cd or mCd), (modified) pollution index (PI or MPI) and sediment quality guidelines. The bioavailability of these elements to ascertain the degree of toxicity was assessed using *C. nodosa* leaves, rhizomes and roots**.** Sediments nearby urban effluents, industrial discharges, and watersheds supplied by agricultural runoff were deemed to be heavily polluted. At these localities, sediment quality guidelines (SQGs) and the modified potential ecological risk index (MRI), predicted a multiple environmental risk with frequent occurrence of adverse biological effects. In turn, *C. nodosa* leaves and roots accumulated greater trace elements in the impacted areas and therefore, reflected efficiently the sediment pollution gradient and featured the availability of these elements to the biota. Both leaves and roots performed equally as good bioindicators of Cu and Cd in sediment whereas leaves are considered as the best bioindicator for Zn contamination and roots for Pb load. Contrastingly, this seagrass does not act as potential bioindicator of Al, Cr and Ni contamination. These results provide an insight on trace element accumulation in *C. nodosa*, that can be applied for the design of biomonitoring programs.

Cymodocea nodosa of Al Hoceima National Park and _Zostera marina_ of Jbel Moussa: First quantitative description

At Al Hoceima National Park, the assessment of *C. nodosa* meadows structural development and population dynamics using the reconstructing techniques reviled that the low rhizome elongation rate and the epiphytes overload reduced the population recruitment rate inducing the decline of these meadows. In fact, the age distributions of *C. nodosa* shoots showed exponential reductions of shoot density since the second year class. Furthermore, there is a critical gap in physicochemical datasets and environmental conditions affecting the meadows growth as a direct result of coastal management and monitoring planning lack in the park. Our result strengthens the marked regression of this species throughout its overall range of distribution even though it is considered to be resilient to natural and anthropogenic stresses.

At Jbel Moussa, the first assessment of the environmental status of *Zostera marina* meadows, the only remained ones along Mediterranean coasts of Morocco, or even of North Africa, and the deepest ones in the entire Mediterranean (lower limit at 17 m depth with patches extending up to 20 m), found low contamination level by trace elements and no inputs of land-derived organic matter and nutrients. Initially, the meadows showed well vegetative development with no macro-effect of the biological and mechanical encountered threats (i.e. *Caulerpa cylindracea* invasion and trawling activities). During the following four years, *Z. marina* meadows experienced rapid expansion of the invasive seaweed *Rugulopteryx okamurae,* high summer temperatures linked to the global climate change, together with evidences of persistent trawling activities until a complete collapse. Concomitantly, amphipods communities inhabiting Jbel Moussa soft bottom decreased significantly in richness, diversity and abundance with total variation up to 70% as a direct result of *Z. marina* disappearance.

Recommended actions for effective conservation

The sustainability of seagrass habitats depends upon the implementation of effective national plans for seagrass management. Complete coastal mapping is the first emerging action to determine the seagrass occurrence and their current aerial extent. The setting up of monitoring surveys are then required to collect detailed information on their habitat structure, health, and environmental conditions conducive for their growth. Concomitantly, it is strongly needed the scientific research on seagrass ecology that will lead to increase the knowledge of how they respond to surrounding environment changes over time and how they can be managed to remain resilient to these changes.

For more effective conservation outcomes, raising recognition among policymakers, managers, costal users, environmental groups, and the general public about the importance of these valuable ecosystems, along with increased awareness about the threats to their survival is crucial to encourage their protection.

Seagrass ecosystems conservation is an opportunity to progress towards the United Nations Convention on Biological Diversity Strategic Plan for Biodiversity and its 20 Aichi Biodiversity Targets with the post-2020 biodiversity framework (particularly Targets 5, 6, 8, 11, 14 and 15 addressing habitat loss, fish and invertebrate stocks, pollution, marine protected areas, ecosystem service provision for livelihoods and well-being, and climate security respectively). In addition to the Sustainable Development Agenda and Sustainable Development Goals since it can help achieve 10 SDGs (1, 2, 6, 8, 11, 12, 13, 14 and 17 related respectively to end poverty, food security, clean water, economic growth, cities protuction, sustainable production, combat climate change, and conserve marine resources).

This recognition may incite policy- and decision makers to develop coastal and marine spatial plans management against anthropogenic seagrass pressures (*e.g.* urban, agricultural and industrial run-off, coastal development, unregulated fishing and boating activities), which impacts have effects beyond the plants themselves, with evidence to alter associated biodiversity and the overall seascape in the context of ecosystem connectivity. Establishing boundaries of fully protected marine area around seagrass meadows may therefore solve their survival and improve their resilience over time. Restoration of seagrass meadows through transplanting techniques is another viable action to enhance seagrass habitats extend. However, successful transplantations require taking place in areas with low levels of human activities. Therefore, reducing anthropogenic disturbance remain the key stone to boost seagrasses recovery.

Les herbiers à phanérogames marine comptent parmi les habitats marins les plus productifs et les plus précieux, pour les biens écologiques et les services bénéfiques qu'ils procurent. Néanmoins, ce patrimoine marin décline dans le monde entier à un rythme effarant. L'augmentation des facteurs de stress humains met en doute leur viabilité future. Seule l'intégration des écosystèmes d'herbiers marins dans des stratégies de gestion efficaces pourrait contribuer à enrayer leur disparition.

L'objectif de la présente thèse était de fournir des connaissances sur le statut des herbiers marins et leurs services écosystémiques au niveau des côtes marocaines, et de proposer des mesures adéquate à leur conservation.

Biosurveillance du statut environnemental des écosystèmes côtiers semi-fermés en utilisant les herbiers de *Zostera noltei* et *Cymodocea nodosa*

Le long du gradient climatique latitudinal de la côte Atlantique Marocaine (climat Méditerranéen, semi-aride et aride), nous avons étudié pour la première fois l'aptitude des feuilles de *Zostera noltei* en tant que bioindicateur de contamination par les éléments traces dans cinq écosystèmes côtiers semi-fermés (Lagune de Moulay Bousselham, lagune de Sidi Moussa, lagune de Oualidia, lagune de Khnifiss et baie de Dakhla) soumis à différentes pressions anthropiques (urbanisme, agriculture, industries et et exploitation minière). En outre, il s'agit de la première étude mondiale de la capacité des feuilles de *Z. noltei* à concentrer 27 éléments chimiques (Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U, Bi, Hg, Na, Mg, K et Ca) provenant de son environnement. Les résultats ont révélé que les concentrations des éléments chimiques pour les sédiments et les feuilles de *Z. noltei* présentaient une variabilité inter-site et intra-site (aval/amont). Ainsi, une occurrence de contamination similaire a été détectée dans la matrice environnementale et sa biodisponibilité aux feuilles de l'herbiers pour Al, Fe, Mn, Zn, Ag, Cd, Ba et Hg tandis que Cd, Mo, Sb, Ag, Zn et U ont été absorbés efficacement par *Z .noltei* à partir des sédiments. En définitive, nous avons conclu que les feuilles de *Z. noltei* sont de puissants bioindicateurs de la surveillance de la contamination par Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba et Hg.

Sur la côte méditerranéenne, le niveau de contamination et le statut de risque écologique de six éléments traces (Ni, Cd, Pb, Cu, Cr et Zn) dans les sédiments de la lagune de Marchica ont été étudiés en utilisant des indices simple et à multi-éléments : facteur de contamination, facteur d'enrichissement, indice de géo-accumulation (I-geo), degré de contamination (modifié) (Cd ou mCd), indice de pollution (modifié) (PI ou MPI), et directives de la qualité des sédiments. La biodisponibilité de ces éléments permettant de déterminer le degré de toxicité a été évaluée en utilisant les feuilles, les rhizomes et les racines de *C. nodosa*. Les sédiments situé à proximité des effluents urbains, des rejets industriels et des bassins versants alimentés par les ruissellement agricoles ont été qualifiés de fortement pollués.

Dans ces localités, les directives sur la qualité des sédiments (SQG) et l'indice de risque écologique potentiel modifié (MRI) ont prédit un risque environnemental multiple avec l’apparition fréquente d’effets biologiques néfastes. En revanche, les feuilles et les racines de *C. nodosa* ont accumulé davantage d'éléments traces dans les zones impactées et, par conséquent, ont reflété efficacement le gradient de pollution des sédiments et ont décrit la disponibilité de ces éléments au organismes vivants. Les feuilles et les racines se sont révélées de bons bioindicateurs de Cu et de Cd dans les sédiments, tandis que les feuilles sont considérées les meilleurs bioindicateur de la contamination par Zn, et les racines pour la charge en Pb. Par contre, cet herbier n'agit pas comme bioindicateur potentiel de la contamination par Al, Cr et Ni. Ces résultats apportent un aperçu de l'accumulation d'éléments traces chez *C. nodosa*, qui peut être appliqué pour la conception de programmes de biosurveillance.

Cymodocea nodosa du Parc National d'Al Hoceima et _Zostera marina_ de Jbel Moussa : Première description quantitative

Au parc national d'Al Hoceima, l'évaluation du développement structurel et de la dynamique des populations des herbiers de *C. nodosa* à l'aide des techniques de reconstruction a révélé que le faible taux d'élongation des rhizomes et la surcharge d'épiphytes ont réduit le taux de recrutement de la population, entraînant le déclin de ces herbiers. En fait, les distributions en fonction de l’âge des pousses de *C. nodosa* ont montré des réductions exponentielles de la densité des pousses depuis la deuxième classe d'âge. De plus, il existe une insuffisance critique dans les ensembles de données physicochimiques et les conditions environnementales qui affectent la croissance des herbiers, résultat directe du manque de planification de la gestion côtière et de la surveillance littoral dans le parc. Notre résultat renforce la régression marquée de cette espèce dans l'ensemble de son aire de répartition, même si elle est considérée comme résistante aux stress naturels et anthropiques.

A Jbel Moussa, la première évaluation du statut environnemental des herbiers de *Zostera marina*, les seuls qui subsistaient le long des côtes méditerranéennes du Maroc, voire de l'Afrique du Nord, et les plus profonds de toute la Méditerranée (limite inférieure à 17 m de profondeur avec des taches s'étendant jusqu'à 20 m), a révélé un faible taux de contamination par éléments traces et aucun apport de matière organique et de nutriments d'origine terrestre. Initialement, les herbiers ont montré un bon développement végétatif sans macro-effets des menaces biologiques et mécaniques rencontrées (c.-à-d. l'invasion par *Caulerpa cylindracea* et les activités de chalutage). Au cours des quatre années qui suivent, les herbiers de *Z. marina* ont connu une expansion rapide de l'algue envahissante *Rugulopteryx okamurae*, des températures estivales élevées liées au changement climatique mondial, ainsi que des évidences d'activités de chalutage persistantes jusqu'à un effondrement complet. Parallèlement, la richesse, la diversité et l'abondance des communautés d'amphipodes habitant le fond meuble du Jbel Moussa ont considérablement diminué avec une variation totale allant jusqu'à 70% en conséquence directe de la disparition de *Z. marina*.

Mesures recommandées pour une conservation efficace

La durabilité des habitats des herbiers marins dépend de la mise en œuvre de plans nationaux de gestion efficaces. La cartographie côtière complète est la première mesure émergente à entreprendre pour déterminer l'occurrence des herbiers marins et leur étendue aérienne actuelle. La mise en place d'enquêtes de surveillance est ensuite nécessaire pour recueillir des informations détaillées sur la structure de leur habitat, leur santé et les conditions environnementales propices à leur croissance. Parallèlement, il est indispensable de mener des recherches scientifiques sur l'écologie des herbiers marins afin d'améliorer les connaissances sur la manières dont ils réagissent aux changements environnementaux au fil du temps et sur la manière dont ils peuvent être gérés pour demeurer résilients à ces changements.

Pour obtenir des résultats de conservation plus efficaces, il est essentiel sensibiliser les décideurs, les gestionnaires, les utilisateurs côtiers, les groupes environnementaux et le grand public à l'importance de ces écosystèmes précieux, ainsi qu'aux menaces qui pèsent sur leur survie, afin d'encourager leur protection.

La conservation des écosystèmes d'herbiers marins est une opportunité de progresser vers le Plan Stratégique des Nations Unies pour la Convention de la Biodiversité et ses 20 objectifs d'Aichi en matière de biodiversité avec le contexte d'après-2020 (en particulier les objectifs 5, 6, 8, 11, 14 et 15 traitant respectivement la perte d'habitat, les stocks de poissons et d'invertébrés, la pollution, les aires marines protégées, la fourniture de services écosystémiques pour les moyens de sbsistance et le bien-être, et la sécurité climatique). En plus des Objectifs de Développement Durable puisqu'il peut aider à accomplir 10 ODD (1, 2, 6, 8, 11, 12, 13, 14 et 17 liés respectivement à la lutte contre la pauvreté, la sécurité alimentaire, l'eau potable, la croissance économique, la protection des villes, la production durable, la lutte contre le changement climatique et la conservation des ressources marines).

Cette reconnaissance peut inciter les responsables politiques et les décideurs à élaborer des plans de gestion de l'espace côtier et marin pour lutter contre les pressions anthropiques exercées sur les herbiers (par exemple, le ruissellement urbain, agricole et industriel, le développement côtier, les activités de pêche et de navigation non réglementées), dont les effets vont au-delà des plantes elles-mêmes, et qui sont susceptibles d'altérer la biodiversité associée et l'ensemble du paysage marin dans le contexte de la connectivité des écosystèmes.

L'établissement de limites d'aires marines entièrement protégées autour des herbiers marins peut donc résoudre leur survie et améliorer leur résilience au fil du temps. La restauration des herbiers marins par des techniques de transplantation est une autre action viable pour améliorer l'étendue des habitats d'herbiers marins. Toutefois, pour que les transplantations soient réussies, ils doivent avoir lieu dans des zones à faible niveau d'activités humaines. Par conséquent, la réduction des perturbations demeure la pierre angulaire du rétablissement des herbiers marins.

Las praderas marinas se consideran entre los tipos de hábitats marinos más productivos y valiosos, por los bienes ecológicos y los servicios que brindan. Sin embargo, este patrimonio marino está disminuyendo en todo el mundo a un ritmo creciente. El aumento de la presión humana está poniendo en riesgo su viabilidad futura. Incluir estos ecosistemas en estrategias de gestión eficaces podría ayudar a revertir su pérdida.

El objetivo de esta investigación fue proporcionar conocimientos sobre el estado de las praderas de fanerógamas marinas en las costas marroquíes, y proporcionar bases para su desarrollar medidas de conservación.

Biomonitoreo del estado ambiental en ecosistemas costeros semicerrados utilizando praderas de *Zostera noltei* y *Cymodocea nodosa*

A lo largo del gradiente climático latitudinal completo de la costa atlántica de Marruecos (clima mediterráneo, semiárido y árido), investigamos por primera vez la idoneidad de las hojas de *Zostera noltei* como bioindicador de contaminación por oligoelementos en cinco ecosistemas costeros semicerrados (Laguna Moulay Bousselham, Laguna Sidi Moussa, Laguna Oualidia, Laguna Khnifiss y Bahía Dakhla) sometidas a diferentes presiones antropogénicas (urbanismo, agricultura, industrias y minería). Además, es el primer estudio mundial de la capacidad de las hojas de *Z. noltei* para concentrar 27 elementos químicos (Fe, Al, Cr, Mn, Co, Ni, V, Cu, Zn, Sr, Li, As, Ag, Cd, Sn, Sb, Mo, Ba, Ti, Pb, U, Bi, Hg, Na, Mg, K y Ca) de su entorno circundante. Los resultados señalaron que las concentraciones de elementos químicos tanto para los sedimentos como para las hojas de *Z. noltei* mostraron variabilidad entre sitios e intra-sitios (aguas abajo / aguas arriba). Por lo tanto, se detectó una contaminación similar tanto en la matriz ambiental como en su biodisponibilidad para las hojas de la fanerógama para Al, Fe, Mn, Zn, Ag, Cd, Ba y Hg, mientras que Cd, Mo, Sb, Ag, Zn y U fueron absorbidos eficientemente por *Z .noltei* de los sedimentos. Finalmente, concluimos que las hojas de *Z. noltei* son un eficiente bioindicador de Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba y Hg con el propósito de monitorear la contaminación.

En la costa mediterránea, se investigó el nivel de contaminación y el estado de riesgo ecológico a partir de seis oligoelementos (Ni, Cd, Pb, Cu, Cr y Zn) en los sedimentos de la laguna de Marchica utilizando índices de uno y varios elementos: factor de contaminación, factor de enriquecimiento, índice de geoacumulación (I-geo), grado (modificado) de contaminación (Cd o mCd), índice de contaminación (modificado) (PI o MPI) y guías de calidad de los sedimentos. La biodisponibilidad de estos elementos para conocer el grado de toxicidad se evaluó utilizando hojas, rizomas y raíces de *C. nodosa*. Se consideró que los sedimentos cercanos a efluentes urbanos, descargas industriales y cuencas hidrográficas abastecidas por la escorrentía agrícola estaban muy contaminados. En estas localidades, las pautas de calidad de sedimentos (SQG) y el índice de riesgo ecológico potencial modificado (MRI) predijeron un riesgo ambiental múltiple con elevada probabilidad de efectos biológicos adversos. A su vez, las hojas y raíces de *C. nodosa* acumularon mayores oligoelementos en las áreas impactadas y, por lo tanto, reflejaron de manera eficiente el gradiente de contaminación sedimentaria y presentaron la disponibilidad de estos elementos para la biota. Tanto las hojas como las raíces se comportaron igualmente como buenos bioindicadores de Cu y Cd en el sedimento, mientras que las hojas se consideran el mejor bioindicador para la contaminación por Zn y las raíces para el Pb. Por el contrario, esta fanerógama marina no actúa como bioindicador potencial de contaminación por Al, Cr y Ni. Estos resultados proporcionan una idea de la acumulación de oligoelementos en *C. nodosa*, que se puede aplicar para el diseño de programas de biomonitoreo.

Cymodocea nodosa del Parque Nacional de Al Hoceima y _Zostera marina_ de Jbel Moussa: Primera descripción cuantitativa

En el Parque Nacional de Alhucemas, la evaluación del desarrollo estructural y la dinámica de la población de las praderas de *C. nodosa* utilizando técnicas de reconstrucción revelaron que la baja tasa de elongación del rizoma y la sobrecarga de epífitas reducían la tasa de reclutamiento de la población, induciendo el declive de estas praderas. De hecho, las distribuciones de edad de los brotes de *C. nodosa* mostraron reducciones exponenciales de la densidad de brotes desde la clase de segundo año. Además, la falta de gestión y de una monitorización adecuada del parque han provocado la ausencia de datos fisicoquímicos y sobre las condiciones ambientales que afectan el crecimiento de las praderas. Nuestro resultado refuerza la marcada regresión de esta especie a lo largo de su rango general de distribución a pesar de que se considera que es resistente a los factores de estrés naturales y antropogénicos.

En Jbel Moussa, la primera evaluación del estado ambiental de las praderas de *Zostera marina*, las únicas que quedan a lo largo de las costas mediterráneas de Marruecos, o incluso del norte de África, y las más profundas de todo el Mediterráneo (límite inferior a 17 m de profundidad con parches que se extienden hasta 20 m), encontró un bajo nivel de contaminación por oligoelementos, materia orgánica y nutrientes de origen terrestre. Inicialmente, las praderas estudiadas mostraron un buen desarrollo vegetativo sin efecto de amenazas biológicas (i.e. invasión por el alga verde *Caulerpa cylindracea*) o mecánicas (pesca de arrastre). Durante los siguientes cuatro años, las praderas de *Z. marina* sufrieron una rápida expansión del alga invasora *Rugulopteryx okamurae*, altas temperaturas de verano vinculadas al cambio climático global, junto con evidencias de actividades de pesca de arrastre persistentes, lo que llevó a estas praderas hasta un colapso total. Al mismo tiempo, las comunidades de anfípodos que habitan el fondo blando de Jbel Moussa disminuyeron significativamente en riqueza, diversidad y abundancia con una variación total de hasta un 70% como resultado directo de la desaparición de *Z. marina*.

Acciones recomendadas para una conservación eficaz

La sostenibilidad de los hábitats conformados por las praderas marinas depende de la implementación de planes nacionales efectivos para su gestión. Su cartografía completa es la primera acción para determinar tanto su presencia como su extensión actual. Se requiere igualmente el establecimiento de programas de monitoreo para recopilar información detallada sobre la estructura de su hábitat, la salud y las condiciones ambientales propicias para su crecimiento. Al mismo tiempo, es muy necesaria la investigación científica sobre su ecología, que permitirá aumentar el conocimiento de cómo responden a los cambios ambientales circundantes a lo largo del tiempo y cómo pueden resistir a estos cambios.

Para obtener resultados de conservación más efectivos, es fundamental aumentar el reconocimiento de estos hábitats por parte de responsables políticos, gestores, usuarios de las zonas costeras, grupos ambientalistas y el público en general, junto con una mayor conciencia sobre las amenazas a su supervivencia.

La conservación de las praderas marinas es una oportunidad para avanzar hacia el Plan Estratégico para la Diversidad Biológica del Convenio de las Naciones Unidas sobre la Diversidad Biológica y sus 20 Metas de Aichi para la Diversidad Biológica en el marco de diversidad biológica posterior a 2020 (en particular, las Metas 5, 6, 8, 11, 14 y 15 que abordan la pérdida de hábitat, stocks de peces e invertebrados, contaminación, áreas marinas protegidas, prestación de servicios ecosistémicos y seguridad climática). Además de la Agenda de Desarrollo Sostenible y los Objetivos de Desarrollo Sostenible, ya que puede ayudar a lograr 10 de los objetivos (1, 2, 6, 8, 11, 12, 13, 14 y 17 relacionados, respectivamente con el fin de la pobreza, la seguridad alimentaria, el agua potable, el crecimiento económico, protección de ciudades, producción sostenible, combate contra el cambio climático y conservación de los recursos marinos).

Este reconocimiento puede favorecer que los responsables políticos y gestores desarrollen planes espaciales de gestión costera para reducir las presiones antropogénicas sobre las praderas marinas tales como escorrentía urbana, agrícola e industrial, desarrollo costero, pesca no reglamentada y actividades de navegación, cuyos impactos tienen efectos más allá de este tipo de hábitats, alterando la biodiversidad asociada y el paisaje marino en general en el contexto de la conectividad de los ecosistemas. Por lo tanto, establecer áreas marinas totalmente protegidas alrededor de las praderas de fanerógamas marinas puede favorecer su supervivencia y mejorar su resiliencia con el tiempo. La restauración de praderas mediante técnicas de trasplante es otra acción viable para mejorar la extensión de este tipo de hábitats. Sin embargo, los trasplantes exitosos requieren que se realicen en áreas con bajos niveles de actividad humana. Por lo tanto, la reducción de la perturbación antropogénica sigue siendo la piedra angular para impulsar la recuperación de las praderas marinas.

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