

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. 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 sediment 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 absorbed 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 with 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

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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 food security and public health concerns (Godwill et al., 2015). Chemical analyses of the environmental matrices, such as water and sediment, remain the most direct approach to detect the levels of TEs. It would be too costly, however, to look for compelling evidence of the 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. It can warn us early on that there might be influences at higher levels as a result of trophic interactions (Bonanno and Di Martino, 2017; Farias et al., 2018).

Seagrasses are marine angiosperms that fulfill important trophic and structural functions in coastal ecosystems (Duffy et al., 2006; Short et al., 2007; Gutiérrez et al., 2011). They are capable of absorbing TEs and major elements from overlying water through leaf surfaces and from the sediment through their roots and rhizomes (Llagostera et al., 2011). Furthermore, as they contribute significantly to the primary production of aquatic ecosystems, they are expected to act as indicators of contaminants at higher trophic levels (Govers et al., 2014). The interest in seagrasses as bioindicators of TE contamination has consequently 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 has 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, thus representing the land-sea interface (Short et al., 2007). *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). While other seagrasses have been studied more regularly, only a few focused on *Z. noltei* and evaluated coastal TE contamination. They concluded that this species can be used as a good bioindicator to monitor some coastal waters (Sanchiz et al., 2000; Bat et al., 2016; Wilkes et al., 2017). 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 a 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 essential, both from a scientific and a stakeholder point of view. This study aimed to be the first to assess the suitability of *Z. noltei* leaves as a bioindicator of TE contamination in semi-enclosed coastal ecosystems along the full latitudinal gradient of the Moroccan Atlantic coast. To achieve this goal, the followed approach was: (1) to study the distribution of 23 chemical elements in seagrass leaves and sediments in five semi-enclosed water-bodies 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 Bouselham 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 Bouselham, Oualidia and Sidi Moussa), harbour activities (Dakhla bay) and mining activities (Sidi Moussa, Oualidia and Khnifiss). 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.

The Moulay Bouselham lagoon (34°47'N–6°13'W and 34°52'N–6°14'W), which is commonly known as Merja Zerga, covers 45 km² 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 ocean, the downstream part's salinity is close to sea water's 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 also showed 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. 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 km² 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) and from 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, while 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 go from 13.9 to 21.7 °C and from 30.1 to 34.7, respectively (Maanan, 2008). A rapid urbanization has occurred in recent years (Maanan et al., 2014). Farms that use 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 essential. During summer, there are numerous touristic activities such as boating, bathing and camping. Wastewaters from rural and urban centers discharge 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 km² surface area and maximum depth of 8.7 m) and the adjacent depression of Guelta El Aouina are included in the 60,000 ha, thus making up an integral natural reserve (Dakki and Ligny, 1988). Water

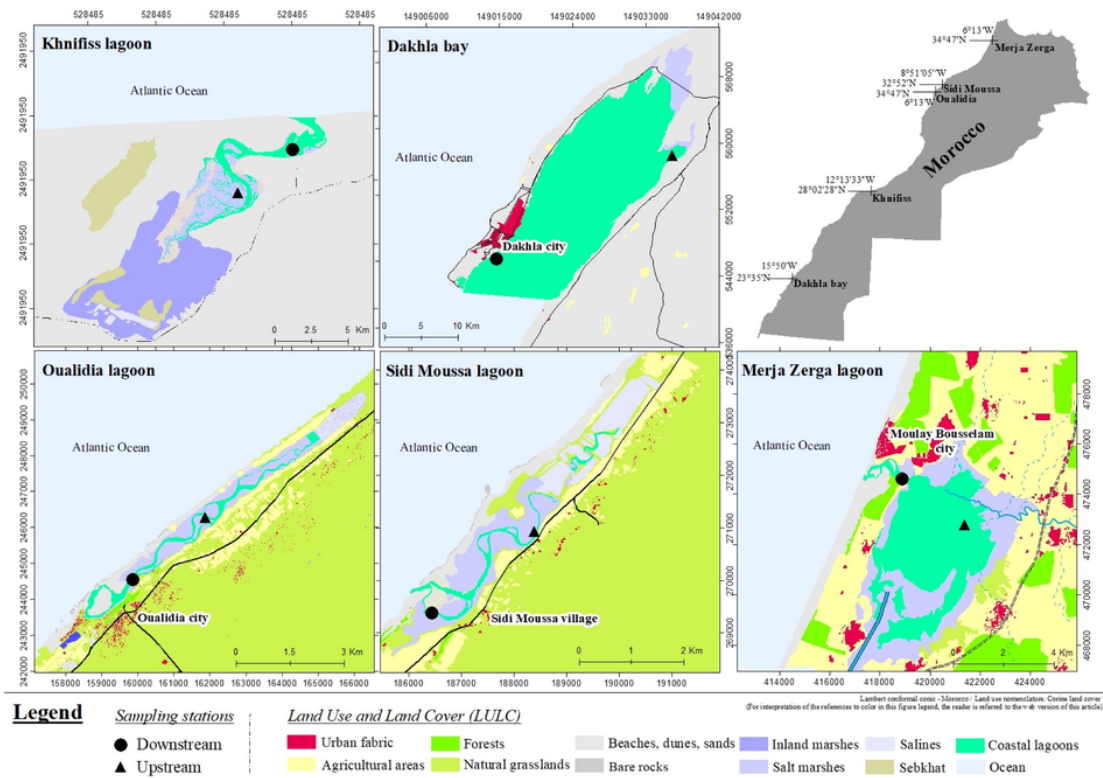


Fig. 1. Maps of the five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. In each site one upstream (U) station and one downstream (D) station were sampled for *Zostera noltei* and sediments.

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 are 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 it was designated as 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 a 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 to 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 which had been 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, they were gently rinsed 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 sediment 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. Sub-samples 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 (4 ml HNO₃/1 ml H₂O₂ for leaves and 2 ml HNO₃/1 ml H₂O₂ for sediment) were used as reagents ('Suprapur' grade, Merck). This partial digestion procedure extracts the adsorbed and organic fractions of chemicals bioavailable to organisms from sediments without digesting their 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 (*Platithypnidium 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% (this was a partial digestion procedure; there was no certified value for Ba, Tl, Bi and U). Vegetal CRM mean recovery was 103% (there was 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:

$$\text{LOD} = \text{mblanc} + 3\text{Sblanc}$$

$$\text{LOQ} = \text{mblanc} + 10\text{Sblanc}$$

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 that were 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:

$$\text{TESVI} = \left[\frac{(x_{\max}/x_{\min})}{(\sum (x_{\max}/x_i) / n)} \right] * \text{SD}$$

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 $\sum(x_{\max}/x_i)/n$. 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:

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

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 is. This index calculates the contamination rate of trace elements, which are considered as potential environmental contami-

nants. This explains why 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:

$$\text{BCF} = \text{mean concentration in a seagrass compartment (in mg kg}_{\text{DW}}^{-1}) / \text{mean co}$$

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 a one-way analysis of variance (one-way ANOVAs). This was followed by a 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. A non-parametric analysis of variance (Kruskal-Wallis test) was performed when assumptions prior to ANOVAs (normality and/or homoscedasticity) were not achieved, followed by a 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. A third Spearman's correlation coefficient analysis was performed on matrices of *Z. noltei* and sediment data to identify further links between TE concentrations and TEPI values in sediments and *Z. noltei* leaves. This was followed by a 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).

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. 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 from 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 waters decreased in the following order: Sidi moussa lagoon > Oualidia lagoon > Merja Zerga lagoon > Khnifiss lagoon > Dakhla bay.

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 |

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). This 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).

According to Cluster Analysis (Fig. 3), the first cluster grouped Dakhla downstream and upstream stations, which were 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 linked Sidi Moussa downstream and upstream stations which 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 highest level of Sr without significant difference with Sidi Moussa lagoon. Li and Zn were also higher in Sidi Moussa lagoon without being significantly different ($p > 0.05$) from 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).

The following results compare numbers between the downstream and upstream stations of each site under study. Regarding the individual analysis of each water body, no significant differences ($p > 0.05$) were observed between the 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. In Oualidia lagoon, the downstream station showed significantly ($p < 0.05$) higher Ca, Sr and Sn levels and lower Pb levels. Dakhla bay's downstream station showed significantly higher concentrations ($p < 0.05$) of Mg, Ca, Mn, Sr, Sn, Pb and Bi. In Sidi Moussa la-

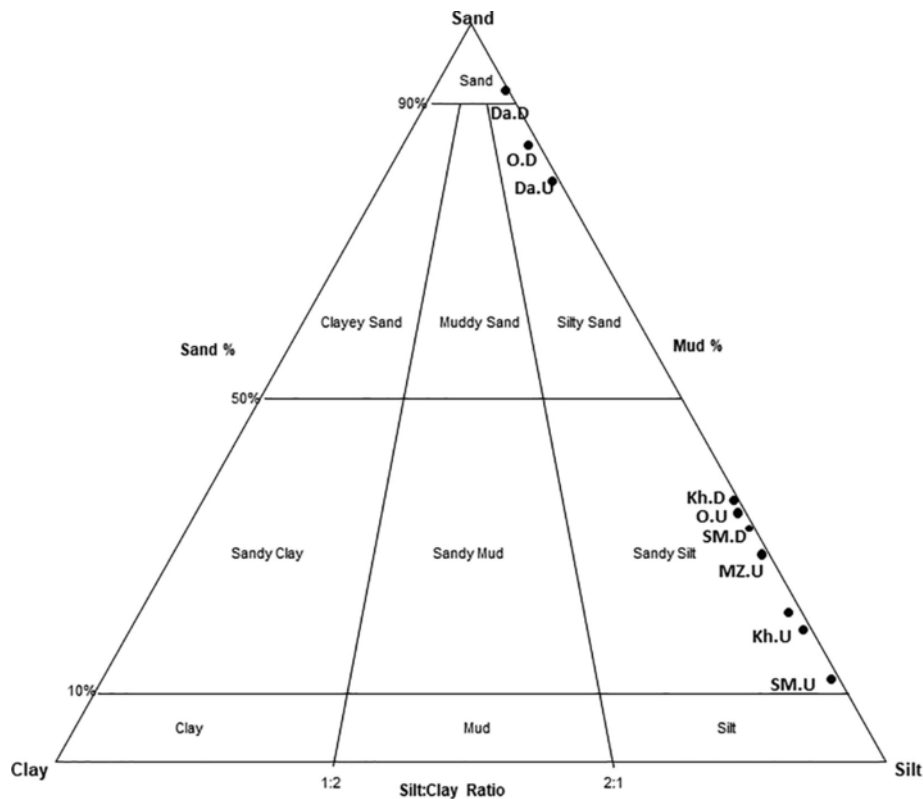


Fig. 2. Textural classification (mean, in %) of sediments (n = 3) sampled from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the Atlantic coast of Morocco. MZ: Merja Zerga. SM: Sidi Moussa. O: Oualidia. Kh: Khnifiss. Da: Dakhla.

goon, the downstream station showed significantly ($p < 0.05$) higher Sb, U and Mo levels and lower Fe levels.

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: $Ca > Mg > Na > Fe > K > Al > Sr > Mn > Zn > V > Ba > Mo > Cu > Cd > U$. 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) was 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 (The four stations in Dakhla and Khnifiss) 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. Sidi Moussa lagoon's downstream station showed significantly ($p < 0.05$) higher levels of Mo, Sb and Hg and lower concentrations of Ag in Oualidia lagoon. Ag and Hg concentrations were significantly ($p < 0.05$) higher downstream. Dakhla's downstream station showed high Pb concentrations while the upstream station had high Mo concentrations. For Khnifiss, there were no significant differences ($p > 0.05$) between the two.

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 \times 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.

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.

Table 2

Chemical element (major and trace elements) concentrations (mean \pm 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 Bioconcentration 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 |
|-------------------|-------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|-------------------|-------------------|--------------------|-------------------|-------|-------|
| | | MZ | | SM | | O | | Kh | | Da | | | |
| | | D | U | D | U | D | U | D | U | D | U | | |
| Na | Sediments | 17429 \pm 585 | 9737 \pm 1188 | 11712 \pm 3930 | 9980 \pm 854 | 11801 \pm 705 | 7997 \pm 1527 | 6971 \pm 1907 | 6067 \pm 906 | 16111 \pm 12524 | 3878 \pm 1279 | 1.60 | 5.20 |
| | Z.n. | 6632 \pm 1981 | 5827 \pm 1682 | 1354 \pm 463 | 1673 \pm 258 | 2407 \pm 145 | 2675 \pm 1291 | 4806 \pm 965 | 5027 \pm 3121 | 10685 \pm 2874 | 10936 \pm 1523 | 3.80 | 13.0 |
| Mg | Sediments | 14036 \pm 303 | 11475.3 \pm 623 | 21772 \pm 1571 | 22685 \pm 2425 | 21889 \pm 3033 | 20515 \pm 1658 | 18265 \pm 949 | 15473 \pm 1973 | 12498 \pm 270 | 4887 \pm 946 | 0.50 | 1.60 |
| | Z.n. | 10932 \pm 654 | 10147 \pm 1444 | 4128 \pm 535 | 5169 \pm 596 | 8452 \pm 988 | 6219 \pm 1678 | 10533 \pm 260 | 8273 \pm 2841 | 10159 \pm 333 | 12138 \pm 1033 | 0.300 | 1.00 |
| Ca | Sediments | 103040 \pm 8321 | 107084 \pm 8893 | 163004 \pm 20810 | 140559 \pm 17665 | 180263 \pm 10239 | 143155 \pm 9166 | 88340 \pm 3379 | 62034 \pm 611 | 85496 \pm 1244 | 5704 \pm 601 | 0.500 | 1.80 |
| | Z.n. | 18037 \pm 3179 | 13599 \pm 1869 | 25314 \pm 3939 | 29355 \pm 2637 | 19128 \pm 1087 | 24650 \pm 2752 | 21142 \pm 1330 | 26115 \pm 3940 | 16803 \pm 3106 | 12747 \pm 1304 | 0.600 | 1.90 |
| K | Sediments | 10794 \pm 9523 | 7815 \pm 1392 | 9385 \pm 1162 | 9960 \pm 1064 | 7337 \pm 1582 | 8469 \pm 1101 | 8025 \pm 1238 | 7185 \pm 1854 | 3948 \pm 551 | 2164 \pm 554 | 1.10 | 3.80 |
| | Z.n. | 1151 \pm 375 | 1196 \pm 494 | 193 \pm 116 | 382 \pm 75.3 | 387 \pm 103 | 610 \pm 470 | 652 \pm 128 | 647 \pm 253 | 1656 \pm 469 | 1224 \pm 271 | 1.40 | 4.80 |
| Fe | Sediments | 41577 \pm 1377 | 35285 \pm 2089 | 18478 \pm 1777 | 25511 \pm 1869 | 18688 \pm 2800 | 22707 \pm 2975 | 27051 \pm 1974 | 24812 \pm 3292 | 9165 \pm 1057 | 6158 \pm 1387 | 5.30 | 18.0 |
| | Z.n. | 5045 \pm 1024 | 3638 \pm 833 | 466 \pm 14.1 | 1522 \pm 567 | 832 \pm 118 | 839 \pm 145 | 2935 \pm 456 | 3372 \pm 721 | 1268 \pm 110 | 1185 \pm 328 | 1.90 | 6.20 |
| Al | Sediments | 45744 \pm 4156 | 33348 \pm 5511 | 27582 \pm 3328 | 30719 \pm 3351 | 21090 \pm 46882 | 26153 \pm 2817 | 25721 \pm 4100 | 23098 \pm 5833 | 11714 \pm 3435 | 7846 \pm 2048 | 0.002 | 0.006 |
| | Z.n. | 2221 \pm 667 | 1256 \pm 388 | 244 \pm 113 | 520 \pm 92.6 | 382 \pm 108 | 317 \pm 44 | 903 \pm 219 | 831 \pm 201 | 628 \pm 138 | 439 \pm 101 | 0.002 | 0.005 |
| V | Sediments | 113 \pm 8.10 | 87.5 \pm 10.9 | 71.8 \pm 7.28 | 67.0 \pm 6.44 | 48.8 \pm 8.56 | 56.6 \pm 6.30 | 61.2 \pm 7.03 | 56.0 \pm 10.3 | 33.2 \pm 4.36 | 20.7 \pm 4.52 | 0.007 | 0.023 |
| | Z.n. | 26.7 \pm 7.31 | 15.9 \pm 6.86 | 7.16 \pm 0.791 | 8.34 \pm 1.14 | 6.41 \pm 1.63 | 8.26 \pm 2.81 | 46.2 \pm 5.47 | 46.8 \pm 7.65 | 19.1 \pm 9.02 | 18.4 \pm 1.80 | 0.006 | 0.021 |
| Cr | Sediments | 95.2 \pm 5.78 | 73.5 \pm 9.01 | 131 \pm 19.0 | 123 \pm 11.8 | 89.3 \pm 50.4 | 73.8 \pm 12.8 | 49.6 \pm 5.68 | 43.5 \pm 8.46 | 35.6 \pm 7.60 | 21.2 \pm 6.79 | 0.600 | 1.90 |
| | Z.n. | 4.08 \pm 1.25 | 2.41 \pm 0.632 | 1.49 \pm 0.448 | 2.27 \pm 0.435 | 1.35 \pm 0.342 | 1.15 \pm 0.087 | 1.67 \pm 0.283 | 1.48 \pm 0.250 | 1.88 \pm 0.259 | 1.35 \pm 0.380 | 2.90 | 9.70 |
| Mn | Sediments | 304 \pm 25.3 | 255 \pm 17.7 | 206 \pm 10.9 | 231.5 \pm 18.1 | 244.9 \pm 31.5 | 236 \pm 16.3 | 236 \pm 11.02 | 210 \pm 26.5 | 95.3 \pm 6.32 | 40.6 \pm 3.42 | 0.007 | 0.024 |
| | Z.n. | 439 \pm 417 | 281 \pm 195 | 62.7 \pm 19.3 | 172 \pm 65.3 | 107.7 \pm 66.9 | 132 \pm 16.1 | 78.3 \pm 13.8 | 167 \pm 65.2 | 28.9 \pm 3.64 | 80.2 \pm 24.1 | 0.025 | 0.085 |
| Co | Sediments | 10.7 \pm 0.088 | 9.01 \pm 0.283 | 5.45 \pm 0.443 | 6.83 \pm 0.501 | 5.88 \pm 1.22 | 6.56 \pm 0.849 | 7.85 \pm 0.378 | 6.77 \pm 0.980 | 3.04 \pm 0.341 | 2.14 \pm 0.307 | 0.006 | 0.019 |
| | Z.n. | 2.62 \pm 1.79 | 1.36 \pm 0.621 | 0.263 \pm 0.091 | 0.683 \pm 0.269 | 0.264 \pm 0.126 | 0.465 \pm 0.010 | 0.756 \pm 0.118 | 1.13 \pm 0.317 | 0.444 \pm 0.036 | 1.15 \pm 0.139 | 0.007 | 0.023 |
| Ni | Sediments | 33.7 \pm 0.756 | 27.1 \pm 1.51 | 25.4 \pm 2.72 | 28.0 \pm 1.92 | 34.9 \pm 23.3 | 26.7 \pm 5.05 | 28.5 \pm 1.99 | 24.7 \pm 3.80 | 11.0 \pm 2.38 | 7.97 \pm 0.675 | 0.018 | 0.060 |
| | Z.n. | 4.78 \pm 2.28 | 3.20 \pm 1.23 | 1.83 \pm 0.396 | 2.19 \pm 0.563 | 1.44 \pm 0.530 | 0.970 \pm 0.089 | 2.33 \pm 0.226 | 2.11 \pm 0.405 | 2.12 \pm 0.069 | 2.26 \pm 0.962 | 0.023 | 0.078 |
| Cu | Sediments | 38 \pm 4.02 | 31.1 \pm 4.70 | 63.3 \pm 5.46 | 72.5 \pm 7.30 | 54.5 \pm 8.78 | 58.0 \pm 6.22 | 76.4 \pm 9.32 | 75.4 \pm 14.5 | 46.6 \pm 5.17 | 35.7 \pm 4.57 | 0.070 | 0.230 |
| | Z.n. | 10.6 \pm 3.68 | 10.1 \pm 4.48 | 5.15 \pm 1.26 | 6.72 \pm 1.45 | 3.62 \pm 0.301 | 3.64 \pm 0.620 | 4.74 \pm 0.593 | 5.70 \pm 1.12 | 6.09 \pm 0.269 | 3.96 \pm 1.02 | 0.078 | 0.260 |
| Zn | Sediments | 95.4 \pm 2.07 | 78.7 \pm 5.27 | 87.8 \pm 10.1 | 92.9 \pm 8.51 | 60.7 \pm 10.6 | 77.2 \pm 8.98 | 69.2 \pm 3.97 | 60.6 \pm 9.03 | 40.6 \pm 5.33 | 24.4 \pm 3.90 | 0.041 | 0.140 |
| | Z.n. | 68.8 \pm 31.1 | 62.3 \pm 28.4 | 192 \pm 22.4 | 180 \pm 21.0 | 99.0 \pm 27.6 | 126 \pm 22.7 | 87.2 \pm 11.2 | 93.5 \pm 30.2 | 56.9 \pm 26.9 | 31.4 \pm 5.67 | 0.085 | 0.280 |
| Sr | Sediments | 356 \pm 43 | 347 \pm 19.6 | 627 \pm 73.6 | 522 \pm 75.2 | 936 \pm 69 | 762 \pm 82.5 | 281 \pm 14.2 | 190 \pm 14.9 | 400 \pm 18.7 | 132 \pm 28.8 | 0.012 | 0.040 |
| | Z.n. | 330 \pm 42.8 | 284 \pm 29.3 | 228 \pm 22.4 | 323 \pm 44 | 283 \pm 12.6 | 277 \pm 17.0 | 325 \pm 7.94 | 291 \pm 24.5 | 344 \pm 27.0 | 287 \pm 14.5 | 0.007 | 0.023 |
| Li | Sediments | 31.7 \pm 2.00 | 25.9 \pm 3.80 | 24.2 \pm 0.980 | 30.1 \pm 1.60 | 21.4 \pm 4.11 | 29.- \pm 2.85 | 26.9 \pm 2.20 | 26.6 \pm 4.66 | 12.1 \pm 4.21 | 6.10 \pm 0.985 | 0.010 | 0.034 |
| | Z.n. | 1.41 \pm 0.498 | 0.821 \pm 0.192 | 0.271 \pm 0.057 | 0.499 \pm 0.050 | 0.387 \pm 0.090 | 0.402 \pm 0.068 | 0.879 \pm 0.194 | 0.858 \pm 0.110 | 0.761 \pm 0.208 | 0.536 \pm 0.117 | 0.011 | 0.037 |
| As | Sediments | 14.7 \pm 1.05 | 12.1 \pm 0.549 | 8.64 \pm 1.25 | 8.50 \pm 0.263 | 7.94 \pm 0.129 | 7.37 \pm 0.877 | 9.88 \pm 1.20 | 8.87 \pm 0.829 | 4.03 \pm 0.456 | 2.49 \pm 0.364 | 4.70 | 16.0 |
| | Z.n. | 3.54 \pm 1.58 | 3.94 \pm 1.93 | 0.601 \pm 0.081 | 1.04 \pm 0.252 | 1.01 \pm 0.104 | 0.746 \pm 0.070 | 2.86 \pm 0.444 | 1.84 \pm 0.286 | 1.93 \pm 0.156 | 2.17 \pm 0.275 | 5.3 | 18 |
| Mo | Sediments | 1.60 \pm 0.363 | 1.15 \pm 0.173 | 4.86 \pm 1.28 | 2.11 \pm 0.137 | 1.62 \pm 1.07 | 2.92 \pm 0.640 | 3.97 \pm 0.338 | 2.69 \pm 0.559 | 2.88 \pm 0.518 | 2.28 \pm 0.510 | 0.009 | 0.028 |
| | Z.n. | 7.58 \pm 2.01 | 7.66 \pm 1.90 | 16.6 \pm 2.32 | 8.25 \pm 1.09 | 12.1 \pm 1.99 | 10.6 \pm 2.04 | 23.6 \pm 1.87 | 19.6 \pm 7.09 | 10.8 \pm 0.699 | 21.7 \pm 2.61 | 0.014 | 0.047 |
| Ag | Sediments | 0.082 \pm 0.010 | 0.073 \pm 0.009 | 2.48 \pm 0.828 | 2.92 \pm 0.214 | 0.44980.164 | 0.752 \pm 0.146 | 0.0730.003 | 0.0630.009 | 0.116 \pm 0.112 | 0.037 \pm 0.005 | 0.027 | 0.088 |
| | Z.n. | 0.178 \pm 0.107 | 0.146 \pm 0.058 | 1.86 \pm 0.717 | 2.47 \pm 0.846 | 2.20 \pm 0.393 | 0.468 \pm 0.256 | 0.122 \pm 0.037 | 0.157 \pm 0.037 | 0.940 \pm 0.014 | 0.092 \pm 0.030 | 0.013 | 0.042 |
| Cd | Sediments | 0.116 \pm 0.001 | 0.97 \pm 0.013 | 1.17 \pm 0.251 | 1.47 \pm 0.186 | 0.523 \pm 0.016 | 0.515 \pm 0.154 | 0.238 \pm 0.045 | 0.215 \pm 0.013 | 0.770 \pm 0.067 | 0.292 \pm 0.027 | 0.006 | 0.020 |
| | Z.n. | 0.803 \pm 0.201 | 0.779 \pm 0.208 | 11.9 \pm 0.538 | 2.52 \pm 5.92 | 6.53 \pm 1.24 | 2.78 \pm 0.157 | 1.12 \pm 0.346 | 1.10 \pm 0.133 | 2.15 \pm 0.50 | 3.01 \pm 0.330 | 0.008 | 0.026 |
| Sn | Sediments | 0.329 \pm 0.077 | 0.169 \pm 0.114 | 0.760 \pm 0.056 | 0.686 \pm 0.050 | 0.538 \pm 0.057 | 0.342 \pm 0.039 | 0.245 \pm 0.082 | 0.263 \pm 0.075 | 0.550 \pm 0.0768 | 0.108 \pm 0.022 | 0.005 | 0.015 |
| | Z.n. | 0.077 \pm 0.027 | 0.052 \pm 0.014 | BLD | 0.046 \pm 0.002 | BLD | BLD | 0.043 \pm 0.010 | 0.040 \pm 0.005 | 0.086 \pm 0.013 | 0.032 \pm 0.005 | 0.004 | 0.013 |
| Sb | Sediments | 0.043 \pm 0.007 | 0.031 \pm 0.056 | 0.598 \pm 0.111 | 0.225 \pm 0.026 | 0.131 \pm 0.008 | 0.071 \pm 0.09 | 0.062 \pm 0.007 | 0.060 \pm 0.009 | 0.083 \pm 0.011 | 0.053 \pm 0.036 | 0.015 | 0.051 |
| | Z.n. | 0.318 \pm 0.069 | 0.258 \pm 0.077 | 0.430 \pm 0.062 | 0.279 \pm 0.053 | 0.306 \pm 0.035 | 0.273 \pm 0.058 | 0.489 \pm 0.018 | 0.354 \pm 0.025 | 0.273 \pm 0.053 | 0.416 \pm 0.015 | 0.026 | 0.085 |
| Ba | Sediments | 68.0 \pm 8.27 | 54.7 \pm 10.1 | 1045 \pm 10.6 | 82.9 \pm 7.26 | 86.2 \pm 9.90 | 76.9 \pm 14.8 | 68.1 \pm 9.95 | 71.2 \pm 16.3 | 62.2 \pm 16.5 | 32.0 \pm 4.80 | 0.013 | 0.044 |
| | Z.n. | 12.8 \pm 2.54 | 10.4 \pm 0.594 | 27 \pm 2.63 | 21.1 \pm 2.16 | 12.75 \pm 2.07 | 16.9 \pm 4.82 | 18.2 \pm 2.53 | 20.9 \pm 9.03 | 10.2 \pm 4.87 | 9.63 \pm 1.24 | 0.006 | 0.019 |
| Ti | Sediments | 0.238 \pm 0.024 | 0.194 \pm 0.032 | 0.245 \pm 0.032 | 0.412 \pm 0.015 | 0.178 \pm 0.031 | 0.238 \pm 0.0333 | 0.238 \pm 0.033 | 0.227 \pm 0.043 | BLD | BLD | 0.006 | 0.021 |

| | | | | | | | | | | | | |
|------|-------------|--------------|--------------|--------------|-------------|--------------|--------------|---------------|--------------|---------------|-------|-------|
| 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 |
|------|-------------|--------------|--------------|--------------|-------------|--------------|--------------|---------------|--------------|---------------|-------|-------|

Table 2 (Continued)

| Chemical elements | Compartment | Sites | | | | | | | | | | LOD | LOQ |
|-------------------|-------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|-------|-------|
| | | MZ | | SM | | O | | Kh | | Da | | | |
| | | D | U | D | U | D | U | D | U | D | U | | |
| 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 |
| | 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 |
| | 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 |
| 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 | --- | --- |
| | 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 | --- | --- |
| 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 | --- | --- |

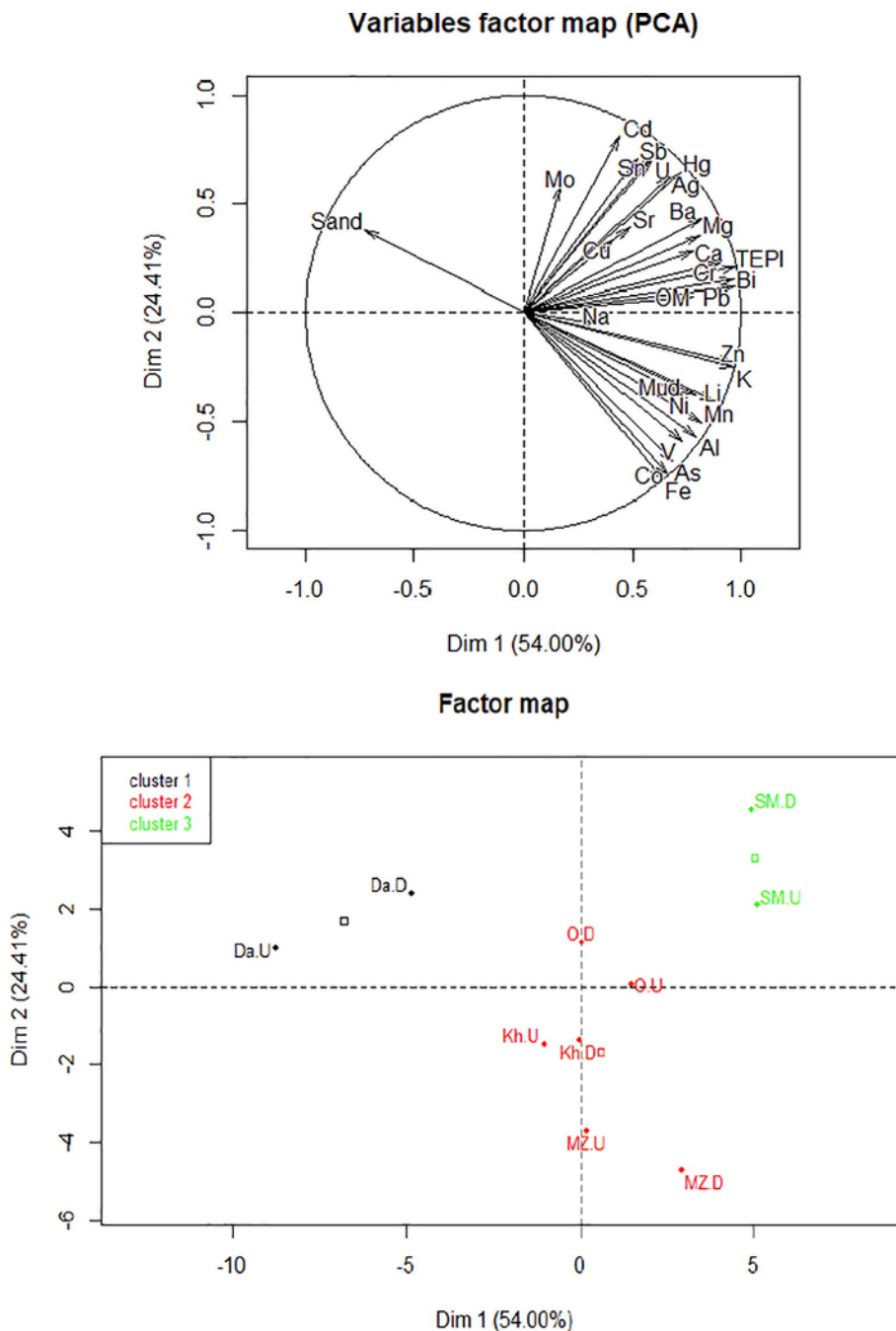


Fig. 3. Factor loading plots for the first two principal components identified in the PCA of chemical element concentrations and Trace Element Pollution Index (TEPI) values for sediments. Samples were collected from downstream (D) and upstream (U) stations in five semi-enclosed water bodies along the 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.

4. Discussion

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

Chemical elements concentrations for both sediments and *Z. noltei* leaves showed inter-site and intra-site (downstream/upstream) variability. This is 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 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 do not only exist between sites but also be-

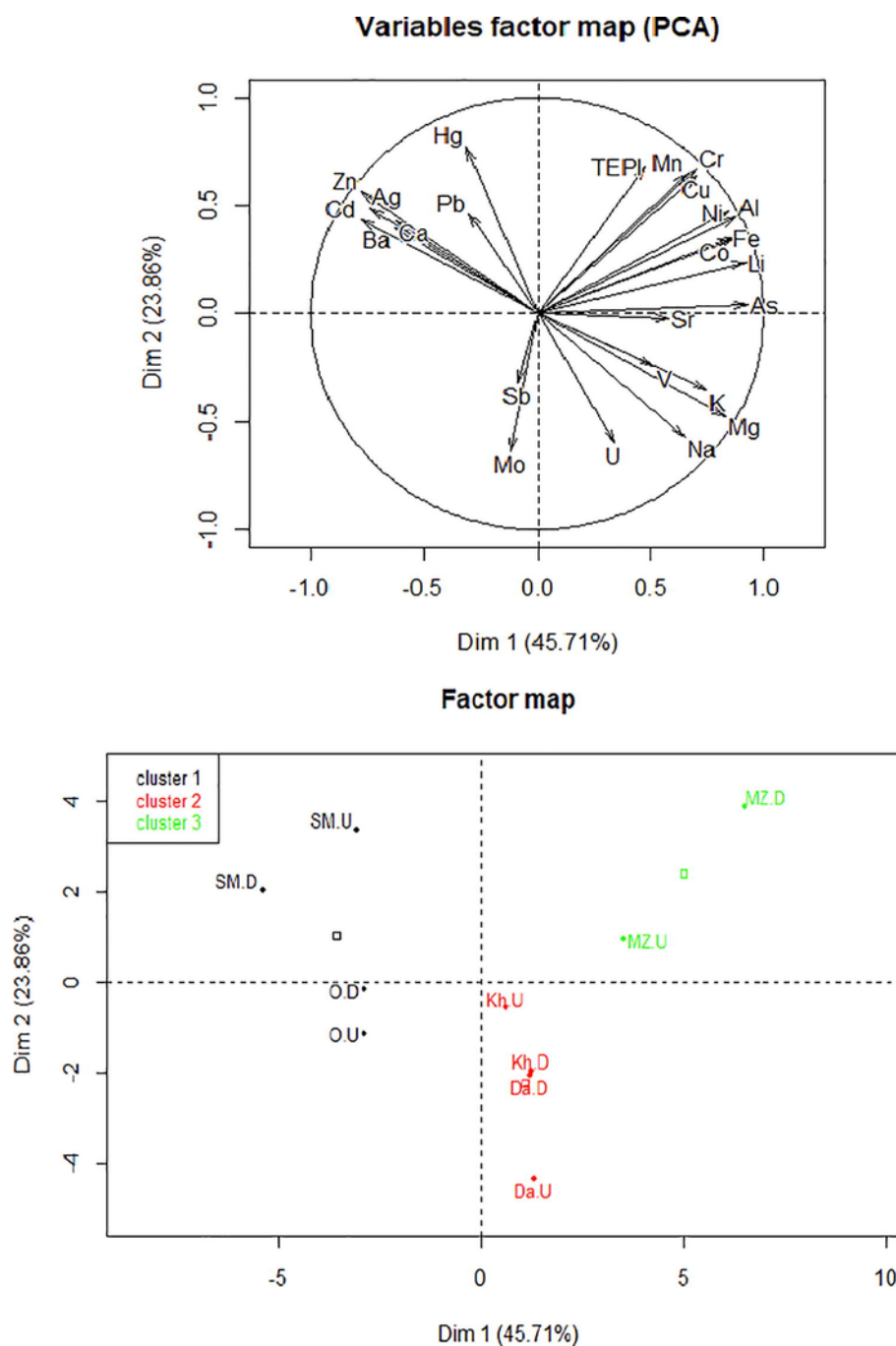


Fig. 4. Factor loading plots for the first two principal components identified in the PCA of chemical element concentrations and Trace Element Pollution Index (TEPI) values for *Zostera noltei* leaves. Samples were collected from downstream (D) and upstream (U) stations in five semi-enclosed water bodies 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.

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 | |

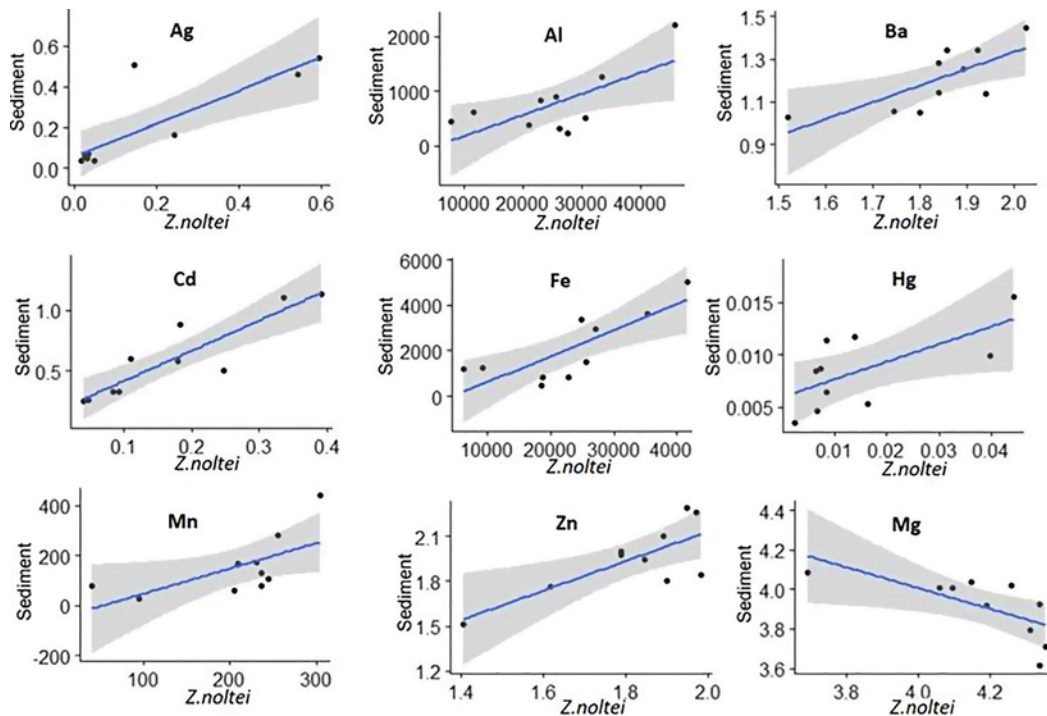


Fig. 5. Mean concentrations of Ag, Al, Ba, Cd, Fe, Hg, Mn Zn and Mg in *Zostera noltei* leaves (in $\text{mg kg}^{-1}\text{DW}$) plotted against mean concentrations measured in sediments (in $\text{mg kg}^{-1}\text{DW}$). Samples were collected from downstream and upstream stations of five semi-enclosed 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.

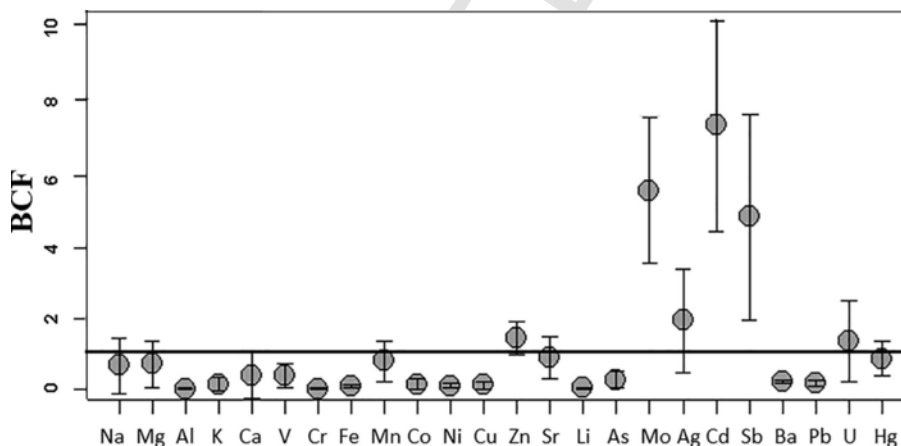


Fig. 6. Mean ($n = 10$) bioconcentration factor (BCF) from sediments to *Zostera noltei* leaves for chemical elements monitored in downstream and upstream stations of five semi-enclosed coastal ecosystems along the Atlantic coast of Morocco. Bars symbolize SD. Solid black line corresponds to the BAF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

tween upstream and downstream stations within transitional water systems. The hydrodynamic situation resulting from small intra-lagoon currents remained the main factor differentiating 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 both 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 the hydrological conditions of the 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).

TE contamination estimated by the Trace Element Pollution Index (TEPI), using *Z. noltei* leaves and sediments, evidenced contrasting situations. While the seagrasses TEPI values varied little among Moroccan Atlantic semi-enclosed water bodies, TEPI estimated from sediment discriminated clearly between the case studies. Indeed, sediments 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 also showed high levels of Sb, U, Cd, Li and Cu. Comparing to the 2001 results given by Maanan et al. (2004, Table 6), Cr, Zn and Cu levels have increased by 1.31, 1.82 and 2.23% respectively. 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 15km northeast

Table 6
Average TEs concentrations recorded in sediments from some of the study cases (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., 2004a,b | 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 | | | | | | |

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., 1999, Kaimoussi, 2001). In addition, agricultural activities near the lagoon use heavy machinery which 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 almost doubled 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 other (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. The Cr sediment concentration was 1.79% lower in 2010 than in our study. The level of As is 44 times higher than the one reported in 2004 (0.3 mg.kg⁻¹DW; 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 also results from modern intensive agriculture, which is mainly located in the lagoon catchment area and at the lagoon outlet where agro-chemicals fertilizers are profusely used. 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, which are 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) reporting high pollution rates along the Nador canal considered this as probably the 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 indicated their additional anthropogenic sources. Therefore, natural processes and anthropogenic inputs are both responsible of TE contamination in Merja Zerga lagoon, which is accentuated by its confinement effect.

Khniouss 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 can be related to the strong hydrodynamic conditions ensuring the homogenization of water masses and their fast renewal rate. It is also linked with the absence of continental freshwater inputs (Lakhdar et al., 2004) which maintain the lagoon's generally good environmental quality. Our results are in agreement with Lefrere et al. (2015) who reported on its status using benthic macrofauna as bioindicators.

Dakhla bay was the last contaminated site. No chemical element levels were significantly higher in this water body compared to the other sites. This bay, however, shared the highest level of Cd content with Sidi Moussa and Oualidia lagoons. The high level of Cd in Dakhla bay was previously considered as from metal and nutrient-rich water upwellings of marine origin (Zidane et al., 2017). Our results showed modest to strong correlations between Cd and Ag, Sn, Sb, U and Hg, thus indicating supplementary industrial sources for this element. Overall, the downstream station was more contaminated than the upstream station. This was 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 TE 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 TE 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 TE 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 TE 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 in the 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). On the other hand, 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 the 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* in Morocco.

4.2. Performance of *Zostera noltei* as bioindicator

There is now enough scientific evidence to support the suitability of seagrasses as bioindicators of TE contamination (Bonanno and Orlando-Bonaca, 2018). For example, *Posidonia oceanica* and *Cymodocea nodosa* can reflect the level of a wide range of TE 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*, and despite its wide distribution, few works have been conducted to highlight its capacity to monitor TEs in Ireland, Mediterranean Spanish coast and the Turkish coast of the Black Sea (Sanchiz et al., 2000; Bat et al., 2016; Wilkes et al., 2017). This study was subsequently 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) They ought to be abundant enough and widely distributed for repetitious sampling and comparison. (3) They have to be easy to identify. (4) They need to be easy to sample and raise in the laboratory. (5) They should occupy an important position in the food chain (Zhou et al., 2008; Polechońska et al., 2018) and they need to be able to absorb high levels of contaminants without dying (Zhou et al., 2008). *Z. noltei* is a widespread and abundant species in coastal semi-enclosed ecosystems along the Atlantic coast of Morocco, which makes it fit most of the abovementioned characteristics for this study.

Additionally, ideal bioindicators are sensitive to change in the surrounding environment. Bioindicators should especially 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 reflected the plants' capability to accumulate them from sediments (Zhang et al., 2011). In this study, a high bioconcentration factor ($BCF > 1$) for Cd, Mo, Sb, Ag, Zn and U indicated their efficient uptake by *Z. noltei* from sediment.

This study consequently 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 (e.g. *P. oceanica*) should be considered as effective short-term bioindicators. They may be 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. These organs are however 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 noltei* 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). Furthermore, the degree of TEs accumulation from sediment to seagrass belowground tissues depends 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 taken into account for further investigations.

5. Conclusion

The present study was carried out at ten different seagrass beds (5 sites and two up- and downstream stations per site) that are submitted to different anthropogenic pressures along a latitudinal climatic gradient on the Atlantic coast of Morocco. The results suggest that *Z. noltei* leaves can be used as a bioindicator for Cd, Mo, Sb, Ag, Zn, U, Al, Fe, Mn, Ba and Hg contamination in the case studies. 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.

Uncited references

Hu et al. (2018), Tchounwou et al. (2012).

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