BIOMONITORING OF HEAVY METAL POLLUTION USING THE BROWN SEAWEED *ERICARIA SELAGINOIDES* ALONG THE ATLANTIC COAST OF MOROCCO

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Abstract. Increased pollution in the coastal areas may cause changes in the biodiversity of marine organisms depending upon their physiological capacity and resilience to thrive under stressing environmental conditions. The present research evaluates the heavy metals pollution degree of coastal waters using the macroalgae *Ericaria selaginoides* as bioindicator along the Atlantic coast of Morocco. Eight stations were chosen: two located near Eljadida city, three nearby Safi city and three around the city of Essaouira. Results showed that the heavy metal content in the thalli of *E. selaginoides*, in seawater and sediment varied seasonally. At the same time, it was negatively correlated with algal biodiversity onsite. However, the Chemical Oxygen Demand was significantly higher at the polluted station S5 than at other stations, while Dissolved Oxygen and Biological Oxygen Demand were lower. *E. selaginoides* accumulated metals in the following order Fe > Zn > Mn > Cu > Ni > Pb > Cr > Cd. In conclusion, *E. selaginoides* is overall more resilient to heavy metal pollution than other marine organisms in the Atlantic coast of Morocco, as indicated by substantially elevated concentrations of heavy metals in some sites. Our results support that *E. selaginoides* would be a suitable bioindicator for monitoring of heavy metals in polluted coastal areas.

Keywords: *bioindicator, ecotoxicology, marine water quality, monitoring, seaweed resilience*

Introduction

Due to anthropogenic pressure resulting mainly from high population densities and the concentration of diverse anthropogenic activities along shorelines many coastal areas are among the most severely degraded ecosystems worldwide (Lotze et al., 2001; Halpern et

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al., 2008). Correspondingly, littoral and sublittoral communities are particularly sensitive to such pressures since they are exposed to a wide range of extreme environmental conditions at the edge of marine, as well as terrestrial realms (Crowe et al., 2000; Martínez et al., 2012).

Heavy metal contamination is an environmental issue of increasing importance in many coastal areas, especially when those metals are found at concentrations higher than natural loads (Islam and Tanaka, 2004; Akcali and Kucuksezgin, 2011; Scherner et al., 2013; Thibaut et al., 2015). In aquatic ecosystems, metals are naturally found at low concentrations, and are distributed among the different compartments of the aquatic ecosystem, such as organisms, water, or sediments (Squadrone et al., 2018; Haghshenas et al., 2020). Brown algae have been reported to be often severely affected by heavy metal pollution (Alahverdi and Savabieasfahani, 2012).

Macroalgae play a fundamental role in structuring benthic ecosystems and maintaining their ecological balance (Umanzor et al., 2019; Wernberg and Filbee-Dexter, 2019). Apart from their valuable ecosystemic services (Cheminée et al., 2013; Cuadros et al., 2013), they are part of the blue economy due to their industrial and biotechnological interest (Radmer, 1996; Bowles, 2007; Mazarrasa et al., 2013). Seaweeds have been widely used in environmental status assessments for more than 50 years (Bryan et al., 1980, 1985; Rainbow and Phillips, 1993). On one hand, macroalgal biodiversity and abundance have been used as general indicators of the health status of coastal areas, due to their tolerance or sensitivity to marine pollutants (Levine, 1984; Norton et al., 1996; Vasquez and Guerra, 1996; de Caralt et al., 2019). On the other hand, seaweeds can be good indicators of micropollution (Gopinath et al., 2011; Sekabira et al., 2011), and have been used to obtain information on the concentrations or availability of heavy metals in their surrounding environment (Melville and Pulkownik, 2006; Akcali and Kucuksezgin, 2011). There is an extensive scientific literature concerning macroalgae as marine organisms that may bioaccumulate metals and micropollutants up to levels many times higher than those found in the surrounding waters (i.e. Jones, 1922; Black and Mitchell, 1952; Farías et al., 2002; Varma et al., 2011; Gubelit et al., 2016). As one of the main primary producers in coastal systems, seaweeds may facilitate the entry of pollutants into aquatic food chains via a biomagnification process, which increases their relevance as bioindicators of the ecological status of aquatic ecosystems (Conti et al., 2007; Conti and Finoia, 2010).

In recent years, Morocco has experienced a tremendous population growth, concomitantly with a significant acceleration in urbanization and land use for industrial and agricultural purposes. All these human-derived processes have led to a huge increase in the discharge of a wide range of pollutants into coastal waters, causing deleterious effects on the various components of the aquatic environment, including macroalgae (Kaimoussi et al., 2001; Mouradi et al., 2014). The present study aims to characterize the heavy metal content and biomonitoring potential of *Ericaria selaginoides* (Linnaeus) Novoa and Guiry (2020) (Ochrophyta; synonym: *Cystoseira tamariscifolia*), a warm temperate brown seaweed widely distributed in the Eastern Atlantic and the Mediterranean Sea (Guiry and Guiry, 2020). Diverse species included in the basal genus *Cystoseira* (Orellana et al., 2019) meet with the pre-requisites proposed by Phillips (1980) for marine bioindicator organisms and are among the most used seaweeds in biomonitoring. Heavy metal pollution occasionally caused disappearance of these species from habitats (Sales et al., 2011; Thibaut et al., 2015; Celis-Plá et al., 2018). A previous study conducted by Boundir et al. (2019) in the same Moroccan coastal section investigated in the present work demonstrated that while populations of *E. selaginoides* exist in the less polluted areas; their physiology seems to be significantly affected by pollution. Moreover, in the highly polluted areas this brown seaweed tends to disappear (Boundir et al., 2019). Based upon these observations we hypothesized that individuals of *E. selaginoides* will present higher heavy metal content in polluted than less polluted or unpolluted areas. In the present contribution, we aim to test this hypothesis and we present heavy metal concentrations and variation of abiotic parameters in *E. selaginoides* collected across a pollution gradient in three different cities in the Atlantic coast of Morocco. Thus, our study also aimed to test the value of this brown seaweed as a bioindicator of coastal pollution.

Materials and Methods

Target species

The brown seaweed *Ericaria selaginoides* (Linnaeus) (Novoa and Guiry, 2020); Synonyms: *Cystoseira tamariscifolia* (Papenfuss, 1950) and *Carpodesmia tamariscifolia*, Orellana et al. (2019), is the main subject of this study, with extant populations in most of the sampling sites. According to Guiry and Guiry (2020), the geographic distribution of *E. selaginoides* includes the coasts of the Eastern Atlantic from Mauritania to The Netherlands, Ireland and the archipelagos of Azores, Canaries and Cabo Verde, as well as the coast of the Mediterranean Sea.

Sampling sites

The coastline in the study area is characterized by a succession of rocky outcrops and sandy beaches. The coordinates of the sampling sites along the Atlantic coast of Morocco are described in *Table 1*. The eight sampling stations are located near to or within the perimeter of three cities. They were selected as control sites or polluted sites as described in the following paragraph and as indicated in (*Table 1 and Fig. 1*):

- In Eljadida city two stations were designated: Sidi Bouzid beach was selected as a control area (S1) and Jorf Lasfar (S2) as a polluted one. Sidi Bouzid beach was selected as a control site as it has been awarded the eco-label “Blue Flag” starting from 2006 (site ID 7167) by The Mohammed VI Foundation for the Protection of the Environment (F.M.6, 2018), this distinction is renovated each year to beaches that fit the international standard norms of cleanliness. In contrast, the Jorf Lasfar area is characterized by the presence of multiple industrial units, including a phosphate production complex and a thermal power plant (Kaimoussi et al., 2001; Essedaoui and Sif, 2001; Ferssiwi et al., 2004).
- In Safi city three stations were designated: Beddouza (S3) was selected as a less polluted control site, as it is located 34 km North from the industrial city of Safi (Goumri et al., 2018). In contrast, Industrial Area (S4) and Phosphate Area (S5), were selected as polluted sites.
- Finally, in (3) Essaouira city three stations were selected: Moulay Bouzerktoun (S6) is located approximately 15 km north from the enclosure of the city and was selected as a control site since it is the less affected by anthropogenic impact, with just some tourism activities during the summer, than the other two sites. These were Bab Doukala (S7) and the Port (S8), which both receive domestic and some industrial wastewater.

Table 1. *Nearest cities and GPS coordinates of the sampling sites of E. selaginoides along the Atlantic coast of Morocco. Type indicates control (C) or polluted (P) stations*

City	ID	Type	Station	Coordinates
Eljadida	S1	C	Sidi Bouzid	33°13’52"N-8°33’17.89"W
	S2	P	Jorf Lasfar	33°08’15"N -8°36’57.28"W
Safi	S3	C	Beddouza	32°32’23.06"N-9°17’2.44"W
	S4	P	Industrial Area	32°17’8.29"N-9°14’50.09"W
	S5	P	Phosphate Area	32°09’53.18"N-9°16’20.83"W
Essaouira	S6	C	Moulay Bouzerktoun	31°38’3.49"N-9°40’31.99"W
	S7	P	Bad Doukala	31°30’56.22"N-9°46’10.89W
	S8	P	The Port	31°30’33.3"N-9°46’30.38W

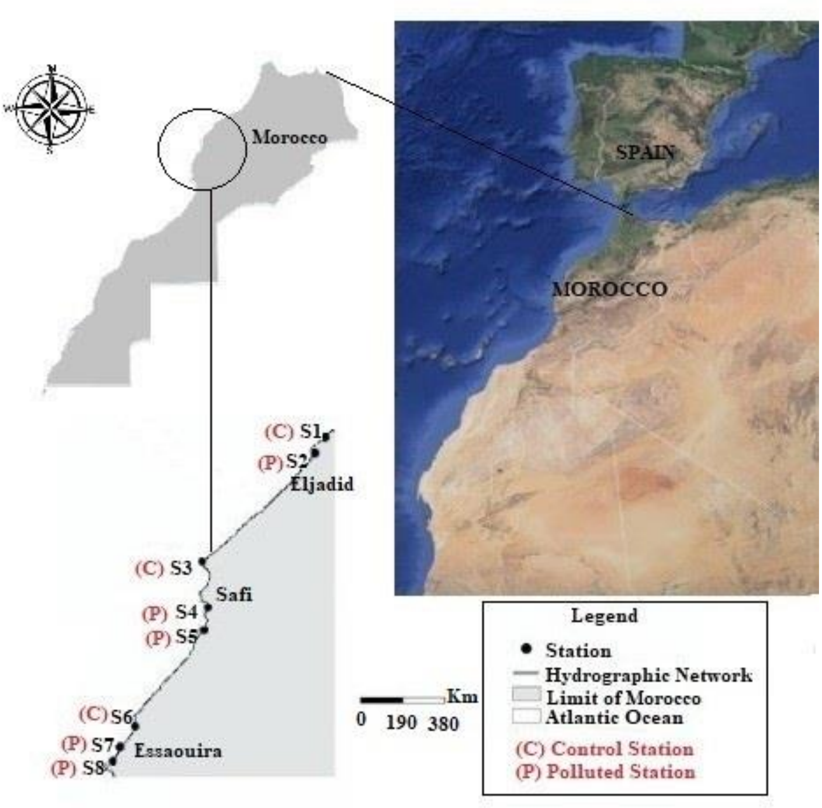

Figure 1. *Study area of E. selaginoides at the Atlantic coast of Morocco with sampling sites located near three cities*

Algal collection, seawater and sediment samples

10 whole individuals of *Ericaria selaginoides* were collected between autumn 2018 and summer 2019, four times at the beginning of each season at eight stations (*Fig. 1*) from hard substrata in the sublittoral zone between 0-5 m below Lowest Astronomical Tide, either by walking from the shoreline at low tide or with mask and fins. The collected specimens were washed in seawater, put in plastic bags and transported in a cooler to the laboratory for later metal analysis.

Hydrographical parameters measurements

The seawater abiotic parameters, Temperature (T), pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Salinity and Total Dissolved Solids (TDS), were measured seasonally in situ at each station, using a multi-parameter probe: Horiba, Model U-5000, Kyoto, Japan. Seawater samples for Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5) analysis were sampled with algae and measured according to AFNOR, 2001 (French Association of Normalization), NF T90-103 for BOD_5 and NF T90-101 for COD, while the Oxidizable Matter (OM) was calculated according to the following equation:

$$OM = \frac{(BOD5 \times 2) + COD}{3} \qquad \text{(Eq.1)}$$

Heavy metals determination in E. selaginoides, seawater and sediment

All samples were analyzed using a SHIMADZU AA-6300 Atomic Absorption Spectrophotometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). Triplicates of dried samples of *E. selaginoides* were digested with concentrated HNO_3. Eight metal elements namely Iron, Zinc, Manganese, Nickel, Copper, Lead, Chromium and Cadmium were determined following the methodology of Conti et al. (2010); atomization was carried out with flame (air/acetylene) as described in the mentioned protocol, heavy metal concentration was expressed as µg of metal per gram of algal dry weight ($\mu g.g^{-1}$ DW). Samples of seawater were collected in glass bottles with stabilizing agent (0.5 ml HNO_3) according to the protocol of Rodier (2009) and heavy metal concentration was expressed as $mg.L^{-1}$. Moreover, heavy metal concentrations in sediment samples were determined following the protocol of Tahiri et al. (2005) and concentration are expressed in $mg.g^{-1}$. To prevent contamination, all bottles were drown in the acid.

Statistical analysis

To determine the potential relationships between the heavy metals studied and the abiotic parameters at the different stations, Pearson correlation was applied, using SPSS version 25 (IBM, USA). One-way ANOVA, Principal Component Analysis (PCA) and Cluster Analysis were performed using Statistica 7 (StatSoft Inc., USA) to identify data clusters among the stations, seasons and heavy metals to visualize the distribution of heavy metals and the hydrographical parameters at the eight studied stations. The broken-stick criterion (Jackson, 1993) was followed to identify the number of significant components in the PCA analysis and was obtained with the software PAST (Hammer et al., 2001).

Results

Ericaria selaginoides distribution in the Eljadida-Essaouira section

Ericaria selaginoides was not regularly found at the different sampling stations along the study area during the 4 seasons of 2018/2019; moreover, it was entirely absent during the overall research period from the industrialized areas at Jorf Lasfar coast (S2) and at Safi coast (S4 and S5) as presented in *Fig. 2*.

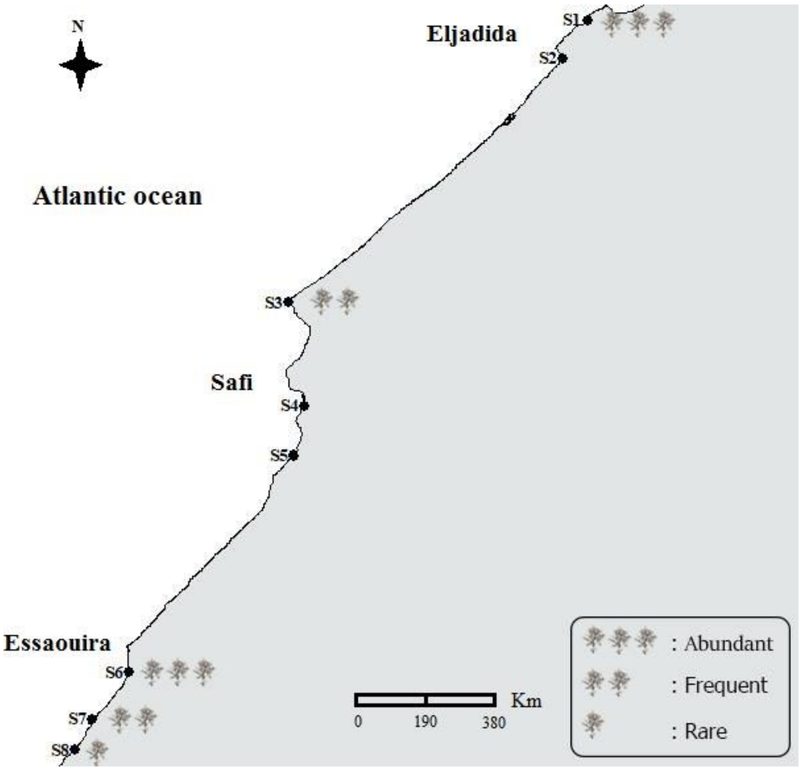

Figure 2. *Overall distribution and abundance of Ericaria selaginoides distribution from Eljadida-Essaouira section during 2019. At S2, S4 and S5 E. selaginoides is absent*

Hydrographical parameters

Temperature (T), pH, electrical conductivity (EC), dissolved oxygen (DO), salinity (Sal) and total dissolved solids (TDS)

The results of the hydrographical parameters, Temperature (T), pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Salinity (Sal) and Total Dissolved Solids (TDS) of seawater measured in situ during the study period are presented in *Table 2*. Temperature values varied between 14.30 °C during winter at S4 and 25.58 °C during summer at S5. pH values ranged between 6.38 at S3 and 9.00 at S1, and both minimum and maximum were reached during summer. The EC, DO, Sal and TDS values reached maxima of 58 mS.cm^{-1}, 9.40 mg.L^{-1}, 27.50 σt and 28.00 g.L^{-1} at the same station S1 during summer, and in the case of TDS only during autumn. The minimum of EC was 23.30 mS.cm^{-1} at S4 during autumn and the minimum of DO 4.20 mg.L^{-1} at S5 during winter. Minima of Sal and TDS were around 15.00 σt and 13.90 g.L^{-1}, respectively, and both minima were reached during autumn at the same station S4 of Safi city (*Table 2*).

Biological oxygen demand (BOD$_5$), chemical oxygen demand (COD) and oxydable matter (OM)

The maximum value of BOD$_5$ was recorded at S3 in summer with 417.34 mg.L^{-1}, while the minimum was reached at S5 with only 3.00 mg.L^{-1} during the same summer season. Furthermore, values of COD ranged from 806.40 mg.L^{-1} at S5 in summer to 135.00 mg.L^{-1} at S1 in winter. OM results reached a maximum of 474.18 mg.L^{-1} at S3 in summer and a minimum of 70.00 mg.L^{-1} at S7 in autumn (*Table 3*).

Table 2. *Spatial variation of abiotic parameters in situ at the different stations during 2019. EC: Electrical conductivity, DO: Dissolved Oxygen, Sal: Salinity, TDS: Total Dissolved solids*

Station	Season	T (°C)	pH	EC (mS.cm^{-1})	DO mg/L	Sal (σt)	TDS (g.L^{-1})
S1	Autumn	19.80±2.96	8.40±0.35	55.00±3.01	7.15±0.80	26.00±1.62	28.00±1.25
	Winter	16.20±3.08	8.10±0.57	54.30±4.89	8.20±1.12	25.60±3.04	27.80±4.02
	Spring	19.40±3.28	8.20±0.69	49.60±4.69	8.44±1.65	23.00±2.87	25.40±3.39
	Summer	24.50±3.29	9.00±0.74	58.00±5.43	9.40±1.71	27.50±3.38	25.40±3.26
S2	Autumn	18.10±1.75	7.40±0.11	45.00±3.77	6.30±0.60	19.40±2.54	17.20±2.54
	Winter	15.50±1.86	7.30±0.61	52.00±3.36	5.24±0.99	23.10±2.28	21.40±2.06
	Spring	18.60±1.16	7.20±0.63	45.00±3.56	5.22±1.05	19.20±2.38	17.90±2.13
	Summer	20.40±1.36	7.10±0.58	53.00±0.25	6.53±0.63	25.20±0.45	23.40±0.45
S3	Autumn	19.60±1.70	8.60±0.88	53.10±0.47	7.60±0.49	24.20±0.38	22.30±0.63
	Winter	17.30±1.72	8.10±0.78	53.50±13.23	7.88±1.08	24.50±4.13	22.50±3.93
	Spring	20.80±2.90	8.40±0.78	53.60±13.79	8.22±1.75	24.00±4.15	22.30±4.15
	Summer	21.87±2.74	6.38±0.67	54.40±11.52	8.90±1.70	25.00±3.91	23.80±4.06
S4	Autumn	19.30±2.74	7.90±0.49	23.30±7.32	6.00±0.56	15.00±2.84	13.90±2.67
	Winter	14.30±2.84	8.10±0.48	30.50±8.52	4.50±0.35	18.00±3.38	16.00±3.37
	Spring	19.20±2.53	7.70±0.41	35.40±7.46	5.00±0.47	16.00±3.74	14.00±3.68
	Summer	21.86±2.50	6.82±0.46	43.40±4.23	5.50±0.48	22.40±1.27	20.50±1.15
S5	Autumn	16.50±3.17	7.80±0.49	53.00±0.86	5.00±0.33	24.50±0.63	22.40±0.50
	Winter	15.30±2.95	7.80±0.50	53.40±2.55	4.20±1.34	25.70±1.80	23.50±1.48
	Spring	18.80±2.74	8.00±0.46	53.10±3.55	4.63±1.38	25.30±2.31	23.10±2.32
	Summer	23.58±2.80	6.74±0.69	51.20±3.14	5.00±1.16	24.10±1.97	22.30±2.09
S6	Autumn	18.60±1.44	7.20±0.53	47.00±1.42	7.64±0.19	21.10±1.18	19.70±1.33
	Winter	16.00±1.46	7.50±0.43	44.00±0.89	7.50±0.44	19.50±2.36	17.20±2.38
	Spring	17.9±1.05	8.60±0.45	43.20±1.77	7.85±0.47	19.10±2.13	17.30±2.10
	Summer	20.00±1.01	8.00±0.39	45.00±1.77	8.00±0.43	22.00±1.19	20.10±1.12
S7	Autumn	18.80±2.04	7.60±0.40	45.50±2.05	6.85±0.23	25.00±1.79	23.00±1.84
	Winter	17.20±2.09	8.70±0.33	41.00±4.09	7.21±0.36	23.00±2.31	21.50±2.51
	Spring	18.20±2.55	8.10±0.20	43.10±3.96	7.10±0.51	24.50±2.23	22.60±2.38
	Summer	22.60±2.53	7.90±0.28	40.20±3.31	7.50±0.69	20.40±1.40	18.30±1.54
S8	Autumn	18.20±2.86	7.90±0.25	32.30±2.71	6.50±0.44	18.50±1.25	16.30±1.19
	Winter	15.50±3.26	8.40±0.05	39.00±2.78	6.20±0.49	19.90±1.42	17.50±1.35
	Spring	18.80±2.33	8.50±0.04	34.00±0.75	5.60±0.60	16.80±0.10	14.20±0.70
	Summer	23.45±2.00	8.50±0.06	32.50±0.33	6.80±0.45	17.00±0.25	15.60±0.61

Heavy metals

Heavy metals in E. selaginoides

The mean concentrations of heavy metals in *E. selaginoides* showed a decrease in the following order: Fe > Zn > Mn > Cu > Ni > Pb > Cr > Cd (*Table 4*). The highest concentrations of Fe, Zn and Mn were recorded at the Sidi Bouzid station (S1) of Eljadida city, while Cu, Ni, Pb, Cr and Cd peaked at the Port station of Essaouira city (S8) (*Table 4*). According to the Kruskal-Wallis H test (non-parametric one-way ANOVA) for internal heavy metal concentration in *E. selaginoides* differed significantly ($p < 0.05$) among the groups for all metals analyzed (*Appendix Table 8*).

Table 3. *Spatial variation of Biological Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD) mean concentrations in seawater along the studied stations in the Moroccan Atlantic coast during 2018/2019*

Station	Season	BOD_5 ($mg \cdot L^{-1}$)	COD ($mg \cdot L^{-1}$)	OM ($mg \cdot L^{-1}$)
S1	Autumn	144.00±33.59	265.00±73.26	184.33±45.37
	Winter	165.00±36.65	135.00±74.70	155.00±44.12
	Spring	186.00±38.01	265.00±66.28	212.33±36.49
	Summer	294.94±39.55	487.87±56.32	359.25±37.68
S2	Autumn	123.00±42.92	362.00±78.97	202.66±53.64
	Winter	147.00±36.17	564.00±89.33	286.00±49.90
	Spring	232.00±27.77	623.00±117.20	362.33±53.62
	Summer	311.34±41.06	739.56±107.78	454.08±54.96
S3	Autumn	176.00±55.24	316.00±72.47	222.66±58.95
	Winter	243.00±80.23	256.00±76.67	247.33±58.64
	Spring	365.00±88.79	412.00±41.53	380.66±60.09
	Summer	417.34±83.38	587.87±27.62	474.18±60.94
S4	Autumn	56.00±31.84	562.00±72.98	224.66±42.54
	Winter	123.00±37.09	461.00±92.38	235.66±54.46
	Spring	86.00±42.90	522.00±90.38	231.33±57.76
	Summer	203.32±44.61	794.88±90.10	400.50±58.19
S5	Autumn	26.00±6.47	364.00±92.67	138.66±27.96
	Winter	17.00±36.60	497.00±130.90	177.00±23.68
	Spring	33.00±35.24	562.00±142.33	209.33±25.73
	Summer	3.00±49.48	806.40±137.97	270.80±35.66
S6	Autumn	162.00±28.80	171.00±54.22	165.00±32.78
	Winter	95.00±48.03	289.00±51.87	159.66±49.26
	Spring	235.63±51.24	422.35±53.71	297.87±48.92
	Summer	173.00±34.41	364.00±65.51	236.66±35.80
S7	Autumn	14.00±14.82	182.00±79.06	70.00±35.59
	Winter	56.00±16.42	388.00±38.01	166.66±14.19
	Spring	52.00±19.01	498.00±20.82	200.66±15.20
	Summer	86.32±19.40	533.23±44.25	235.29±22.83
S8	Autumn	6.64±1.89	561.48±61.01	191.58±20.87
	Winter	9.23±1.96	465.79±82.62	161.41±28.66
	Spring	10.47±2.59	362.41±141.42	127.78±48.87
	Summer	15.66±1.89	645.25±61.01	225.52±20.87

Heavy metals in sediment and seawater

All mean concentrations of heavy metals in sediment revealed significantly higher values at sites S4 and S5, the polluted stations of the industrial and phosphate zones at Safi city, than at other sites. The average concentrations of Zn, Cu, Pb and Cr do not exceed the standards of the French norm for sediments (FNHMIS, 2000) in all the studied stations. However, the Cd concentration at S5 exceeded the norm threshold value (3.97>2.4 $mg.g^{-1}$) (*Table 5*). However, all mean concentrations of heavy metals in seawater were under the Moroccan Norm (FAO 2006). Concentrations of Cu, Pb, Cr and

Cd in seawater were the highest concentrations of toxic metals recorded in the two polluted stations near to Safi coast S4 and S5. At the industrial discharges S4 with 1.18±0.09 µg.L^{-1} for Cd, 3.26±0.83 µg.L^{-1} for Pb, 0.25±0.04 µg.L^{-1} for Cu and 1.12±0.15 µg.L^{-1} for Cr. The phosphate discharge area S5 with a concentration of 1.10±0.08 µg.L^{-1} for Cd, 1.63±0.22 µg.L^{-1} for Pb, 0.75±0.13 µg.L^{-1} for Cu and 0.92±0.05 µg.L^{-1} for Cr (*Table 6*). Furthermore, Fe, Mn and Ni were not detectable in seawater using the spectrophotometer.

Table 4. *Mean concentration (Mean ± SD) in (µg.g^{-1} DW) of Fe, Zn, Mn, Ni, Cu, Pb, Cr and Cd in E. selaginoides collected from the Atlantic coast of Morocco during 2018/2019*

Station	Season	Fe	Zn	Mn	Ni	Cu	Pb	Cr	Cd
S1	Autumn	1668.67±2.31	46.65±0.38	20.58±0.64	1.33±0.04	0.22±0.15	2.30±0.08	0.12±0.03	0.10±0.02
	Winter	1201.67±77.36	36.43±1.95	**25.82±2.99**	2.45±0.39	3.77±0.27	1.23±0.02	0.32±0.06	0.23±0.02
	Spring	**1718.67±24.85**	37.82±1.47	16.74±0.67	1.60±0.04	0.38±0.04	1.36±0.12	0.39±0.08	0.35±0.05
	Summer	1056.67±5.13	**49.16±0.32**	20.47±0.34	1.24±0.03	0.16±0.05	0.49±0.03	0.15±0.02	0.11±0.03
S3	Autumn	446.33±30.99	11.46±0.61	6.20±0.17	1.42±0.27	4.04±0.19	2.48±0.03	0.12±0.03	0.20±0.02
	Winter	504.67±16.65	26.88±0.36	16.10±0.42	3.01±0.32	2.60±0.68	2.12±0.06	0.24±0.04	0.43±0.05
	Spring	449.67±10.50	19.50±0.47	15.08±0.12	2.35±0.20	0.44±0.05	1.26±0.04	0.25±0.06	0.13±0.02
	Summer	445.67±5.13	27.67±1.61	17.38±0.09	2.60±0.30	1.83±0.05	1.24±0.07	0.32±0.06	0.22±0.06
S6	Autumn	751.67±27.61	12.83±1.34	7.14±0.62	2.56±0.25	2.55±0.39	1.12±0.09	0.23±0.02	0.48±0.06
	Winter	457.67±15.50	9.15±0.08	3.50±0.08	1.53±0.08	4.54±0.34	2.18±0.07	0.61±0.08	0.60±0.03
	Spring	675.00±6.24	46.47±1.38	14.21±0.25	1.52±0.27	4.09±0.02	1.11±0.03	0.25±0.06	0.23±0.06
	Summer	878.67±10.50	36.44±0.85	12.37±0.23	1.64±0.07	6.10±0.00	1.09±0.06	0.14±0.06	0.10±0.02
S7	Autumn	164.00±24.25	19.15±1.22	11.96±1.19	4.74±0.34	4.87±0.48	3.10±0.15	**2.88±0.91**	1.22±0.04
	Winter	243.33±11.37	7.96±0.37	3.60±0.46	4.37±0.34	5.68±0.44	1.30±0.20	0.85±0.23	1.24±0.08
	Spring	256.67±11.68	35.85±0.75	4.74±0.55	5.61±0.43	7.55±0.38	2.20±0.07	1.94±0.61	1.82±0.13
	Summer	263.00±8.54	37.08±0.74	14.81±0.56	4.74±0.20	8.13±0.32	2.20±0.02	1.83±0.69	1.74±0.28
S8	Autumn	181.67±2.52	18.28±2.92	7.09±0.52	4.61±0.15	4.99±0.56	**4.09±0.09**	2.73±0.64	2.45±0.36
	Winter	244.00±38.00	19.22±0.22	11.54±1.88	**5.44±0.42**	5.32±0.99	1.92±0.56	1.30±0.17	**2.75±0.44**
	Spring	248.00±9.54	31.53±1.21	11.22±0.23	4.54±0.14	**11.14±0.87**	2.20±0.08	2.22±0.19	2.55±0.37
	Summer	275.00±24.25	47.15±1.89	14.01±0.88	5.43±0.42	10.19±0.23	1.64±0.28	2.60±0.34	1.49±0.33

NB: At S2, S4 and S5 E. Selaginoides is absent

Table 5. *Mean concentrations of heavy metals in sediment (Mean ± SD) of Fe, Zn, Mn, Ni, Cu, Pb, Cr and Cd expressed in mg·g^{-1} during 2018/2019*

Station	Fe	Zn	Mn	Ni	Cu	Pb	Cr	Cd
S1	2965±23.30	123±0.32	51±1.65	-	9.12±0.53	-	-	2.15±0.05
S2	1695±23.00	56±32	23±2.03	-	10.75±0.65	-	-	2.22±0.22
S3	3231±15.32	-	16±0.26	-	8.19±0.25	-	-	2.18±0.05
S4	2563±12.03	173.62±2.35	62±0.45	-	29.10±0.58	49.5±0.33	14.22±0.55	3.77±0.04
S5	2361±6.32	148.1±1.22	21±0.32	-	15.06±0.62	21.27±0.51	12.32±0.87	3.97±0.02
S6	1563±3.26	64±0.32	45±1.38	-	10.25±0.56	-	-	1.55±0.23
S7	1233±5.32	146±1.31	22±0.56	-	13.45±0.75	-	-	2.32±0.55
S8	865±3.12	166±0.22	36±0.55	-	14.24±0.65	-	-	1.66±0.65
French Norm (FNHMIS, 2000)	-	552	-	74	90	200	180	2.4

Table 6. *Mean concentrations of heavy metals in seawater (Mean ± SD) of Fe, Zn, Mn, Ni, Cu, Pb, Cr and Cd expressed in µg.L^{-1} during 2018-2019*

Station	Fe	Zn	Mn	Ni	Cu	Pb	Cr	Cd
S1	-	1.90±0.56	-	-	-	-	-	-
S2	-	2.55±0.96	-	-	0.17±0.14	0.88±0.41	0.11±0.08	0.14±0.02
S3	-	-	-	-	0.15±0.12	-	-	0.10±0.01
S4	-	1.82±0.33	-	-	0.25±0.04	3.26±0.83	1.12±0.15	1.18±0.09
S5	-	1.93±0.18	-	-	0.75±0.13	1.63±0.22	0.92±0.05	1.10±0.08
S6	-	1.70±0.44	-	-	-	1.17±0.38	-	-
S7	-	0.88±0.23	-	-	0.19±0.05	0.68±0.23	0.08±0.01	0.12±0.07
S8	-	0.56±0.22	-	-	-	0.57±0.15	0.06±0.01	0.11±0.03
Moroccan Norm. (FAO, 2006)		500		500	500	500	2000	200

Statistical sampling sites grouping

Pearson correlation results (*Fig. 3*) were supported by the PCA ordination (*Figs. 4, 5*) and the dendrogram (*Fig. 6*). Indeed, the Principal Component Analysis (PCA) performed on the eight heavy metals (Cd, Pb, Zn, Cu, Fe, Ni, Cr and Mn) and the physico-chemical parameters (T, pH, EC, DO, Salinity, TDS, BOD, COD and OM) under study showed two principal components that have been extracted by covering 60% of the cumulative variance (*Appendix Tables 9, 10*).

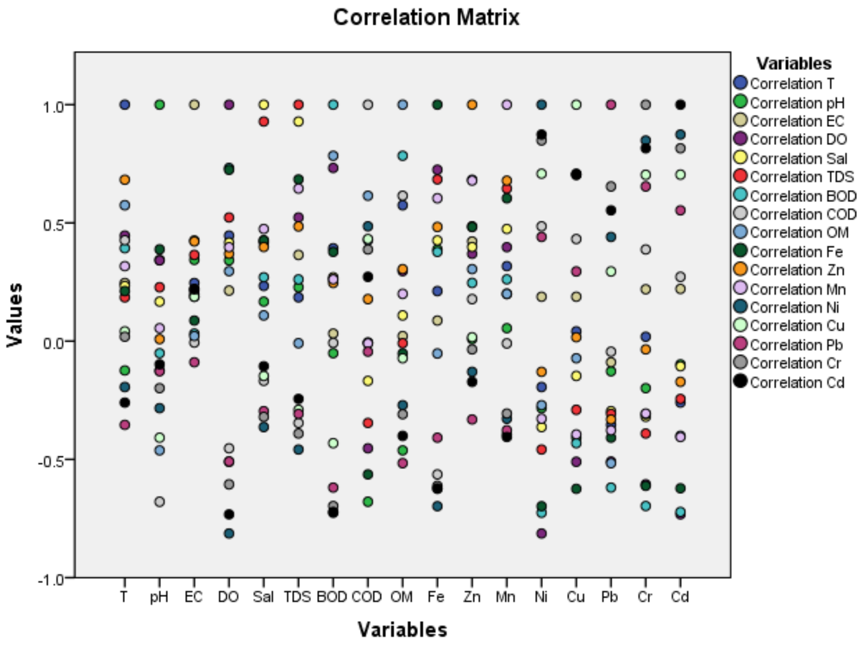

Figure 3. *Pearson correlation for all the studied hydrographical parameters and heavy metals in E. selaginoides*

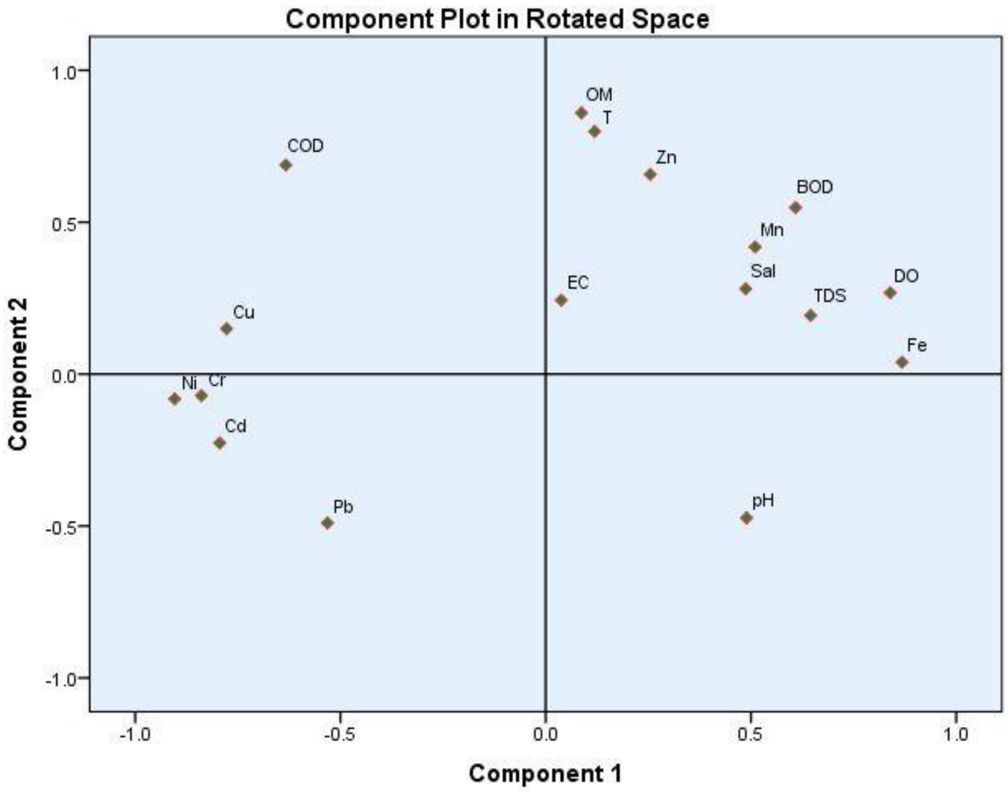

Figure 4. *PCA Analysis regrouping all the studied hydrographical parameters and heavy metals in E. selaginoides*

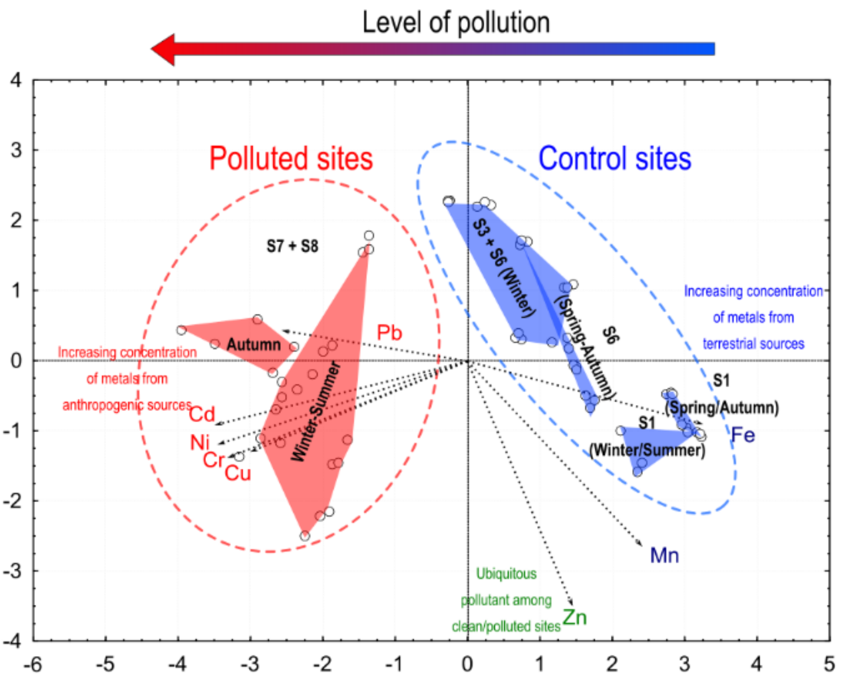

Figure 5. *Component plot representing PC1 and PC2 in rotated space for the eight studied heavy metals in E. selaginoides from the Atlantic coast of Morocco*

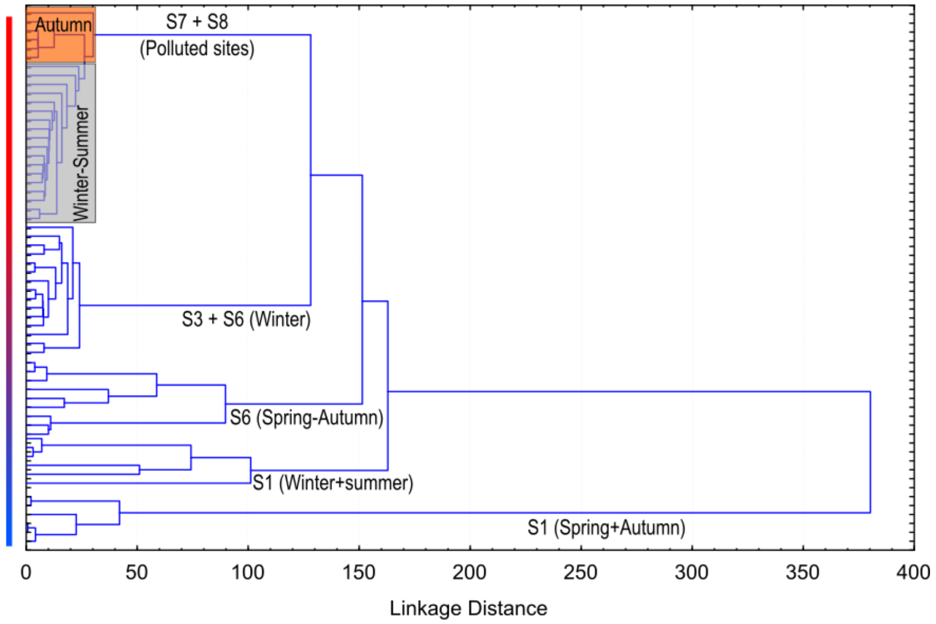

Figure 6. *Dendrogram using average linkage (between groups) obtained by hierarchical clustering analysis for the eight heavy metals studied in E. selaginoides from the Atlantic coast of Morocco*

Eigenvalue percentages of PC1 and PC2 were above broken-stick random forest model analysis, which confirmed that both principal components were suitable for interpretation (*Appendix Fig. 7*). Based on PCA results, PC1 explained a lower percentage of total variance than PC2 (38.286% and 59.208%, respectively). Fe and Mn were the dominant metals increasing along PC1, component 1 with values of 0.868 and 0.510, respectively, while Ni, Cu, Pb, Cr and Cd were the dominant metals decreasing along this component with values around -0.904, -0.777, -0.532, -0.839 and -0.794 respectively. However, Zn was the only dominant metal increasing along PC2, component 2 with a value of 0.657. These metals were the most informative heavy metals to identify the environmental quality of a site based on the bioindicator role of *E. selaginoides*, given their higher weight on the first component (*Appendix Table 9*). In contrast, the hydrographical parameters of DO, TDS, BOD were the dominant ones increasing along PC1 with 0.839, 0.645 and 0.609, respectively; while COD was the only decreasing one with -0.633. Moreover, T, COD and OM were the dominant parameters increasing along PC2, represented with values around 0.799, 0.689 and 0.860, respectively.

Clustering analysis allowed us to represent data clusters in the PCA plot by means of convex hulls (*Fig. 5*). The statistical significance among groups was confirmed by K-W ANOVA from the metals with higher factor loadings in each case (*Appendix Table 8*). The metals PCA and clustering analysis also allowed to group sampling stations along PC1, which represented a pollution gradient (*Figs. 5, 6*). Polluted sites (S7, S8) were located towards negative values of PC1, due to the presence of higher concentrations of Cd, Pb, Cu, Ni and Cr. On the other hand, control sites were placed at positive PC1 values, given their lower concentrations of heavy metals from polluted sites and their higher content in Fe and Mn (*Figs. 5, 6*). For this second analysis, only PC1 was suitable for interpretation, as the eigenvalue percentage of PC2 was below the values generated by the broken-stick random forest model (*Appendix Fig. 7*).

Discussion

The comparison of the present heavy metals results with those previously studied by other authors revealed that iron predominates at all sites (Caliceti et al., 2002; Akcali and Kucuksezgin, 2011) (*Tables 4, 5*). The concentrations of Cadmium (2.75 $\mu g.g^{-1}$ DW) were lowest among the 8 heavy metals analyzed in *E. selaginoides*, but that value was higher than that reported by Akcali and Kucuksezgin (2011) from the Aegean Sea, Turkey (0.18 $\mu g.g^{-1}$); Schintu et al. (2010) from Sardinia, Italy (1.72 $\mu g.g^{-1}$); Calisti et al. (2002) from Venice lagoon, Italy (0.2 $\mu g.g^{-1}$); Al-Masri et al. (2003) from the Syrian Coast (0.1-0.5 $\mu g.g^{-1}$) and Conti et al. (2010) from Linosa island (1.07 $\mu g.g^{-1}$). In the case of Chromium concentrations, Calisti et al. (2002) and Conti et al. (2010) detected lower values in Venice lagoon and Linosa island (Italy) (1.5 and 0.32 $\mu g.g^{-1}$ DW, respectively) than that recorded in the present study (2.9 $\mu g.g^{-1}$ DW). The same result was found for nickel concentrations by Calisti et al. (2002) (2.7 µg g-1 DW) which is lower than that found in this study (5.24 $\mu g.g^{-1}$ DW). With respect to lead concentration, the values found in the present study (4.43 $\mu g.g^{-1}$ DW) were higher than those found by Akcali and Kucuksezgin (2011) from the Aegean Sea, Turkey (0.003 $\mu g.g^{-1}$ DW) and Al-Masri et al. (2003) from the Syrian Coast (1.31 $\mu g.g^{-1}$ DW), and lower than those found by Conti et al. (2010) and Schintu et al. (2010) from Italian coastlines (4.78 and 10.3 $\mu g.g^{-1}$, respectively) (*Table 7*).

Table 7. *Comparison of heavy metals concentrations ($\mu g.g^{-1}$ DW) in diverse species of the genus Cystoseira complex from other coastal locations*

Location	References	Fe	Zn	Mn	Ni	Cu	Pb	Cr	Cd
Bulgarian black sea coast	Jordanova et al., 1999	-	-	10-100	-	-	-	-	-
Venice lagoon, Italy	Caliceti et al., 2002	609	88	-	2.7	21	5.6	1.5	0.2
Syrian Coast	Al-Masri et al., 2003	-	14.37	-	-	7.21	1.31	-	0.1-0.5
Linosa Island, Sicily	Conti et al., 2010	-	26.2	-	-	6.78	4.78	0.32	1.07
Sardinia, Italy	Schintu et al., 2010	-	52.4	-	-	1.80	10.3	-	1.72
Aegean Sea, Turkey	Akcali and Kucuksezgin., 2011	271.42	51.25	-	-	6.00	0.003	-	0.18
Atlantic coast of Morocco	Present study. 2019	1718.67	49.16	25.82	5.61	11.14	4.09	2.88	2.75

The PCA ordination method allowed us to identify a first group represented by Fe, Zn and Mn, being the elements with the highest abundance in *E. selaginoides*, and a second group including the rest of them at lower concentrations (*Table 2, Fig. 2*). The classification obtained in our study strongly agreed with the sources for each element: Fe and Mn are commonly related to terrestrial inputs whereas Pb, Cu, Zn and Cd gave evidence of anthropogenic contamination (Harris et al., 1998). In general, brown algae are one of the frequently and firmly affected algae among the algal source (Kaviarasan et al., 2018). We found that elements from the first group were more abundant in the most pristine site (S1, Eljadida control station), and they come from terrestrial runoff in the case of Fe and Mn. However, the presence of high concentrations of Zn at that control site suggest potential inputs from some industrial activities inland that contribute to its presence (FAO, 2019).

Seasonality had a significant effect in the Fe content of *E. selaginoides* in S1 (Spring> Autumn> Winter> Summer), being consistent with the clustering and the PCA results, demonstrating that seasonal differences were also significant at a lower clustering level. Furthermore, in S3, Fe concentrations were similar throughout the year, whereas in S6 there was a significant increase towards summer (S6 spring to autumn as shown in the PCA and the dendrogram) and minimum values in winter (*Figs. 5, 6*), which were similar to that of S3, which explain why S6-winter was in same cluster than S3. Irrespective of the season, Fe concentrations were always significantly higher in S1 than in the rest of sites, followed by S6, S3 and then the polluted sites. In the case of Mn, such differences were more progressive and there was more overlapping among the groups. Concentrations of Ni, Cr and Cd were significantly greater at the polluted sites. Nevertheless, S3 at some seasons presented some similar concentrations than in polluted areas, which in the PCA was represented by its location closer to zero values in the first component (*Fig. 5*). This would indicate that S3 could be considered as the "less clean" from the control sites. Therefore, it is possible to say that a "pollution gradient" may also be found among the three control sites. Besides, the increase in Pb in autumn would explain why polluted sites in that season lie in a different cluster than the rest of the year. That is because it was the only element for which that value was significantly higher than in spring-winter. For the other heavy metals, seasonal differences were not marked and neither the differences between stations S7 and S8.

E. selaginoides seemed to take up higher concentrations of these heavy metals, which are used inside physiological processes like Na, Ca, Mg and Fe, whereas lower concentrations of those which do not participate in these processes, for example Cd and Pb (Malea, 1994). Copper is essential for organisms' life and its toxicity is connected with the concentrations of other essential elements. The main sources of pollution in this area in the Atlantic coast of Morocco are the two-phosphate chemical complex (OCP) of Jorf lasfar (20 km south of El Jadida) and the cannery fish factory at Safi city (5 km south of Safi) (Boundir et al., 2019). The concentrations of the eight (8) heavy metals in the tissue of *E. selaginoides* were variable depending on the sampled stations and seasons. Given the toxicity of heavy metals, it is important to know the source and what happens to them in the environment.

For a long time in Morocco, seaweeds have remained absent from the debate on the impact and effects of anthropogenic activities in the marine biodiversity. Heavy metals in the Moroccan waters have been studied more recently than other chemical parameters. Research focused on water and sediment compartments, as well as some coastal marine areas (FAO, 2019). Worldwide, the use of algae (including brown seaweeds such as *Cystoseira* complex) in biomonitoring has been well established. Metal content and accumulation in seaweed is recognized as a suitable bioindicator for assessing the degree of contamination in marine ecosystems (Alahverdi Savabieasfahani, 2012). Nevertheless, until now there were no studies focusing on the bioaccumulation of these heavy metals in macroalgae, especially *Cystoseira* complex species from the Atlantic coast of Morocco.

In general, studies on the levels and distribution of contaminants, including heavy metals, in Morocco have focused on urban and industrial areas. As a result, the actual ground levels of water, sediment and biota, may not be exactly known, which can also distort the interpretation of the data. Heavy metals entering the aquatic environment come from natural and anthropogenic sources (Ghorab, 2018). Their entry can be the result of either direct discharges into marine ecosystems and fresh waters or indirect pathways such as dry and wet dumps and agricultural runoff. In addition, the mining activities are responsible for significant contributions of heavy metals to the Moroccan coastlines. For most heavy metals, anthropogenic emissions are equal to or greater than natural emissions. The combustion of leaded gasoline in cars, for example, is responsible for the wide spread of lead around the world (FAO, 2019).

In the next future, seaweeds will have to adapt to the new environmental conditions in the projected scenarios derived from global change and the increase of local stressors (Viejo et al., 2011; Bellard et al., 2012) or, conversely, to suffer local extinction, as it has been documented with increasing frequency in the scientific literature (e.g. Lima et al., 2007; Wernberg et al., 2011; Scherner et al., 2013; Thibaut et al., 2015; Mineur et al., 2015; Valdazo et al., 2017). The ecosystem services provided by brown macroalgal forests, such in the case of *Cystoseira* sensu lato, seems to be affected by diverse environmental and anthropogenic factors (Mineur et al., 2015). As demonstrated by Cheminée et al. (2013) the nursery value of *Cystoseira* complex forest changes not only as function of the depth gradient, but more importantly by the height of the plant canopy; comparatively, *E. selaginoides* forest hosted richer and three-fold more abundant juvenile fish assemblages. Therefore, the loss of *E. selaginoides* biomass and/or its complete extinction in some of the studied localities along the Atlantic Moroccan coasts are probably affecting negatively the richness and abundance of the local juvenile fish populations.

Conclusion

The reduction in biomass and eventually the extinction of *E. selaginoides* populations from the studied sites along the Atlantic coasts of Morocco seems to be related to the increasing pressure of anthropogenic activities, mainly leading towards high levels of heavy metal pollution. *E. selaginoides* accumulate heavy metals differently in the different localities studied and hence, can be used to monitor heavy metals levels in seawaters. Moreover, we suggest using it as a model for coastal pollution studies in Morocco and worldwide. The highest concentrations of Fe, Zn and Mn were recorded at Sidi Bouzid station (S1) of Eljadida city, while those of Cu, Ni, Pb, Cr and Cd were detected at the Port station of Essaouira city (S8). The concentrations of Chemical Oxygen Demand were significantly higher at the polluted station S5, while Dissolved Oxygen and Biological Oxygen Demand were lower. To rationally manage and control marine pollution in the Atlantic coast of Morocco, it is necessary to study everything related to the inputs, distribution and destiny of contaminants, including land-based heavy metals that flow into aquatic ecosystems. It is essential to address their effects on seaweed biodiversity, especially in Safi city where the growth of phosphate industry is accelerating. In conclusion, the loss of brown seaweed populations in the studied locations seems to be affecting the biodiversity richness and abundance of marine life; this biological effect needs further studies in the future. Moreover, the analysis of heavy metals in *E. selaginoides* gives a reasonable indication of water environmental quality at discrete points along the study area in the Atlantic coast of Morocco, providing a comparative methodology for monitoring purposes in subsequent years or with other coastal areas that could be implemented through the implementation of coastal biomonitoring programs.

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APPENDIX

Table 8. *Kruskal-Wallis H test (non-parametric one-way ANOVA) for internal heavy metal concentration in E. selaginoides. Combination of sites and seasons was used as categorical grouping variable in the analysis*

	Fe	**Zn**	**Mn**	**Cu**	**Ni**	**Pb**	**Cr**	**Cd**
Total N	60	60	60	60	60	60	60	60
H	57.41	57.52	58.05	57.77	56.1	55.11	56.07	57.76
df	19	19	19	19	19	19	19	19
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 9. *Factor loadings of the variables analysed in the PCA. Rotated Component Matrix, Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in three iterations*

Parameter	**Component**	
	1	**2**
T	0.119	0.799
pH	0.489	-0.473
EC	0.038	0.243
DO	0.839	0.268
Sal	0.487	0.281
TDS	0.645	0.193
BOD	0.609	0.548
COD	-0.633	0.689
OM	0.087	0.860
Fe	0.868	0.039
Zn	0.255	0.657
Mn	0.510	0.418
Ni	-0.904	-0.082
Cu	-0.777	0.149
Pb	-0.532	-0.490
Cr	-0.839	-0.071
Cd	-0.794	-0.227

Table 10. *Percentage of total variance explained by the first two components of the PCA analysis*

Component	**Initial Eigenvalues**			**Extraction Sums of Squared Loadings**			**Rotation Sums of Squared Loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	6.937	40.807	40.807	6.937	40.807	40.807	6.509	38.286	38.286
2	3.128	18.401	59.208	3.128	18.401	59.208	3.557	20.922	59.208
Extraction Method: Principal Component Analysis.									

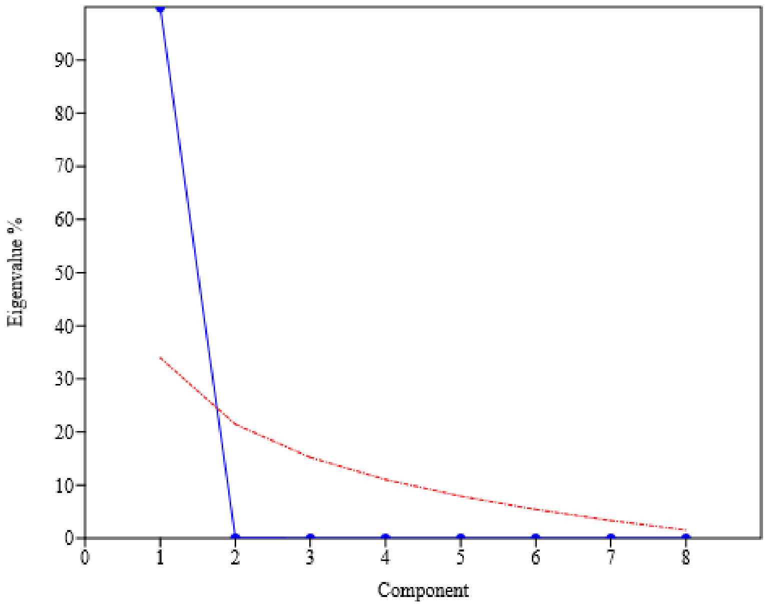

Figure 7. *Scree plot of PCA analysis of heavy metals in E. selaginoides, representing ordination eigenvalues for each component (blue line) and broken-stick random-forest model (red dashed line)*