FIRST ASSESSMENT OF POLLUTION IMPACT AT ESSAOUIRA COAST (MOROCCO) USING BIOTIC AND ABIOTIC PARAMETERS AND THE RED ALGAE *ELLISOLANDIA ELONGATA* AS POTENTIAL BIOINDICATOR OF ORGANIC POLLUTION

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Abstract. Environmental pollutants might significantly affect the ecological integrity of coastal waters. Biological indicators like seaweeds have been used globally to assess water pollution. In the present work, the seaweed *Ellisolandia elongata* was used to evaluate the pollution status in coastal waters around Essaouira city. Three sites were chosen: One as reference station (S1) and two polluted ones (S2 and S3). Seaweed biodiversity, physiologic parameters of *E. elongata*, as well as, abiotic parameters were studied. Results showed that at the polluted stations, seaweed biodiversity was significantly lower than in S1. However, the concentrations of Total Suspended Solids, Electrical Conductivity, Biological Oxygen Demand, Chemical Oxygen Demand, Ammonium, and Orthophosphates at S2 were significantly higher than at S1. Metal content of *E. elongata* remained below detection limit at all stations except for Zn and Cu at S2 considered the most polluted station. With respect to the physiologic parameters, Proline, Glycine Betaine and Polyphenol contents at S2 were above, whereas, Chlorophyll a content and axis length were below those determined at the reference station. From the results it can be concluded that seaweed *E. elongata* could be a good indicator to determine organic pollution in marine ecosystems.
Keywords: *seaweed, organic pollution, physicochemical parameters, Ellisolandia elongata physiology, biomonitoring*

Introduction

Boarding to the Atlantic Ocean and the Mediterranean Sea, the Moroccan coastlines are exposed to various types of anthropogenic stress. This environment is constantly exposed to human influence through domestic and industrial waste waters, which often introduce high quantities of pollutants without pre-treatment. In addition, the habitats undergo physical destruction which produces a significant impact on the coastal

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environment and lead to significant ecological deterioration (Islam and Tanaka, 2004; Moore et al., 2004; Gu et al., 2007; Amin et al., 2009; Er-Raioui et al., 2012; Hong et al., 2020), causing serious changes in marine organisms, including macrofauna, seagrasses, algae and others (Sivadas et al., 2010; Sabri et al., 2017; Boundir et al., 2019). In fact, algae are the basis of many marine food webs. Their composition fluctuates depending on several conditions, such as temperature, light, salinity, pH, and nutrients (Huertas et al., 2011). According to literature, it is known that the amount of macronutrient of coastal areas are the main factors which control the structural composition of algae communities (McGlathery et al., 2007; Leterme et al., 2014; Nassar et al., 2015). Celis-Plá et al. (2014) demonstrated that Nitrate, ammonium and orthophosphate are essential macronutrients for algae growth. However, these nutrients can have a serious negative impact on algal communities if they reach high concentrations near urban areas (Guerra-García and Koonjul, 2005; Nassar et al., 2015). In addition, urbanization and industrialization which increases due to population expansion is often coupled to a release of heavy metals into coastal waters which are considered among the most serious contaminants of aquatic ecosystems due to their high potential to enter and accumulate in food chains (Erdoğrul and Erbilir, 2007).

However, only limited published information on organic and inorganic pollutants is available for the Moroccan coast, and in particular for its Atlantic section (Kaimoussi et al., 2002; Mouradi et al., 2014; Rezzoum et al., 2016). At certain concentrations, these compounds could have an impact on seaweeds, causing disappearance of some species or declining or shifting biodiversity (DÍez et al., 1999; Nassar et al., 2015; El-adl et al., 2017; Sabri et al., 2017). Many authors used seaweeds to evaluate the degree of pollution, as they are considered to be excellent indicators of environmental change (Harley et al., 2006; De Faveri et al., 2010; Benkdad et al., 2011; Reis et al., 2014; Shams El-Din et al., 2014; García-Seoane et al., 2018). When seaweeds are exposed to stress (e.g. heavy metals or pathogens) they may respond in characteristic ways, such as decrease of axis length or chlorophyll content. Also overproduction of specific metabolic products, such as proline, glycine betaine (GB) or total phenolic compounds (TPC) may compensate the cellular imbalances caused by environmental stress (Häder et al., 1997; Coelho et al., 2000; Di Martino et al., 2003; Koivikko et al., 2005; Liang et al., 2013). Thus, these metabolites allow certain seaweeds to acclimatize to some degree and to ensure survival (Fatma et al., 2007; Lamalakshmi et al., 2017). Previous studies already reported that algal size and the content of molecules like chlorophyll, TPC, GB and proline may be used as bioindicators to monitor air pollution (Anbazhagan et al., 1988; Agbaire, 2016; Khairallah et al., 2018; Mukherjee et al., 2019), salt stress (Ali et al., 1999; Carillo et al., 2008; Amirjani, 2010; Fariduddin et al., 2013), drought stress (Si et al., 2015; Mirshad and Puthur, 2016; Mao et al., 2019) and freezing stress (Nomura et al., 1995), as well as inorganic (Alia and Saradhi, 1991; Amna et al., 2015; Varun et al., 2015; Saif and Khan, 2018; Boundir et al., 2019) and organic pollution (Abdel and Abdel, 2015; Bibi et al., 2019).

A precondition to use seaweeds as a bioindicator is that they are present in the areas under study. This is the case for the Corallinaceae which exist in almost every habitat type, even in polluted areas, within the photic (Adey and Macintyre, 1973; Steneck, 1986; DÍez et al., 1999). Likewise, *Ellisolandia elongata* which occurs along the Atlantic intertidal zone of Essaouira and its surroundings was chosen in the present study to investigate its applicability as a bioindicator. For doing so, the species and numbers of macroalgae present were determined at two polluted sites and compared to

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those at an unpolluted site during different seasons in 2017/2018. In addition, variations in the content of selected metabolic substances and heavy metals of the tissue of *Ellisolandia elongata* samples were determined and compared together with the seasonal differences of the physicochemical parameters of the waters at the study sites.

Materials and methods

Study area

The study area is located along the Atlantic Coast west of Central Morocco in the Essaouira Province, which is administered under the Marrakech-Safi Region government (Morocco). It extends longitudinally among 9°67' and 9°77' West and latitudinally among 31°51' and 31°63' North (*Fig. 1*).

The study area has a diverse and exceptional climate, shaped by its geographical location between the Sahara Desert and the Atlantic Ocean. The aridity increases from west to east. The western narrow coastal fringe around Essaouira is reached by cold currents coming from the Canary Islands, which generate a microclimate with a relatively homogeneous average temperature of about 16.7 °C throughout the year. As a consequence, the difference between average temperatures in the hottest and the coldest month is relatively small (Mwambo, 2007; Bazairi et al., 2010). Agriculture, fishing, craft, mining, trade, tourism, energy production and some other industrial and recreational activities are the most dominant economic sectors in Essaouira city.

Three stations, located on rocky substrates along the Essaouira coast, were chosen for this study (*Fig. 1*).

- Moulay Bouzerktoune Station (S1), located approximately 30 km away from the city (31°63'N-9°67'W), is considered as a reference station, affected by anthropogenic activities only during ephemeral tourism activities at the end of spring and during summer.
- Bab Doukkala Station (S2), the industrial district of Essaouira, located in the city, north of the port (31°51'N-9°76'W), allows us to assess the impact of the discharges of Essaouira city on marine macroalgae. Indeed, this urban coast receives significant domestic and some industrial wastewater, as well as limited solid discharges that are mainly composed of household waste.
- Port Station (S3), also located within the city (31°51'N-9°77'W), receives some industrial discharges from the port, as well as domestic discharges from the city districts.

Sampling

In order to study the phytobenthos biodiversity, seaweed samples were collected seasonally (once per season) during 2017 to 2018 in autumn, winter, spring and summer at low tide (0 to 1.5 m) along the Essaouira coast at the three stations (S1, S2, S3), following the quadrat method (Ar Gall and Connan, 2004). 30 quadrats were provided for each bathymetric level because of the irregularity of algal distribution in the foreshore. Samples were transferred to the laboratory and stored at 4 °C in a cooler. The species of the seaweeds were identified *in situ* and at the laboratory using a magnifying glass and a binocular microscope.

A large quantity of the Corallinaceae *Ellisolandia elongata* (J. Ellis and Solander) K. R. Hind and G.W. Saunders with basionym *Corallina elongata* (Guiry and Guiry, 2020)

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was collected in order to study its physiology and metal uptake. The algal material sampled was carefully washed with seawater, in order to omit epiphytes, sediments and associated fauna, and was cleaned thereafter with distilled water to remove excess salt. The algal material thus prepared was lyophilized, ground to powder in a dust-free atmosphere and using metal-free equipment to avoid contamination and finally stored at -20 °C for toxicological and physiological analysis.

In addition, water was sampled in glass bottles (500 ml) and transported to the lab in a cooler on ice (4 °C) to reduce adsorption, precipitation, contamination or evaporation (Rodier, 2009).

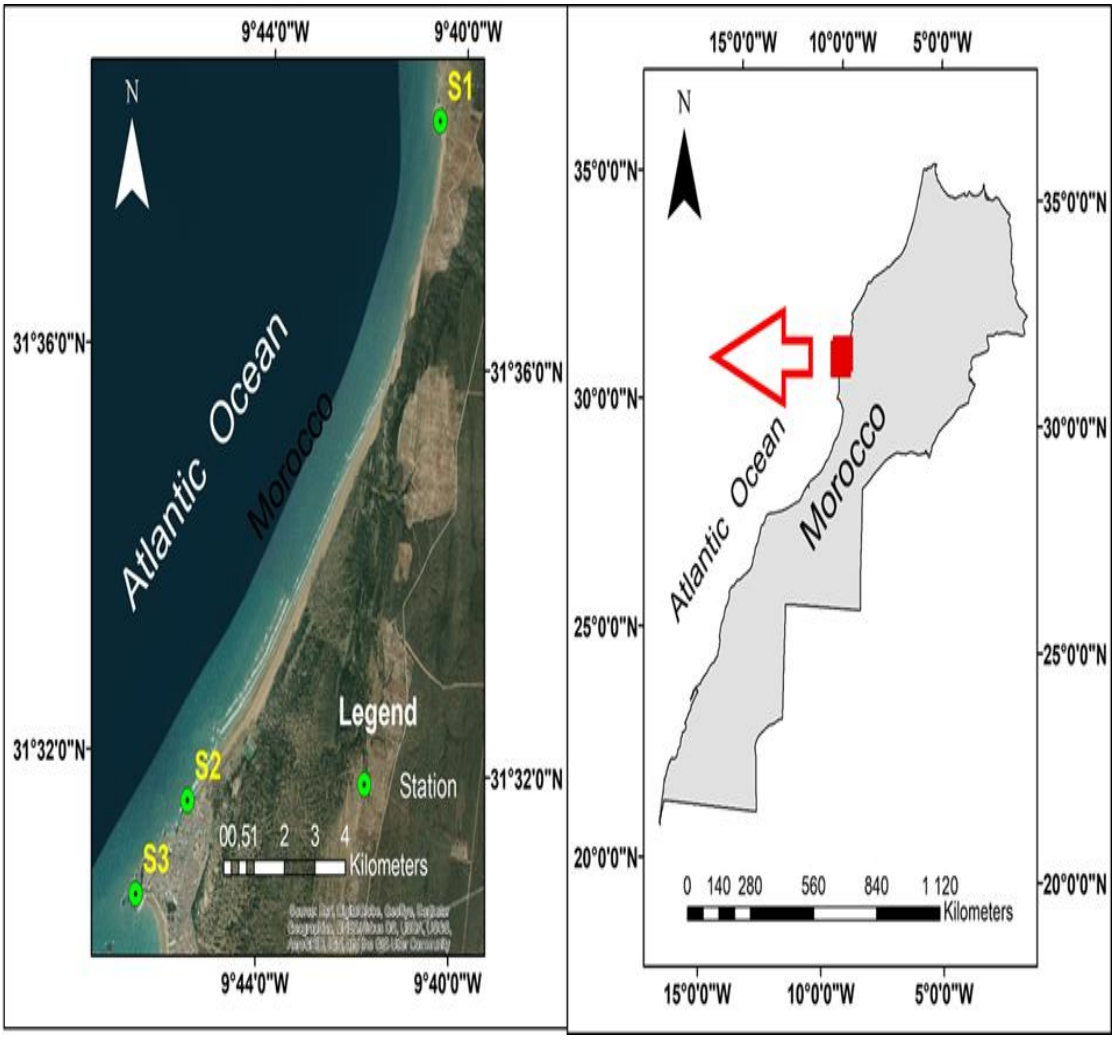

Figure 1. *Geographical position of the study sites along the coast of Essaouira. (S1) My Bouzerktoune: 31°63'N-9°67'W – (S2) Bab Doukkala: 31°51'N-9°76'W – (S3) The port: 31°51'N-9°77'W*

Physicochemical parameters

The water temperature, electrical conductivity (EC) and pH were determined on site with a multimeter instrument (Orion 4 Star), while dissolved oxygen (DO) was measured by a digital pocket oxymeter (HANNA-HI9829). All parameters were always measured *in situ* during collection of the samples at all sites and in all seasons.

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Concentrations of Total Suspended Solids (TSS) in water were determined by filtration of 200 ml of water sample through glass filters (0.45 µm mesh), which was subsequently dried and weighed. Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), and concentrations of Nitrogenous and Phosphorus compounds were measured according to AFNOR norms (Association Française de NORmalisation) reported in *Table 1*. The oxidizable matter concentrations were calculated using *Equation 1:*

$$\text{Oxidizable matter} = \frac{2 \times BOD_5 + COD}{3} \quad \text{(Eq.1)}$$

Table 1. *AFNOR norms of the studied parameters*

Parameters	AFNOR norms
BOD_5	NF T90-103
COD	NF T90-101
Total nitrogen	NF T90-061
Nitrate	NF T90-012
Ammonium	NF T90-015-2
Total phosphorus	NF T90-023
Orthophosphate	NF T90-022

Physiological parameters

Chlorophyll a

Chlorophyll a (Chla), was determined according to the method of Jeffrey and Humphrey (1975), based upon extraction of 10 g of fresh algae in 10 ml of acetone 90%. The tubes were incubated in the dark at 4 °C for 24 h and subsequently centrifuged at 19,000 g for 30 min. Absorbance of the resulting supernatant was measured at 662 and 644 nm, using a spectrophotometer (Boeco Germany S20).

Proline

The algal proline content was quantified following the method of Monneveux and Nemmar (1986). 100 mg of dried tissue was homogenized in 2 ml of methanol (40%) and then placed in a water bath at 85°C for 1 h. After cooling the samples were centrifuged at 4000 g for 10 min at 4 °C. 1 ml of supernatant was added to a mixture composed of 1 ml of acetic acid, 25 mg of ninhydrin and 1 ml of a solution composed of distilled water, glacial acetic acid and ortho-phosphoric acid of density 1.7 (120, 300, 80: v/v/v). The mixture was heated again for 30 min at 100 °C in a water bath and subsequently allowed to cool at room temperature, and then added to 5 ml of toluene. The upper phase was collected and dehydrated with a pinch of anhydrous Na_2SO_4. The absorbance was read at 520 nm.

Glycine betaine (GB)

The GB essay was performed according to the protocol of Grieve and Grattan (1983). 0.5 g DW of seaweed was mechanically shaken with 20 ml of deionized water at 25 °C for 48 h. After filtration, 0.5 ml of extract was mixed with 0.5 ml of 2N sulfuric

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acid. Then 0.2 ml of KI-I_2 reagent (containing 15.7 g iodine and 20 g KI in 100 ml) was added and shaken in ice cold water for 1 h. The tubes were stored at 0-4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was carefully drawn off and the pellet was dissolved in 9 ml of 1,2-dichloroethane (chilled at -10 °C). The absorbance was measured after 2 h at 365 nm.

Total phenolic compounds (TPC)

TPC were measured according to Silvia Taga et al. (1984). A quantity of 0.5 g of dried algae was extracted in 60:40 acidified methanol/water (0.3% HC1). Supernatant at 100 µl was mixed with 2 ml of Na_2CO_3 (2%). After 2 min 100 µl of Folin-Ciocalteu's phenol reagent (50%) were added and the mix was allowed to stand at room temperature for 30 min. The absorbance was then read at 750 nm within 2 h. The results were expressed as gallic acid equivalents (GAE), based upon measurement of gallic acid standards ranging in concentration from 10 mg mL^{-1} to 200 mg mL^{-1}.

Morphometry

The main axis length of seaweeds was determined using a non-destructive method by Murray et al. (2002), which does not require detaching the seaweed. The lengths of individual axes were measured *in situ* with a ruler.

Heavy metals

The method of Topcuoğlu et al. (2003) was adopted to quantify trace metals after digestion with hydrogen peroxide, sulfuric and nitric acids. If necessary, samples thus obtained were diluted depending on their metal concentrations to allow for analysis with an Atomic Absorption Spectrophotometer (AA-6300 SHIMADZU).

Statistical analysis

Morphometric measurements were conducted on 30 algal samples, while *in situ* measurements and laboratory analysis were established in triplicate and mean values and standard deviations were calculated. For statistical data analysis, Kruskal-Wallis and Wilcoxon tests were applied to analyze differences among the three sampling stations. Linear correlations between parameters were analyzed with the Pearson coefficient. The regression coefficient was calculated between concentrations of different compounds in wastewater, and Principal Component Analysis was used as a classification tool. The SPSS Statistics version 23 package (IBM, USA) was used for data analysis. All statistical hypothesis testing was based upon use of a probability value p of 0.05.

Results

Algal diversity

A total of 309 taxa was recorded at the three sampling stations located along the coastline of Essaouira city. The maximum of specific richness was found at S1 with 221 species, followed by S3 with 140 species and S2 with only 111 species (*Fig. 2*). Generally, most species belonged to the Rhodophyceae (68%), followed by the Chlorophyceae (17%) and the Phaephyceae (16%). The green algal species at S2 and

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S3 contributed 29% and 21%, respectively, while this percentage was lower at S1 (13%). On the other hand, the red and brown algae contributed 73% and 14%, respectively, at reference station S1 and where thus more dominant at this station than at the polluted stations S2 and S3. The dominant species at S1 were *Hypnea musciformis*, *Gelidium corneum*, *Carpodesmia tamariscifolia* and *Bifurcaria bifurcata*. The brown seaweeds were associated with epiphytic species (e.g. *Aglaothamnion tripinnatum*, *Polysiphonia atlantica*, *P. elongata*, *Lomentaria articulata*, *Plocamium raphelisianum*, *P. cartilagineum*). The S2 and S3 stations exhibited mostly common species, which were generally dominated by the Chlorophyceae (*Ulva* and *Codium* genera), by some epiphytic species (e.g. *Derbesia lamourouxii*, *D. tenuissima*, *cladophora polifera*, *Chaetomorpha aerea*) and by the red alga *Caulacanthus ustulatus*.

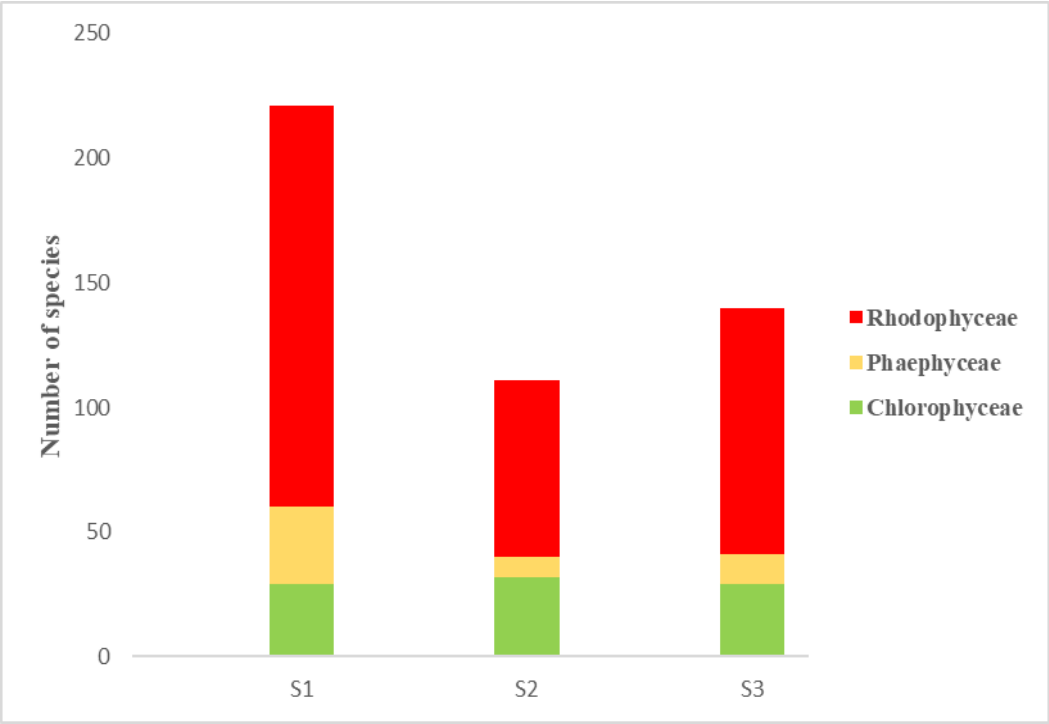

Figure 2. *Algal diversity in the studied areas*

Physicochemical parameters

The results of the physicochemical parameters of seawater measured *in situ* during the study period are presented in *Table 2*.

Temperature values vary between 15.5 °C during winter and 23.46 °C during summer all recorded at S3. For this parameter, statistical analyses did not show clearly a significant difference between the study sites, while the other parameters showed a significant difference between the control station and the polluted ones, especially between S1 and S2 ($p < 0.05$). pH and DO contents ranged from 7.2 to 8.7 and from 5.6 to 8 mg L^{-1}, respectively, and were significantly higher at S1 in comparison to the other stations (S2 and S3). In contrast, the highest values of EC were recorded at the two polluted stations. Their maxima reached 45500 µs cm^{-1} at S3 and 47000 µs cm^{-1} at S2, but only 39000 µs cm^{-1} at S1.

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Table 2. *Seasonal variation of temperature (°C), pH, electrical conductivity (µs cm^{-1}), and dissolved oxygen (mg L^{-1}) in the seawater at the three studied areas*

Season	Station	T	pH	EC	DO
Autumn	S1	18.6 ± 0.00^{AB}	7.9 ± 0.00^{A}	32500 ± 0.00^{A}	7.64 ± 0.00^{A}
	S2	18.8 ± 0.00^{A}	7.2 ± 0.00^{B}	45000 ± 0.00^{B}	6.50 ± 0.00^{B}
	S3	18.2 ± 0.00^{B}	7.6 ± 0.00^{AB}	40100 ± 0.00^{AB}	6.85 ± 0.00^{AB}
Winter	S1	16.0 ± 0.00^{AB}	8.7 ± 0.00^{A}	32000 ± 0.00^{A}	7.50 ± 0.00^{A}
	S2	17.2 ± 0.00^{A}	7.5 ± 0.00^{B}	43100 ± 0.00^{B}	6.20 ± 0.00^{B}
	S3	15.5 ± 0.00^{B}	8.4 ± 0.00^{AB}	34000 ± 0.00^{AB}	7.21 ± 0.00^{AB}
Spring	S1	17.9 ± 0.00^{A}	8.6 ± 0.00^{A}	39000 ± 0.00^{A}	7.85 ± 0.00^{A}
	S2	18.8 ± 0.00^{B}	8.1 ± 0.00^{B}	44000 ± 0.00^{B}	5.60 ± 0.00^{B}
	S3	18.2 ± 0.00^{AB}	8.5 ± 0.00^{AB}	41000 ± 0.00^{AB}	7.10 ± 0.00^{AB}
Summer	S1	20.0 ± 0.00^{A}	8.5 ± 0.00^{A}	32300 ± 0.00^{A}	8.00 ± 0.00^{A}
	S2	22.6 ± 0.00^{AB}	7.9 ± 0.00^{B}	47000 ± 0.00^{B}	6.80 ± 0.00^{B}
	S3	23.46 ± 0.00^{B}	8 ± 0.00^{AB}	45500 ± 0.00^{AB}	7.5 ± 0.00^{AB}

Different upper-case letters in the same column indicate differences between the studied stations for the four seasons ($p < 0.05$)

The spatial variation of TSS, BOD_5, COD, and OM in sea water are given in *Table 3*. The statistical analysis of these data revealed a significant difference between the control and the polluted stations, especially between S1 and S2 ($p < 0.05$).

Table 3. *Seasonal variation of total suspended solids, biological oxygen demand, chemical oxygen demand, and oxidizable matter (mg L^{-1}) in the seawater at the three studied areas*

Season	Station	TSS	BOD	COD	OM
Autumn	S1	42.00 ± 1.00^{A}	05.66 ± 0.57^{A}	13.00 ± 1.73^{A}	08.11 ± 0.83^{A}
	S2	55.66 ± 1.15^{B}	45.00 ± 5.00^{B}	83.00 ± 4.35^{B}	57.67 ± 4.58^{B}
	S3	53.33 ± 0.57^{AB}	32.66 ± 2.51^{AB}	59.00 ± 1.00^{AB}	41.44 ± 2.01^{AB}
Winter	S1	31.57 ± 0.51^{A}	03.66 ± 1.15^{A}	8.33 ± 2.08^{A}	05.22 ± 0.19^{A}
	S2	127.33 ± 0.57^{B}	31.33 ± 1.53^{B}	75.67 ± 3.51^{B}	46.11 ± 1.95^{B}
	S3	71.16 ± 0.29^{AB}	25.33 ± 2.52^{AB}	41.00 ± 1.73^{AB}	30.56 ± 1.71^{AB}
Spring	S1	26.41 ± 0.37^{A}	3.66 ± 0.58^{A}	34.00 ± 1.00^{A}	13.78 ± 0.69^{A}
	S2	86.26 ± 0.12^{B}	37.00 ± 1.73^{B}	70.67 ± 1.53^{B}	48.22 ± 0.84^{B}
	S3	66.66 ± 0.01^{AB}	30.33 ± 0.75^{AB}	36.33 ± 2.08^{AB}	32.33 ± 1.00^{AB}
Summer	S1	52.21 ± 0.03^{A}	6.33 ± 0.58^{A}	16.33 ± 1.53^{A}	09.67 ± 0.88^{A}
	S2	40.00 ± 0.01^{AB}	56.67 ± 1.53^{B}	94.67 ± 4.16^{B}	69.33 ± 2.31^{B}
	S3	33.77 ± 0.22^{B}	41.33 ± 1.53^{AB}	69.00 ± 2.00^{AB}	50.56 ± 1.68^{AB}

Different upper-case letters in the same column indicate differences between the studied stations for the four seasons ($p < 0.05$)

The TSS values varied between 26.41 and 127.33 mg L^{-1}. Particularly high levels were recorded at S2, especially during winter and spring, reaching 127.33 and 86.26 mg L^{-1}, respectively. Medium concentrations were recorded at S3 that varied between 33.77 and 71.16 mg L^{-1}, while the lowest concentrations were observed at S1

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(*Table 3)*. The highest BOD values were recorded at S2 (56.67 mg L^{-1}) and S3 (41.33 mg L^{-1}) both recorded during summer, while considerably lower values were recorded at S1 especially during winter (3.66 mg L^{-1}). Likewise, maximum COD values were recorded during summer at S2 (94.67 mg L^{-1}), and the lowest values were found at S1 (*Table 3*). The same was true for OM which exhibited a minimum at S1 of 5.22 mg L^{-1} and a maximum at S2 (69.33 mg L^{-1}) recorder during winter and summer, respectively (*Table 3*).

Concentrations of nutrients (TN, NH_4-N, NO_3-N, TP and PO_4-P) exhibited significant differences, Also, between control station S1 and the other polluted station S2 ($p < 0.05$) (*Table 4*).

Total nitrogen concentrations (TN) ranged from 6 mg L^{-1} at S1 to 12.97 mg L^{-1} at S2. The minimum value of ammonium was also recorded at S1 (0.12 mg L^{-1}) and the maximum at S2 (0.53 mg L^{-1}). However, elevated nitrate concentrations were found at S1 (Maximum: 4.41 mg L^{-1}) in comparison to the other stations (*Table 4*).

For phosphorus compounds, the lowest concentrations of PO_4-P were found at S1 in season autumn (0.02 mg L^{-1}), whereas the highest concentration was measured at S2 in spring (0.21 mg L^{-1}) (*Table 4*).

Table 4. *Seasonal variation of TN, NH_4-N, NO_3-N, TP and PO_4-P (mg L^{-1}) in the seawater at the three studied areas*

Season	Station	TN	NH_4-N	NO_3-N	TP	PO_4-P
Autumn	S1	6.25 ± 0.09^{A}	0.32 ± 0.01^{A}	3.53 ± 0.01^{A}	0.39 ± 0.01^{A}	0.02 ± 0.01^{A}
	S2	9.27 ± 0.04^{B}	0.53 ± 0.01^{B}	2.20 ± 0.01^{B}	0.52 ± 0.01^{B}	0.06 ± 0.01^{B}
	S3	8.06 ± 0.08^{AB}	0.50 ± 0^{AB}	2.69 ± 0.01^{AB}	0.42 ± 0.01^{AB}	0.03 ± 0.01^{AB}
Winter	S1	6.00 ± 0.04^{A}	0.06 ± 0^{A}	4.41 ± 0.02^{A}	0.38 ± 0.01^{A}	0.06 ± 0.01^{A}
	S2	12.97 ± 0.03^{B}	0.23 ± 0^{B}	2.85 ± 0.02^{B}	0.60 ± 0.01^{B}	0.12 ± 0.01^{B}
	S3	7.91 ± 0.05^{AB}	0.18 ± 0^{AB}	3.52 ± 0.01^{AB}	0.50 ± 0.01^{AB}	0.07 ± 0.01^{AB}
Spring	S1	8.77 ± 0.03^{A}	0.14 ± 0^{A}	2.09 ± 0.02^{A}	1.92 ± 0.07^{A}	0.13 ± 0.01^{A}
	S2	10.87 ± 0.13^{B}	0.23 ± 0^{B}	1.33 ± 0.02^{B}	7.20 ± 0.05^{B}	0.21 ± 0.01^{B}
	S3	8.96 ± 0.07^{AB}	0.17 ± 0^{AB}	1.40 ± 0.01^{AB}	2.90 ± 0.05^{AB}	0.14 ± 0.01^{AB}
Summer	S1	9.64 ± 0.10^{A}	0.12 ± 0^{A}	1.79 ± 0.01^{A}	5.20 ± 0.03^{A}	0.04 ± 0.01^{A}
	S2	11.98 ± 0.24^{B}	0.22 ± 0^{B}	1.69 ± 0.01^{B}	7.38 ± 0.02^{B}	0.06 ± 0.01^{B}
	S3	10.3 ± 0.14^{AB}	0.18 ± 0.01^{AB}	1.70 ± 0.01^{AB}	5.73 ± 0.05^{AB}	0.05 ± 0.01^{AB}

Different upper-case letters in the same column indicate differences between the studied stations for the four seasons ($p < 0.05$)

Heavy metals

Pb, Cr, Ni and Cd contents of *E. elongata* were always below the detection limit at the different sites during the four seasons. In contrast, Cu and Zn were detected and their concentrations differed significantly between the studied stations throughout the study period ($p < 0.05$) (*Table 5*). The highest values were always recorded at S2, with up to 2.64 μg g^{-1} DW and 0.99 μg g^{-1} DW of Zn and Cu, respectively.

Physiological parameters

Table 6 shows the physiological parameters of the seaweed *E. elongata*. The statistical analysis detected significant differences for all the parameters between the

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control station S1 and the station S2 ($p < 0.05$), except for TPC and axis length during winter.

Table 5. *Seasonal variation of zinc and copper (µg g^{-1} dry weight) in E. elongata at the three studied areas*

Season	Station	Zn	Cu
Autumn	S1	1.13 ± 0.003^{A}	0.04 ± 0.002^{A}
	S2	2.02 ± 0.00^{B}	0.12 ± 0.001^{B}
	S3	1.41 ± 0.001^{AB}	0.07 ± 0.00^{AB}
Winter	S1	1.31 ± 0.002^{A}	0.07 ± 0.002^{A}
	S2	2.64 ± 0.003^{B}	0.14 ± 0.003^{B}
	S3	1.50 ± 0^{AB}	0.13 ± 0.002^{AB}
Spring	S1	1.94 ± 0.002^{AB}	0.09 ± 0.001^{A}
	S2	2.03 ± 0.001^{A}	0.17 ± 0.001^{B}
	S3	1.13 ± 0.001^{B}	0.17 ± 0.002^{AB}
Summer	S1	0.96 ± 0.001^{A}	0.09 ± 0.00^{A}
	S2	1.79 ± 0.170^{B}	0.99 ± 0.001^{B}
	S3	1.31 ± 0.001^{AB}	0.13 ± 0.003^{AB}

Different upper-case letters in the same column indicate differences between the studied stations for the four seasons ($p < 0.05$)

Samples from station S1 exhibited both the highest content of chlorophyll a (Maximum: 433.68 µg g^{-1} FW during winter) and the longest thalli (maximum: 5.77 cm during summer), respectively. Lower values were found at S2, with 86.67 µg g^{-1} FW during spring and 1.53 cm during summer, respectively. In contrast, the highest values of proline and GB were recorded at S2 (54.72 mg g^{-1} DW and 869.39 µg g^{-1} DW, respectively), while their minimum was always recorded at S1 (*Table 6*). With the exception of spring TPC also always reached its minimum at S1 (absolute minimum: 477.17 µg g^{-1} DW). Seasonal maxima were always observed at S2 or S3 (absolute maxima: 2116.58 and 2794.83 µg g^{-1} DW, respectively).

Table 6. *Seasonal variation of Chla (µg g-1 fresh weight), proline (mg g-1 dry weight), GB and TPC (µg g-1 dry weight) and axis length (cm) of E. elongata at the three studied areas*

Season	Station	Chla	GB	Proline	TPC	Axis length
Autumn	S1	315.85 ± 0.44^{A}	291.16 ± 10.27^{A}	21.35 ± 0.47^{A}	477.17 ± 15.62^{A}	5.60 ± 0.26^{A}
	S2	182.83 ± 0.25^{B}	704.76 ± 06.23^{B}	54.72 ± 0.99^{B}	657.81 ± 21.29^{A}	3.60 ± 0.10^{B}
	S3	216.53 ± 0.42^{AB}	518.37 ± 08.16^{AB}	32.10 ± 0.72^{AB}	671.44 ± 15.62^{A}	4.57 ± 0.35^{AB}
Winter	S1	433.68 ± 0.80^{A}	303.40 ± 13.12^{A}	06.67 ± 0.13^{A}	1960.53 ± 35.91^{A}	5.47 ± 0.50^{A}
	S2	180.94 ± 0.19^{B}	855.78 ± 08.50^{B}	17.07 ± 0.26^{B}	2116.58 ± 46.86^{AB}	4.43 ± 1.04^{A}
	S3	232.92 ± 0.41^{AB}	629.93 ± 06.23^{AB}	10.09 ± 0.27^{AB}	2794.83 ± 56.31^{B}	4.03 ± 0.15^{A}
Spring	S1	254.04 ± 0.36^{A}	521.09 ± 06.23^{A}	14.08 ± 1.61^{A}	961.15 ± 27.05^{AB}	5.00 ± 0.50^{A}
	S2	86.67 ± 0.56^{B}	869.39 ± 08.16^{B}	39.77 ± 1.61^{B}	1428.09 ± 50.44^{A}	2.57 ± 0.12^{B}
	S3	189.20 ± 0.31^{AB}	606.80 ± 08.50^{AB}	23.94 ± 0.61^{AB}	576.01 ± 56.31^{B}	3.50 ± 0.50^{AB}
Summer	S1	328.75 ± 1.26^{A}	533.33 ± 10.27^{A}	12.79 ± 1.38^{A}	562.38 ± 30.68^{A}	5.77 ± 0.38^{A}
	S2	121.37 ± 0.40^{B}	858.50 ± 04.71^{B}	39.18 ± 1.38^{B}	749.83 ± 51.46^{AB}	1.53 ± 0.38^{B}
	S3	157.74 ± 0.56^{AB}	801.36 ± 04.71^{AB}	21.99 ± 0.69^{AB}	940.70 ± 30.68^{B}	2.60 ± 0.26^{AB}

Different upper-case letters in the same column indicate differences between the studied stations for the four seasons at the level ($p < 0.05$)

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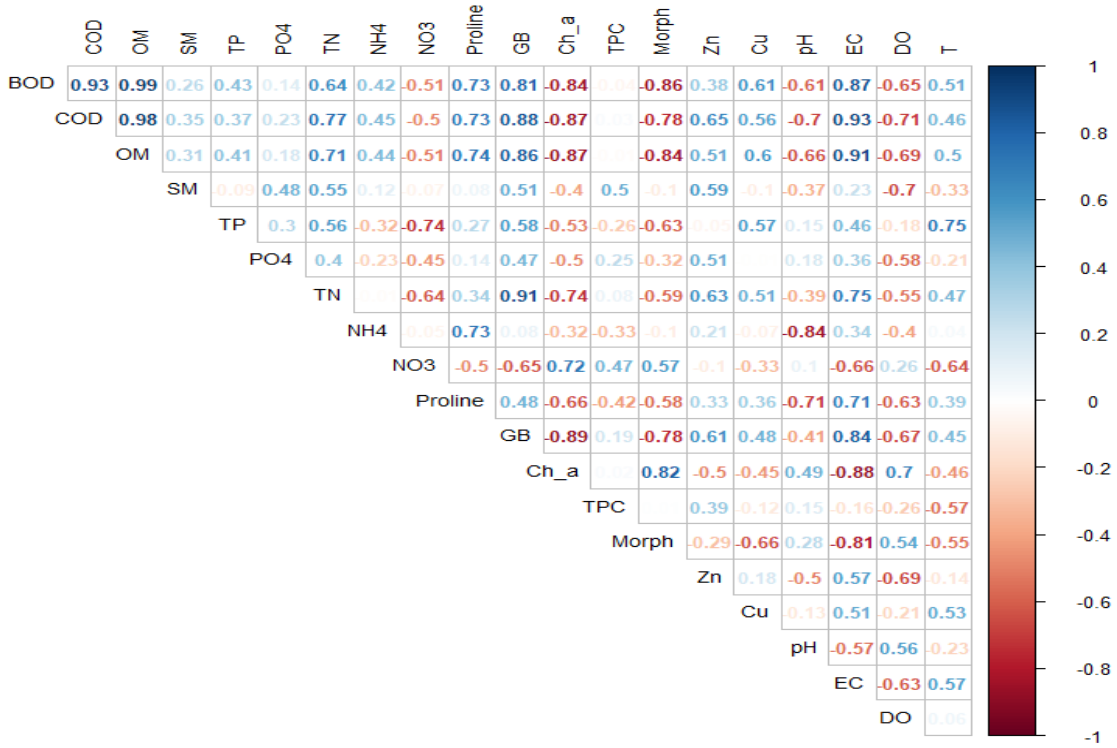
Statistical analysis

As shown on the Pearson correlation matrix (*Table 7*), parameters that reflect organic pollution (BOD, COD and OM) exhibit strong positive correlations with TN, GB and proline ($r > 0.7$), but negative correlations with Chla and morphometry ($r > -0.7$). However, the organic parameters show mainly weak relationships with the remaining parameters (e.g., TPC, PO_4).

Table 7. *Pearson correlation matrix between the parameters studied*



Principal Component Analysis (PCA) of the physiological parameters (Morphometry, Chlorophyll a, Proline, and Glycine betaine (GB) combined with the physico-chemical parameters (T, pH, EC, TSS, DO, BOD, COD, and OM) and heavy metal concentrations (Cu and Zn) allowed the identification of two principal components that together explain more than 75.72% of the overall variability of samples (*Table 8)*. Our PCA analysis allows to identify the most representative variables that distinguish the studied stations (*Fig. 3*). It demonstrates that BOD, COD, OM, Proline, GB, and EC are correlated positively with the PC1 (0.941; 0.975; 0.974; 0.775; 0.892; 0.934, respectively). In contrast, the algal axis length, Chla and DO are negatively correlated with the PC1 (0.834; 0.891; 0.779, respectively). TSS and Cu together correlate with PC2, while T and Zn together correlate along PC1. However, both heavy metals did not correlate particularly well with the algal physiological parameters (*Table 8*).

Discussion

Our study aimed for the first time to use physiological parameters of *Ellisolandia elongata* in combination with macroalgal biodiversity and physico-chemical

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parameters for the assessment of coastal pollution. Three stations were investigated for this purpose. S1 is the reference site, while S2 and S3 received domestic and industrial wastewaters, containing organic and inorganic pollutants. The impact of pollution was partly reflected by the algal biodiversity existing in the studied area. Likewise, we found at the reference station S1 which suffers only small anthropogenic impacts the highest biodiversity of seaweeds. Genera such as *Cystoseira*, *Bifurcaria* and *Saccorhiza* were present at this site, which typically indicate small anthropogenic impacts or non-affected areas (Munda, 1980; Riadi, 1998; DíEz et al., 1999; Arévalo et al., 2007; Ballesteros et al., 2007; Orfanidis et al., 2011; Biskup et al., 2014; Rubal et al., 2014; Celis-Plá et al., 2016; Boundir et al., 2019). In contrast, a dramatic decrease of diversity was observed in sites S2 and S3, located in the urban area close to places where domestic and some industrial waste waters are discharged. Several authors have proven the disappearance of mostly perennial seaweeds including the associated or epiphytic species due to pollution impact (Munda, 1982; Schramm, 1999; Sava et al., 2011). Our finding is in agreement with these earlier reports from other areas and it also generally supports the work of Chouikh et al. (2019) that was based on the assessment of polychaete distribution and conducted in the same study area. Indeed, many epiphytic algae inventoried at S1 (e.g. *Aglaothamnion tripinnatum, Polysiphonia atlantica, P. elongata, Lomentaria articulata, Plocamium raphelisianum, P. cartilagineum*) were not found at S2 and S3. In contrast, it has been noticed that species known to indicate pollution, like *Caulacanthus ustulatus*, *Codium tomentosum*, and other species belonging to the *Cladophora* and *Ulva* genera, were dominant at the last two stations. These findings are in accordance with literature (Jupp, 1976; DÍez et al., 1999; Godeh et al., 2010; Orfanidis et al., 2011). In order to support our conclusions an established indicator of pollution was calculated, which is the mean ratio of red algal and green algal species diversity in a given area (the so-called corrected R/C index). It is based on the fact that the number of Rhodophyceae is generally higher in less polluted areas, whereas Chlorophyceae are more diverse in polluted habitats, pollution is coupled to a decrease of the ratio (Sfriso et al., 2006; Orfanidis et al., 2011). At S1, the R/C ratio was always above 2 (on average 3.84), indicating a high percentage of Rhodophyceae, while at the stations S2 and S3 it never exceeded 0.91 and 1.37, respectively. Moreover, a stronger dominance of opportunistic species was observed at S2 (78%) and S3 (66%), as compared to S1 (42%). Again, similar observations at differently impacted sites have been reported from other coastal areas (Sfriso et al., 2009).

Nitrate, ammonium and orthophosphate are essential macronutrients for algal growth and stress management (Celis-Plá et al., 2014). However, excess concentrations can have a negative impact, as they can generate additional stress and thus inhibit growth (Guerra-García and Koonjul, 2005; Nassar et al., 2015). The macronutrients of coastal areas are often a main factor that drives changes in the structural composition of algal communities (McGlathery et al., 2007; Leterme et al., 2014; Nassar et al., 2015). Such nutrients are present in natural environments, but their concentration near urban areas is usually elevated (Nixon, 1995; Scavia and Bricker, 2006). In the present study, the physiology of *E. elongata* did not correlate significantly with most of the inorganic parameters. This indicates that the state of this seaweed is not really affected by the nutrient concentration at the study site varied between 6 and 12 and 0.3 and 7 for TN and TP mg L^{-1}, respectively, which has also been observed by some other authors (Schaffelke, 1999; Boundir et al., 2019).

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Table 8. *Loading vectors of physiological parameters and heavy metal concentrations in E. elongata and physicochemical parameters in the study areas along principal component axes 1 and 2*

Parameter	Component	
	1	2
BOD	0.941	0.136
COD	0.975	-0.011
OM	0.974	0.071
TSS	0.383	-0.724
Proline	0.775	0.135
GB	0.892	-0.091
Chla	-0.891	0.031
Morphometry	-0.834	-0.295
Zn	0.526	0.554
Cu	0.607	-0.540
pH	-0.658	0.229
EC	0.934	0.091
DO	-0.779	0.472
T	0.473	0.676
Variance (%)	41.85	33.86
Cumulative (%)	41.85	75.72

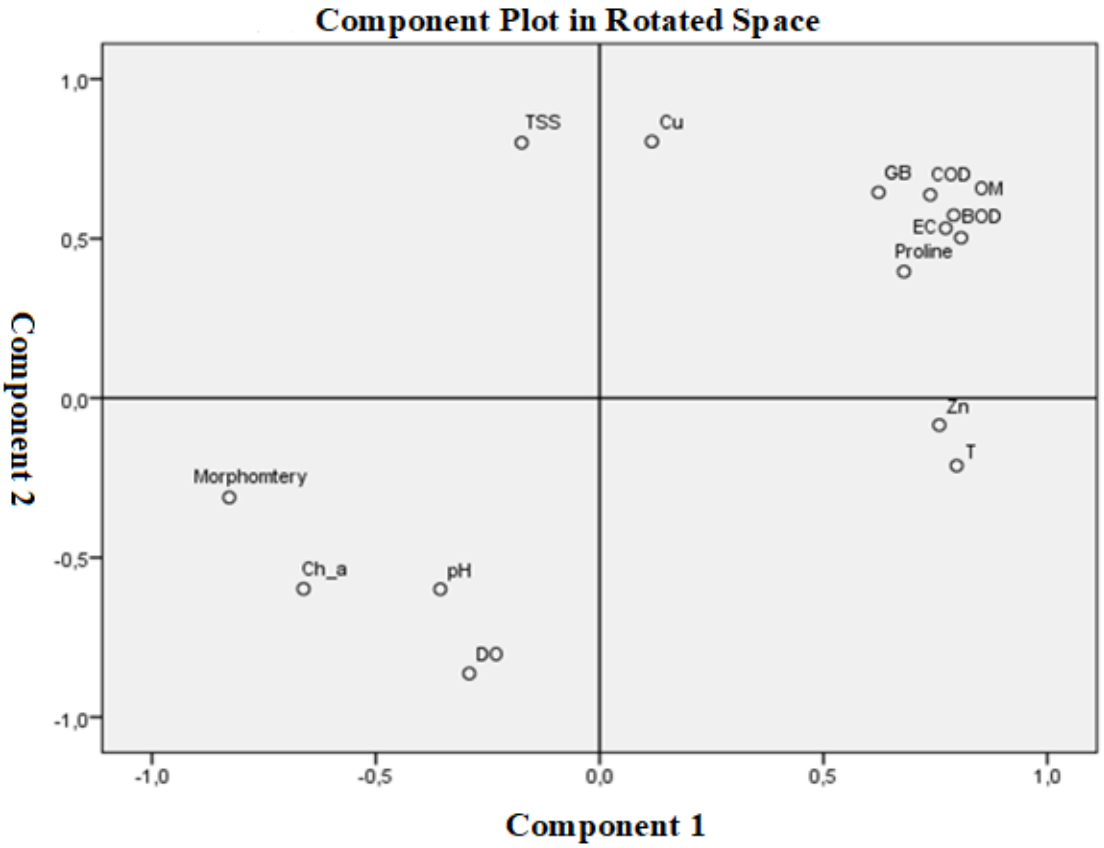

Figure 3. *Component plot in rotated space for the parameters studied. TSS: total suspended solids; T: temperature; EC: electrical conductivity; DO: dissolved oxygen; BOD: biological oxygen demand; COD: chemical oxygen demand; OM: oxidizable matter; Ch a: chlorophyll a; GB: glycine betaine; Zn: zinc; Cu: copper*

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A significant increase of tissue concentrations of GB and proline in *E. elongata* and a decrease of Chla content, DO and algal axis length was observed together with an increase in concentrations of EC, BOD, COD and OM at the polluted stations S2 and S3. These results of our PCA analysis are in accordance with our expectation of an impact of organic pollution on *E. elongata's* physiology. Overall, algal length, chla and DO values were higher in S1 than in S2 and S3, which shows that *E. elongata* was affected by pollution in the two last stations.

Previous studies have demonstrated that pollution stress causes a decrease of chlorophyll contents, reducing algal photosynthetic activities, and correspondingly a reduction of production of dissolved oxygen, which can be decreased if pollution comes along with increased input of organic matter and subsequent degradation processes (Sawyer and McCarty, 1978). Additionally, some authors have shown that the pollution affects also the algal size (Doblin and Clayton, 1995; Coelho et al., 2000; Morin, 2006). However, toxic heavy metals (Cd, Pb and Cr) were not detected in *E. elongata*. Moreover, the Zn and Cu concentrations observed are much lower than those reported by other authors in calcareous red seaweeds (Malea et al., 1994; Abdallah et al., 2006; Dadolahi-sohrab et al., 2011; Khaled et al., 2014) and cannot be expected to be toxic. Working in our study area, some authors already demonstrated that brown algae such as *Carpodesmia tamariscifolia* (as *Cystoseira tamariscifolia*) and *Saccorhiza polyschides* are significantly more contaminated with heavy metals at sites S2 and S3 than at site S1 (Cherifi et al., 2018; Boundir et al., 2019). However, certain studies have already shown that calcareous Rhodophyceae have a reduced capacity for biosorption of metals as compared to other algal groups (Jordanova et al., 1999; Benguedda-Rahal, 2012). Bioaccumulation of heavy metals may be affected by many factors, such as pH, the binding capacity of various sediment components and the specific affinity of species for heavy metals. This binding capacity is largely dependent on the nature and chemical composition of cell wall components. The latter might be a main reason for the findings at the study area, given that brown algae generally exhibit a greater ability to accumulate metals than red and green algae (Leonardi and Vasquez, 1999; Davis et al., 2000; Kaimoussi et al., 2002; Abdallah et al., 2006; Benkdad et al., 2011; Mouradi et al., 2014).

While the inorganic pollution parameters had no impact on the physiological parameters of *E. elongata* at the study sites there was a clear correlation with the parameters indicating organic pollution like BOD_5, COD, OM. The highest concentrations of BOD_5, COD, and OM were always recorded in the polluted areas S2 and S3 which were elevated by a factor 5-10 compared to the unpolluted site S1. That organic matter is an indicator for pollution by discharge of wastewater was shown before (Sawyer and McCarty, 1978; Broecker and Peng, 1983; El-Sonbati et al., 2012; Er-Raioui et al., 2012; El-zeiny et al., 2016). This does not explain, why and how the organic matter affected the seaweed. There are two possible explanations: the organic matter load included substances which were toxic or the organic matter enhanced heterotrophic degradation processes which affected the primary production. The latter can be ruled out because although > 50% of the COD was biologically degradable the parameters like temperature, pH, DO and conductivity did not differ significantly at all study sites and during all season. It is thus highly possible that toxic organic pollutants entered the coastal areas with the discharge of waste water. Because this kind of pollution would have otherwise remained undetected, our approach to use physiological responses of *E. elongata* as indicators of variable kinds of pollution was verified. This is

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in accordance with a growing interest in using seaweeds for the biomonitoring of different kinds of pollution (Pereira et al., 2009; García-Seoane et al., 2018, 2019; Roleda et al., 2019). A number of researchers identified that stressful conditions of seaweeds induce the decrease of chlorophyll contents, algal size and also proline and GB accumulations (*Table 9*). Emphasis should be put on the development of methods which allow to indentify the type of pollution too.

Table 9. *Main factors causing algal stress*

Stress factors	Algal species	References
Solar radiation	*Ellisolandia elongata*	Häder et al. (1997)
Thermal stress	*Ellisolandia elongata*	Nannini et al. (2015)
Acidification effect	*Lithophyllum incrustans*	Noisette et al. (2013)
Light and desiccation stress	*Ulva linza*	Guan et al. (2016)
Heavy metals	*Cystoseira tamariscifolia*	Boundir et al. (2019)
Organic pollution	*Ellisolandia elongata*	Current study

Conclusion

The present work evaluated the pollution degree in the Essaouira city coastal area (Morocco) by using, for the first time, the calcareous seaweed *Ellisolandia elongata* (Rhodophyta) as a bioindicator. Our results show that organic pollution is the main factor that affects the macroalgae diversity at the Essaouira coast. A dramatic decrease of seaweed biodiversity has been noticed in the Bab Doukkala (S2) and Port (S3) stations where urban wastewater is discharged without any treatment, causing pollution. Several species reflecting these polluted conditions have been identified in these two stations and not in the reference station of My Bouzerktoune (S1). The pollution impact is more evident at S2 than at S3. Physico-chemical parameters and inorganic pollution did not show a real impact on the algal biodiversity and the physiological state of *E. elongata*. Thus, this red alga could be used as a tool to monitor organic pollution in Morocco and might be applicable as a bioindicator also in other areas. Further studies should be undertaken on other macroalgae and on other stressful conditions in order to support these results.

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