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The use of seaweeds and abiotic parameters for the biomonitoring of Moroccan coastal areas: case study of Safi City

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ABSTRACT

The establishment of an effective monitoring and assessment system is an important basis for the rehabilitation and restoration of coastal marine aquatic ecosystems. The present work aims to apply, for the first time in Safi area (Morocco), seaweed biotic indices and the physiological parameters of a green seaweed *Ulva lactuca*, in order to assess the capacity of algal communities to reflect the quality of the aquatic ecosystem in the Atlantic coast of Safi. Additionally, some abiotic parameters were analyzed. We considered three stations for the present study (S1, S2 and S3). Four sampling campaigns were conducted during the year 2018, and 164 algal taxa were identified. The study of the algal biodiversity and physiological analysis (the content of proline and photosynthetic pigments) of a selected cosmopolitan alga reveals a state of stress and indicates instability in this macroalgal ecosystem. Indeed, the values of the specific diversity indices of Shannon (H') and equitability (E) show an increase in pollution at the polluted stations S2 and S3, the industrial and the phosphate station, respectively compared to the reference station S1 of Beddouza. The physico-chemical changes of the coastal waters observed in the impacted sites (pH, nitrogenous and phosphorus) could be due to the anthropogenic pollutants resulting from the intense urban and industrial activities that characterize this industrial city. This observation illustrates the interest of phytobenthos in the biomonitoring and bioassessment of coastal marine ecosystems.

Keywords: Atlantic coastline of Morocco; Biomonitoring; Physico-chemical parameters; Biotic indices; Macroalgal ecosystem

1. Introduction

The assessment of anthropogenic impact on coastal zones is crucial, especially in industrial and urban areas that introduce large amounts of pollutants into the marine environment. In Morocco, most wastewaters of factories are dumped into the ocean without any treatments. Consequently, significant disturbances and ecological degradation are induced. The biomonitoring is a valuable tool and receives increased use in assessing water pollution worldwide [1].

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It involves the use of different kind of indicators such as fish and benthic species. Many studies have shown that algae are a precise monitoring tool for quantifying and determining pollutants in aquatic ecosystems, especially heavy metals [2,3]. In Morocco some species have already been used to assess marine water quality such as *Carpodesmia tamariscifolia* [4] and *Laminaria saccorhiza* [5]; and more recently, the red alga *Ellisolandia elongate* [6]. This should enable Moroccan benthic algae to reflect the quality of coastal waters, acting as efficient bioindicators. Indeed, they can be used to characterize environmental conditions in a given place and for a specific period [7]. Safi City is among the industrial cities, which is the seat of significant urban and industrial activities developed on the coastal fringe. Ephemeral studies were done in this threatened coastal area and were limited in the use of one algal species [4]. This survey is a timely approach to assess the pollution level in Safi City based on: (i) the determination and the comparison, for the first time, algal biodiversity at 3 sites along the Safi coastline (control and polluted sites); (ii) the assessment of the quality of this coastal area using abiotic parameters; (iii) the study, for the first time in Morocco, the physiology of a cosmopolitan green seaweed *Ulva lactuca* widely used to monitor coastal areas with other species belonging to the same genus [8–10].

2. Materials and methods

2.1. Description of sampling stations

The sampling stations are located according to the following coordinates:

- S1, located at the beach of Beddouza 30 km north of the town (32°54′N-9°27′W). This station is not exposed to pollution sources from any industrial activities [11]. It is labeled blue flag according to Mohammed VI fondation for the protection of the environment [12]. Thus, this site is considered as reference station;
- S2, station located in the industrial district (32°28′ N-9°24′W), close to a sewage outfall for domestic and industrial activities in the agglomeration of Safi City [13]. These industries concerne mainly those of fish meal. The latter contains essential substances (e.g., carbon, nitrogen and minerals that are essential for the growth of microorganisms [14];
- S3, station located near a phosphogypsum discharge point that is a residual product resulting from the treatment of phosphates from the large phosphate complex of Safi City (32°18′N-9°26′W) (Fig. 1). This industry constitutes one of the major production platforms of phosphoric acid worldwide, and their discharges (mineral and chemical products) are released as liquid form or dumped as solid without prior treatment [11].

2.2. Abiotic parameters

At each sampling site, abiotic parameters (water temperature (TW), hydrogen potential (pH), electrical conductivity (EC), dissolved oxygen (DO)) were measured in situ using a portable device (Orion Star A329, USA.). Seawater samples were collected in plastic jars and placed in a cooler (at 4°C) and returned to the laboratory. Biological oxygen demand (BOD_5), chemical oxygen demand (COD), and concentrations of nitrogenous and phosphorus compounds were measured according to the Association Française de Normalisation (AFNOR) norms: T90-015-2 for ammonium [15], T90-061 for total nitrogen [16], NF T90-012 for nitrate [17], T90-022 for orthophosphate [18] and T90-023 for total phosphorus [19].

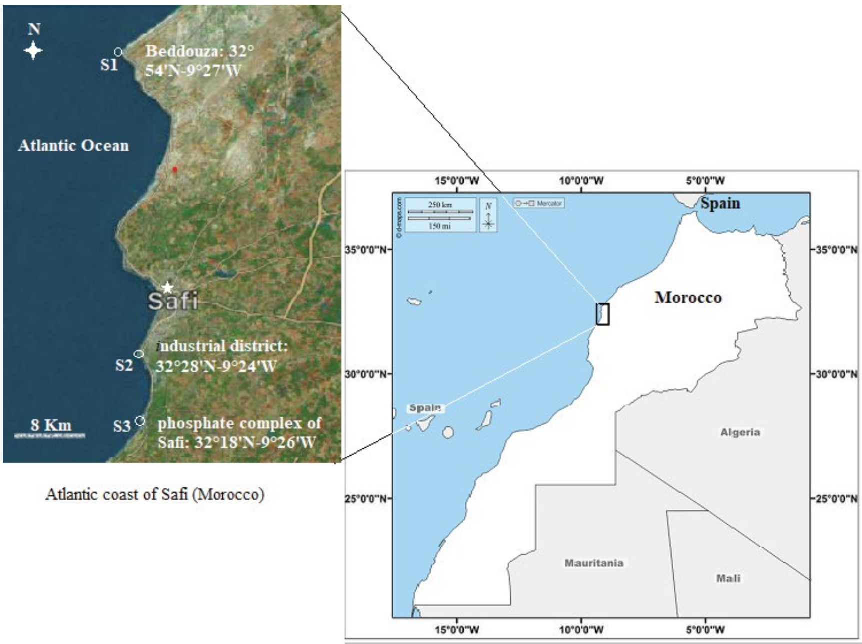

Fig. 1. Geographical position of the study sites along the coast of Safi. (S1) Beddouza: 32°54′N-9°27′W – (S2) industrial district: 32°28′N-9°24′W – (S3) phosphate complex of Safi: 32°18′N-9°26′W

2.3. Benthic macroalgae

Algal samples were taken from submerged rock surfaces on a seasonal basis during the year 2018. Surveys samples were obtained by taking the entire rock and stand by scraping with a hammer and chisel over an area of 0.1 m^2 (33/33 cm). Three quadrats were taken randomly by replicate. Three replicates (n = 3) per station are deployed to finally covering an area of 0.9 m^2 [20].

The abundance of each algal species was estimated by its coverage (percentage of the sampling surface area covered horizontally).

On one hand, samples for systematic identification were placed in jars, preserved and immediately fixed in a 10% solution of alcohol and seawater. The identification of macroalgae was carried out at the laboratory, under magnifying glass and microscope (Motic Digital Microscope PM-143). The nomenclature adopted was that used in algaebase.org [21]. On the other hand, a large quantity of a common seaweed between the 3 sampling sites, the green algae *Ulva lactuca* was collected in order to study its physiology. For this, the algal sample was washed from salt and sediments with seawater and carefully cleaned in order to eliminate epiphytes and associated fauna. Then, it was washed with distilled water. Subsequently, the green seaweed was lyophilized and ground to powder for physiological analysis.

2.4. Analytical and synthetic parameters of phytobenthos

2.4.1. Cover (R)

The overlap (R_i) is defined as the percentage of the substrate surface covered in projection by a specie (i). The assessment of this overlap is based exclusively on observation [22]. The total overlap (R_t) of a survey is the sum of the overlaps (R_i) of each taxon (i):

$$R_t = \Sigma R_i \quad (1)$$

The cover can take values between 0.01%, an arbitrary value attributed to algae with R_i less than 0.1%, and 100% that corresponds to the total cover of the survey. The Braun-Blanquet abundance-dominance scale was used (Table 1).

2.4.2. Quantitative dominance

The dominance by cover quantitative dominance (DRi) of a species (i) in an array of n species is the ratio of its overall mean cover (OMC) to the sum of the OMCs of the n species in the array, multiplied by 100 [22].

For a single survey, Σ DR group = 100%.

$$\text{DR groupe} = h\left(\sum R_i \text{ groupe} \frac{du}{R_t}\right) \times \left(\sum R_i \text{ groupe} \frac{du}{R_t}\right) \quad (2)$$

2.4.3. Overall mean cover

The overall mean cover (OMC) for species (i) in a series of N surveys (P) is the ratio of the sum of the recoveries R_i to the number of surveys at the station level. The sum of the OMC can be greater than 100%.

Table 1
Classification grid of algae recovery index and the corresponding cover % values according to Braun-Blanquet (1959)

Percentage of recovery (%)	Index
0.01	*r* (rare)
<1	+
1–5	1
5–25	2
25–50	3
50–75	4
75–100	5

At each step (class) of the cover coefficient, that R_i assigns to the (n) species (i) in a study. There is a conventional mean value (class center) called the mean cover.

$$\text{OMC} = \left(\sum_{pi=2}^{N} R_i\right) \times 100 \quad (3)$$

where R_i: cover of species i.

2.5. Shannon-Weaver diversity index

Diversity takes into account not only the number of species, but also the distribution of individuals within those species. One of the most widely used indices in the study of populations is given by the expression of H' index:

$$H' = -\sum\left[P_i \times \log 2P_i\right] \quad (4)$$

where P_i (relative overlap of species i) = R_i/R_t; R_i: cover of species i; R_t: total cover.

2.5.1. Equitability (E)

Equitability [23] or regularity [24] (Frontier and Pichod-viale, 1992) is also used to compare the structure of several stands.

It is defined as the relationship between the actual diversity of the community and its theoretical maximum diversity:

$$E = \frac{H'}{\log 2 \times T} \quad (5)$$

where T: total number of species recorded.

When it tends towards one (1), it gives information on an equi-distribution of dominance between all the species surveyed; specific overlaps are unevenly distributed when (E) tends towards zero (0).

2.6. Determination of physiological parameters

2.6.1. Chlorophyll and carotenoids

Pigment concentrations were estimated using the method of Jeffrey and Humphrey [26]. The pigments were extracted from 1 g of fresh algae using 10 mL of acetone 90%, then put in the dark at 4°C. After 24 h, it was centrifuged at 19,000 g for 30 min. Absorbance of the supernatant was measured at 470, 630 and 664 nm, using a spectrophotometer (Selecta VR-2000, SPAIN).

The levels of chlorophyll (a) is expressed as µg g^{-1} fresh weight and is calculated according to the following equation:

$$\text{Chlorophyll (a)} = 11.47\ (\text{O.D. }664) - 0.40\ (\text{O.D. }630) \tag{6}$$

Total carotenoids (µg g^{-1} fresh weight) were calculated according to the equation of [27]:

$$\text{Carotenoids} = \frac{1{,}000\left(\text{O.D. }470\right) - 1.9\ \text{chlorophyll (a)} - 63.14\ \text{chlorophyll (b)}}{214} \tag{7}$$

2.6.2. Proline

The protocol used is the one of Monneveux et al. [28]. 100 mg of the algal powder was placed in a test tube to which 2 mL of 40% methanol were added. The solution was heated in a water bath at 85°C for 60 min. After cooling, the samples were centrifuged and 1 mL of the supernatant was mixed with 1 mL of acetic acid, 25 mg of ninhydrin and 1 mL of a solution composed of 120 mL of distilled water, 300 mL of glacial acetic acid and 80 mL of ortho-phosphoric acid of density 1.7. The whole was heated again in a water bath at a temperature of 100°C for 30 min. After cooling, 5 mL of toluene were added and then the content mixed using a vortex. After this operation, two phases form: an upper phase containing proline and a free-of-proline lower phase. The upper phase was recovered and dehydrated by introducing a pinch of Na_2SO_4. The absorbance was measured at 528 nm.

2.7. Statistical analysis

The analyzed parameters were established in triplicate and mean values. Standard deviation was also calculated. The values were tested for normality, homogeneity of variance and for significance between parameters using a one-way analysis of variance with Excel 2016 and IBM SPSS Statistics 19 (USA), Statistical hypothesis testing was based upon use of a probability value p of 0.05. Principal component analysis (PCA) was used as a classification tool. For data analysis, the SPSS Statistics version 19 package (IBM, USA) was used.

3. Results

3.1. Abiotic parameters

The temperature oscillated between 14.3°C in winter and 23.58°C in summer (Fig. 2). The lower and higher of conductivity values were 42.8 and 53.4 mS cm^{-1} recorded in S1 and S2, respectively (Fig. 2). The concentrations of dissolved oxygen ranged from 6.39 to 11.87 mg L^{-1} (Fig. 2). For the pH, the lower and higher values were 8.65 and 6.02 recorded in S2 and S3, respectively (Fig. 2).

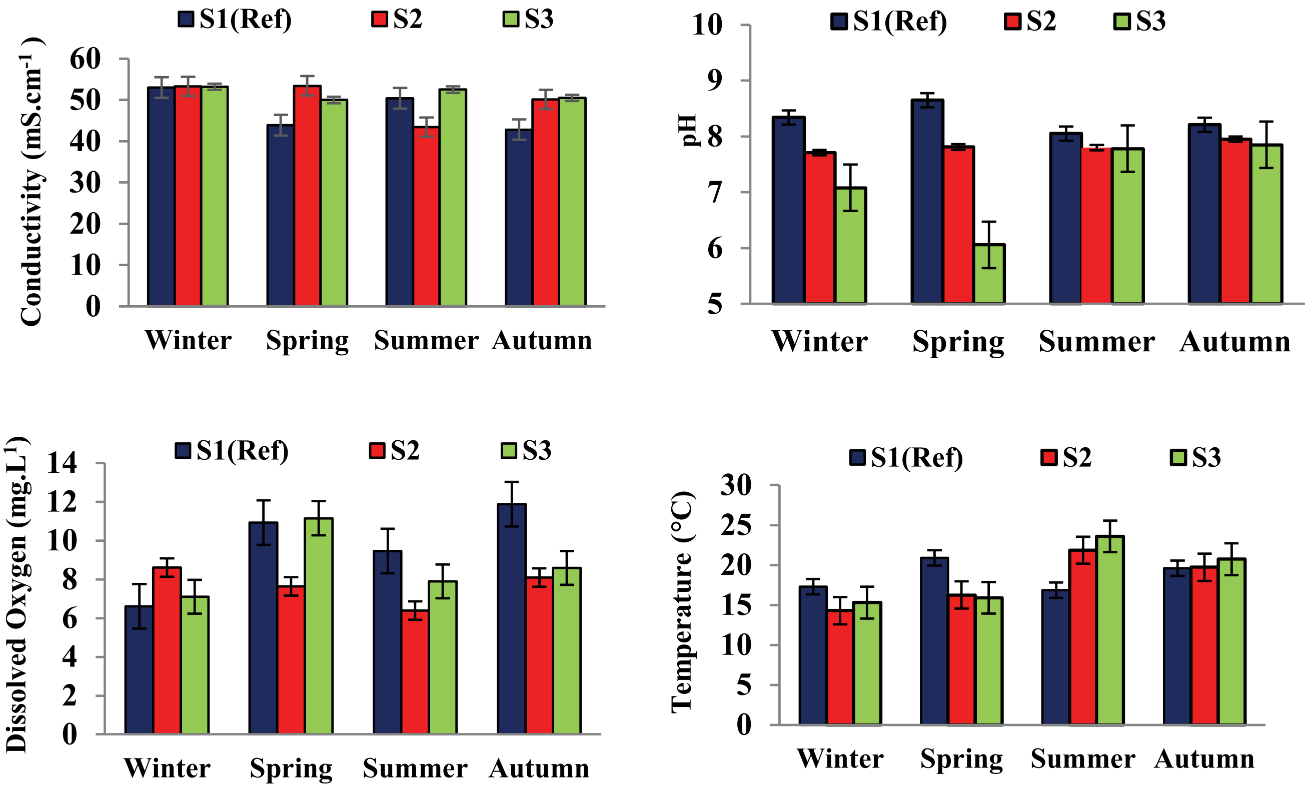

Fig. 2. Seasonal variation of physico-chemical parameters in seawater at the 3 stations during 2018.

The high concentrations of total phosphorus were recorded in S3. They vary between 2.01(winter) and 6.67 mg L^{-1} (spring). However the concentrations of this element didnot exceed 0.5 mg L^{-1} at S1 and S2. For total nitrogen, the higher concentrations were recorded during summer in all the study sites. They vary between 3.49 (S1) and 4.24 mg L^{-1} (S2). While the lowest values were recorded mainly during autumn at all the study sites. With respect to nitrogen–phosphorus (N/P) ratio, it was lower at S3 in comparison with the other sites. The maximum N/P ratio in seawater was recorded at S2 during summer (31.51), while the minimum ratio was noted at S3 during spring (0.13) (Table 2).

3.2. Biotic parameters

3.2.1. Specific richness

164 seaweed taxa were identified along the studied coastline, including 29 *Chlorophyceae*, 27 *Phaeophyceae*, and 108 *Rhodophyceae* (Fig. 3).

In comparison to S1, a noticeable reduction of algal diversity was recorded at S2 and S3 where more than 50% of the species had disappeared. Also, a sharp fall in brown algae, especially *Cystoseiraceae* was recorded at these last stations suspected being impacted (Fig. 4).

3.2.2. Ecological indices

Quantitative dominance (DRi) values of the different systematic groups showed that the rocky intertidal zone was mostly covered by *Chlorophyceae* in stations S2 and S3 with values of 83.56% and 61.57%, respectively. Whereas the station S1 was mostly covered by *Rhodophyceae* (40.21%) and *Phaeophyceae* (44.26%) (Table 3).

At Safi coastline, the obtained results of the Shannon diversity index (H') varied between 3.54 and 4.60, while the equitability index (E) was between 0.47 and 0.62 (Table 4).

3.2.3. Proline and chlorophyll pigments

The contents of proline and the variations of chlorophyll "a" and chlorophyll "b" concentrations in *Ulva lactuca thallus* are represented in Table 5 and Fig. 5, where it is possible to observe a clear increase in the content of proline of the samples collected in the station S2 (52.17 ± 7.81 µg g^{-1} DW) compared to the samples collected in the control station S1 (13.61 ± 1.92 µg g^{-1} DW), and moderately (30.94 ± 6.43) in the station S3. The carotenoid contents followed the same evolution as that of proline. However, the total content of chlorophyll pigments (a+b) showed a respectively reversed evolution compared to proline: 8.91 µg g^{-1} FW in S2, 20.64 µg g^{-1} FW in S1, and 17.15 µg g^{-1} FW in S3.

3.3. Statistical analysis

In order to analyze differences between the three sampling sites, Kruskal–Wallis and Wilcoxon tests were applied. The sampling sites of Safi coastline were aggregated in a PCA bi-plot based on eight physical, chemical and ecological parameters: dissolved oxygen (DO), EC, nitrogen–phosphorus ratio (N/P), specific richness (SR), Shannon diversity index (H'), equitability (E), algal flora coverage (OMC%) and its quantitative dominance (DRi%). Based on these eight parameters, three groups of sites were distinguished with respect to the first two factors F1 and F2, which alone restore 100% of the cloud dispersion, thus, neglecting the other factors. The PCA eigenvalues of the two components F1 and F2 and their contribution to the total inertia are presented in (Table 6).

4. Discussion

This study aimed to determine the state of health of Safi coastal area using seawater physico-chemical parameters, algal biodiversity, its analytical, synthetical and physiological parameters all combined.

Among the parameters measured in situ, only the pH showed significant difference between S1 (reference station) and the other pollutes sites (S2 and S3) ($p < 0.05$).

The variation of water temperature at the stations S1, S2 and S3 was mainly related to seasonal climatic factors; it was relatively higher during summer and autumn than during other seasons (Fig. 2).

Table 2
Seasonal variation of total phosphorus (TP), total nitrogen (TN) (mg L^{-1}) and N/P ratio in seawater at the three study area

Stations	Seasons	TP (mg L^{-1})	TN (mg L^{-1})	N/P ratio
S1	Winter	0.38 ± 0.020	2.90 ± 0.297	8.82 ± 0.724
	Spring	0.14 ± 0.003	1.98 ± 0.123	10.04 ± 1.979
	Summer	0.36 ± 0.056	3.49 ± 1.008	14.43 ± 4.200
	Autumn	0.05 ± 0.010	0.85 ± 0.623	12.26 ± 2.699
S2	Winter	0.30 ± 0.025	2.57 ± 0.268	6.07 ± 1.273
	Spring	0.50 ± 0.173	1.49 ± 0.366	9.60 ± 4.058
	Summer	0.13 ± 0.008	4.24 ± 0.402	23.95 ± 4.326
	Autumn	0.10 ± 0.008	0.93 ± 0.681	4.27 ± 2.243
S3	Winter	2.00 ± 0.093	3.11 ± 0.363	0.85 ± 0.363
	Spring	6.66 ± 0.033	0.89 ± 0.158	0.40 ± 0.161
	Summer	2.89 ± 0.571	3.52 ± 0.27	0.91 ± 0.147
	Autumn	1.80 ± 0.178	0.85 ± 0.643	0.40 ± 0.142

The pH value recorded at station S1 (reference station) remained normal compared to the norms falls between 8.2 and 8.6 [29]. However, the two other stations were influenced by industrial discharges, the pH was slightly lower than the standards, it was 6.02 in S3 (Fig. 2). Indeed, the latter is considered as a heavily human-stressed area since it is impacted by phosphoric acid and chemical products discharges coming from three industrial phosphate plans [11]. In comparison with Essaouira coastline, the pH vary between 8.03 and 8.47 in polluted and unpolluted stations [30]. In coastal areas, the pH values depend on continental water fluxes and on wastewater discharges [31]. According to [32], low pH can reduce marine biodiversity and can allow toxic elements to become more available for uptake by aquatic organisms.

The values of conductivity were slightly higher than the standard established for seawater which is 50 mS cm^{-1} [29]. This reflects excessive mineralization at the three stations, especially at S2, where it reached 53.4 mS cm^{-1}. This increase could be related to the discharges of anthropic origin in S2 and S3.

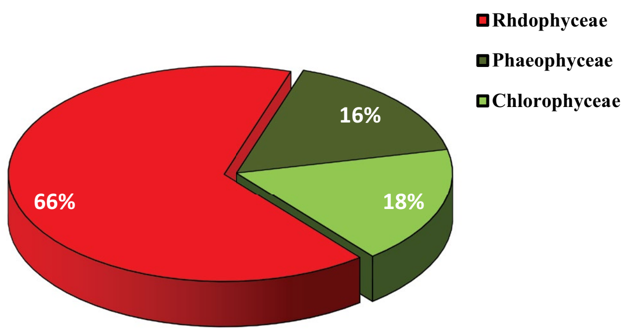

Fig. 3. Global specific richness of phytobenthos at the study area.

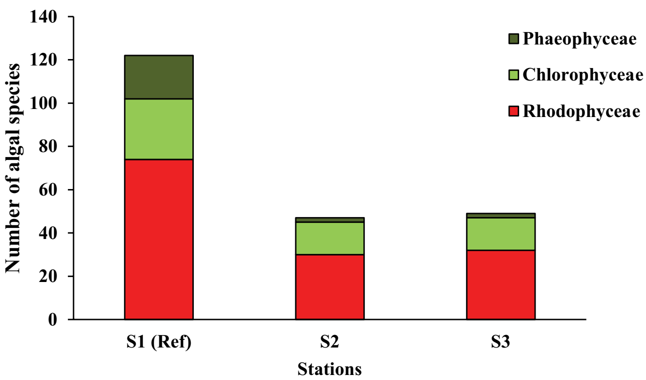

Fig. 4. Algal specific richness in the study area (2018).

The difference in DO content observed between the stations could be related to the load of biodegradable organic matter from industrial and urban origin discharged and accumulated at the station of the industrial district, which contribute to a significant drop in oxygenation of the environment. At S2, the excessive inputs of fermentable organic matter requiring the consumption of oxygen, discharged by the sewers of the urban agglomeration of Safi, contributes in this case to a drop in the water oxygenation. The BOD_5 confirms this biological oxygen demand at S2 and the COD recorded at S3 (Fig. 6). Such a result have already been reported by many authors that had worked on the same study sites [11,33] and seems to persist over time.

Statistical analysis showed significant difference between total phosphorus ($F = 111.087$, $p < 0.05$), total nitrogenous ($F = 6.024$, $p < 0.05$) and N/P ratio ($F = 8.469$, $p < 0.05$) between the study sites. The high concentrations of total phosphorus are related to phosphogypsum wastes released without any prior treatment as reported by many authors [11,34]. For total nitrogen concentrations the highest concentration was recorded at S2 (4.24 mg L^{-1}). This could be due to nitrogen compounds discharges coming from fish meal industries established in this area and from domestic wastwater. Fragallah et al. (2009) add that wind movement is an important factor, influencing the discharge of drainage water containing nitrogen compounds. High concentrations of nitrogenous were noted during summer and could be related to organic matter decomposition that increase during hot season.

The variation of the N/P ratio observed between our sites is related to the urban discharge that is rich in

Table 4
Total number of species (T), specific diversity index (H'), equitability (E) in the average records of the sampling stations

Index/Stations	S1	S2	S3
Number of species	122	47	49
Shannon index (H')	4.6	3.7	3.54
Equitability (E)	0.62	0.5	0.47

Beddouza (reference station S1); industrial district (S2); phosphate complex (S3).

Table 3
Overall mean cover (OMC%) according to the three groups of algae and their quantitative dominance (DRi%)

Stations	Annual OMC values (%)			Quantitative dominance (DRi) values (%)		
	S1	S2	S3	S1	S2	S3
$\sum R_i$ *Rhodophyceae*	18.06	5.20	14.17	40.21	7.69	38.30
$\sum R_i$ *Chlorophyceae*	6.98	56.48	22.78	15.54	83.56	61.57
$\sum R_i$ *Phaeophyceae*	19.88	6.85	0.06	44.26	10.13	0.16
OMC	44.92	67.59	37.00	100.00	100.00	100.00

Beddouza (reference station S1); Industrial district (S2); Phosphate complex (S3).

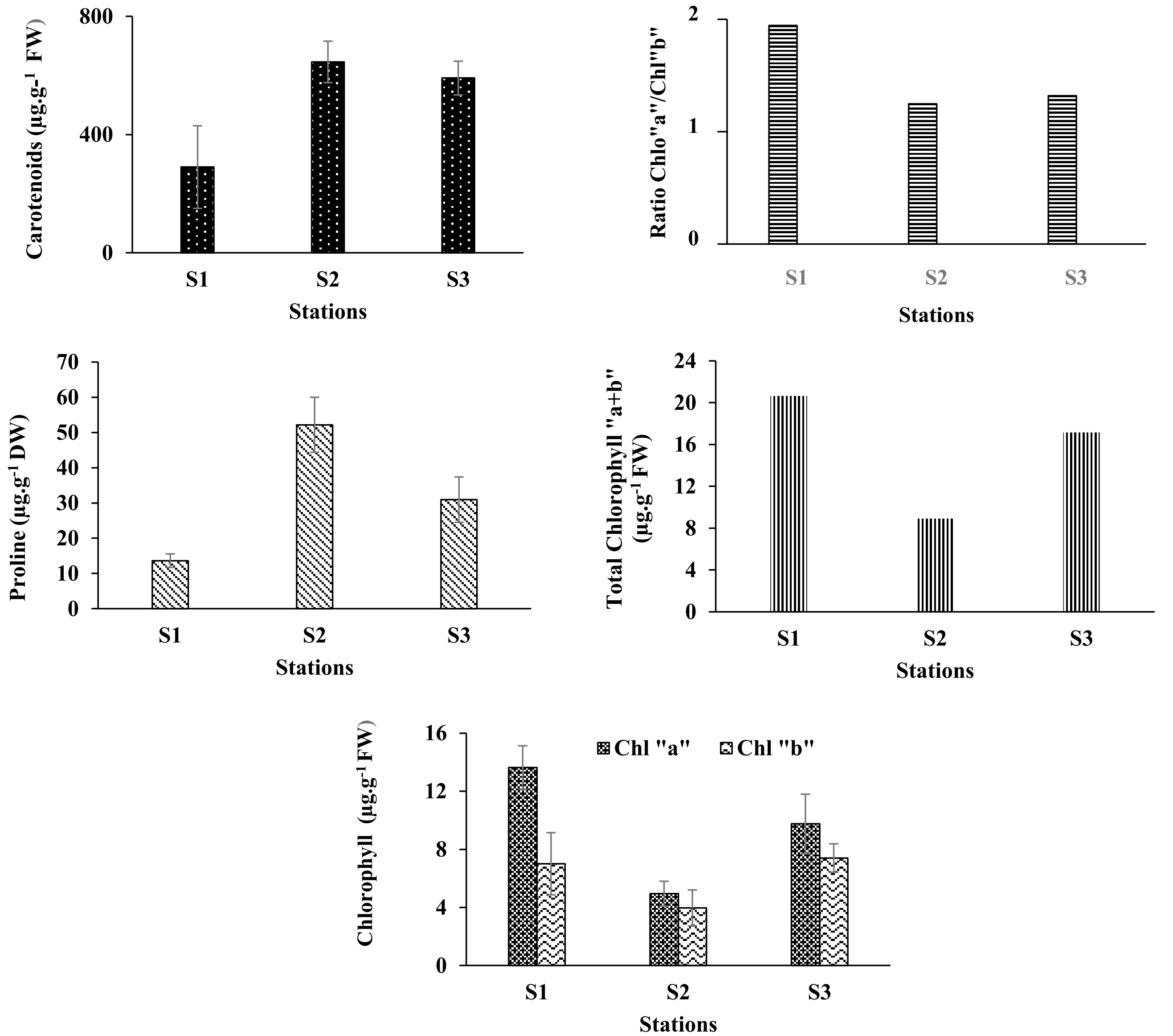

Fig. 5. Annual variation of photosynthetic pigments and proline concentrations of *Ulva lactuca thallus* in the study area during 2018.

Table 5
Concentrations of Chlorophyll a, Chlorophyll b, carotenoids (µg g^{-1} fresh weight) and proline (µg g^{-1} dry weight) of *Ulva lactuca* in the study sites during 2018

Stations	Chl "*a*"	Chl "*b*"	Chl (a+b)	Ratio Chl (a/b)	Carotenoids	Proline
S1	13.63 ± 1.49	7.01 ± 2.14	20.64	1.94	290.05 ± 139.68	13.61 ± 1.92
S2	4.94 ± 0.85	3.96 ± 1.24	8.91	1.25	645.62 ± 70.19	52.17 ± 7.81
S3	9.75 ± 2.05	7.39 ± 0.99	17.15	1.32	591.30 ± 57.11	30.94 ± 6.43

nitrogen (S2), and to the compensation of the contribution of nitrogen by the discharge of phosphorus from the phosphogypsum dumped by the phosphate complex of Safi (several hundreds of tons per day dumped in S3).

In coastal waters, where anthropogenic inputs of nitrogen and phosphorus are high, cases of eutrophication have been identified in a number of coastal areas, due to urban and industrial wastewater, effluent and agricultural discharges. In each case, it is nitrogen that limits the development of algae. The high concentrations of nitrates and nitrites in S2 where the N/P ratio in seawater was high, favors the development of nitrophilic species such

Table 6
Loading vectors of physico-chemical parameters. Overall mean cover (OMC%) and specific diversity index (H') in the study areas along principal component axes 1 and 2

Parameters	Code	Component	
		1	2
Reference station	S1	0.908	0.419
Industrial district	S2	0.091	0.996
Phosphate complex	S3	0.817	0.577
Annual overall mean cover (OMC%) _*Rhodophyceae*	OMC_R	0.381	0.925
Annual overall mean cover (OMC%) _*Chlorophyceae*	OMC_C	0.398	0.917
Annual overall mean cover (OMC%) _*Phaeophyceae*	OMC_P	0.996	0.088
Quantitative dominance (DRi%) _*Rhodophyceae*	DRi_R	0.143	0.990
Quantitative dominance (DRi%) _*Chlorophyceae*	DRi_C	0.729	0.685
Quantitative dominance (DRi%) _*Phaeophyceae*	DRi_P	0.977	0.213
Specific richness	SR	0.898	0.440
Shannon index (H')	*H*	0.958	0.288
Nitrogen–phosphorus quotient	N/P	0.769	–0.639
Electrical conductivity	EC	0.627	0.779
Equitability	*E*	0.971	0.240
Biological oxygen demand	BOD	0.104	0.995
Chemical oxygen demand	COD	0.913	0.408
	Variance (%)	69.585	30.415
	Cumulative (%)	69.585	100.000

as *Ulva* sp. [35,36]. Indeed, *Ulva lactuca* was dominant in this site where the highest N/P ratio recorded was 31.51. According to a laboratory study, it has been reported that tissular N/P ratio of *Ulva lactuca* was much higher in comparison to other studied brown and red algae [37].

In addition to domestic and agricultural activities, there is also significant chemical pollution in this industrialized sector, especially in S3 where the phosphate complex is edified. Due to the high concentrations of phosphorus resulting from the treatment of phosphates, the N/P ratio in seawater was very low (ranging from 0.13 to 1.55) (Fig. 2). In the same station, the green alga *Cladophora* species was dominant, behaving as opportunistic macroalgae. Indeed, many authors reported a relationship between *Cladophora* species and phosphorus content in aquatic ecosystems [35,36]; In contrast, the absence of sensitive species belonging to *Cystoseira* genus such as *Carpodesmia tamariscifolia* in the polluted stations S2 and S3, has been noticed, which was already reported in a previews study. Some authors have mentioned the sensitivity of this group of brown algae [4,38,39]. Thus, the industrial areas had a great impact on algal biodiversity in Safi regions.

Shannon Weaver's index (H'), is used to assess the stability of a stand in its environment. It may also reflect temporal changes in diversity related to the increase or decrease of pollution [40]. Large values of the diversity index (H') reflect an important degree of organization a community [41]. In the literature, the Shannon index varies between 1 and 4.5; for values of (H') below 3, the stand is not considered stable and evolved. The equitability index (E) reflects the distribution of species abundance in the stand; equitability is increasing with the total number of species and complementing the index (H') while a value of 0.5 for (E) is considered as low [42].

In this study, the Shannon-Weaver diversity index (H') showed a high value in S1 ($H' > 4.5$). In S2 and S3, the recorded values were at the limit of the thresholds characterizing an impacted environment, indicating an unstable stand ($3 < H' < 4$). These low indices would reflect the fact that the species are not only installed in a stable way, each with its own ecological niche, but also that they are in competition for the occupation of these niches [43]. Indeed, the station of the industrial district (IQ) presented a particular profile with a very homogeneous biodiversity compared to the other stations.

Furthermore, the equitability (E) values were below 0.65 in all sampling stations. S1 was characterized by a significantly high (OMC) and (DQ) of *Phaeophyceae* compared to *Rhodophyceae* and *Chlorophyceae*. This difference was well illustrated by the dominance of *Cystoseira* species known for their sensitivity to anthropogenic stress [4,44,45]. S1 would be relatively cleaner, and could even be confirmed as a reference station ($H' > 4.5$). Nevertheless, stations S2 and S3 can be considered as noticeably polluted ($H' < 4$) with the absence of *Cystoseira* species (OMC is null). As a result, species that are highly tolerant to pollution colonized any surface left free on the rocky shore of the stations S2 and S3. *Chlorophyceae* such as *Ulva rigida*, *Ulva intestinalis* established a dominant structure in the environment and limited the living areas of other algae.

When environmental conditions become unfavorable, the plants such as alga develop a resistance to abiotic stress by accumulating proline [46]. On the other hand, the presence of this amino acid is reduced in sensitive plants [47].

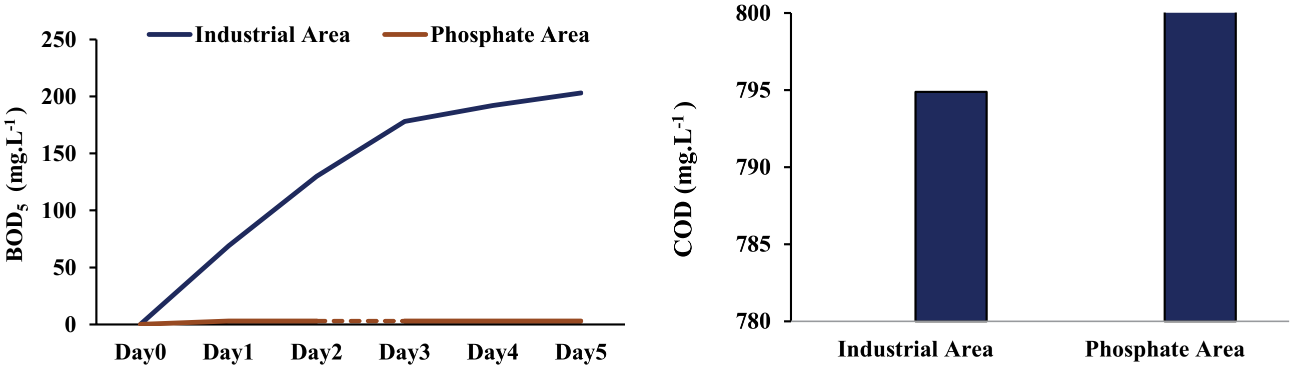

Fig. 6. Evolution of BOD_5 and COD values in the studied stations.

According to [48], the proline is involved in the osmotic regulation and the maintenance of turgidity, as well as the protection of membranes and enzyme systems, mainly the protection of the electron transport complex II [49].

Our results showed that the high contents of proline were found in *Ulva lactuca* at S2 and S3 (Fig. 5). This shows that this specie develops a stress-resistant character due to the accumulation of proline in S2 and S3. Some authors mentioned that a simple stress is defined as an external disturbance exceeding the resistance and regulation capacities of the ecosystem [24]. In our study, the accumulation of proline coincided with a decrease in the content of chlorophyll pigments (Table 5) of the targeted specie *Ulva lactuca*. In algae and higher plants, intracellular proline was also considered as osmoprotectant that accumulates as a function of intracellular potassium ion levels. Wu et al. [50] reported that intracellular proline accumulation upon exposure to heavy metals was related to a protective mechanism against osmotic changes, the specific richness falls clearly in this site with an almost total disappearance of *Cystoseira* species. As the physico-chemical conditions of the environment are constantly changing, the more suitable species develop to the detriment of sensitive species, leading to instability of the algal population. Indeed, the seawater in S2 is characterized by excessive mineralization and a decrease in dissolved oxygen content (Fig. 2), which could be due to the discharge of urban and industrial waste.

By performing the PCA on the studied sites combined with the biological indices (OMC%, DRi%, SR, *H* index, *E*, N/P, EC, BOD and COD), two principal components were extracted by covering 69.585% of the cumulative variance (Fig. 7).

The resultant PCA presenting the loading of the variables on the two principal components demonstrated that S1, S3, OMC_P, DRi_C, DRi_P, SR, *H*, NP, *E* and COD were the dominant correlated positive variables on the PC1 (0.908, 0.817, 0.996, 0.729, 0.977, 0.898, 0.958, 0.769, 0.971 and 0.913, respectively), while S2, OMC_R, OMC_C, DRi_R, EC and BOD the correlated ones on PC2 (0.996. 0.925. 0.917. 0.990. 0.779 and 0.995, respectively). The ratio N/P was systematically opposed to station S3. It is negatively correlated with axis 2 (–0.639).

The global analysis (Fig. 7) allows defining a typology dominated by the individualization of the 3 sites. This spatial organization reveals the exact position of the zones in relation to their situation:

S2 was located in a zone of high pollution due to a decrease in dissolved oxygen, and a high BOD. This is explained by a discharge of intense urban waste of organic nature.

S3 was located in an area characterized by a maritime industrial activity and a high COD and a very low ratio N/P.

These two stations were characterized by the dominance of the following species: *Ulva rigida*, *Ulva lactuca*, *Ulva intestinalis*, *Cladophora* sp. These opportunistic seaweeds grow well in polluted areas. Such indicator species give information on water quality [51–53].

Additionally, a study conducted in Persian Golf, reported that *Ulva intestinalis*, with *Cystoseira myrica* can be used in the biomonitoring to assess seawater and sediment quality [54]. Moreover, many authors try to use green macroalgae to monitor coastal areas worldwide as a reliable tool [10,55,56].

S1 was characterized by a great algal biodiversity referred to the ecological indices. Indeed, there was a positive correlation between S1 and the specific richness of the different groups of algae. In this stand, S1 (species = 122, *H′* = 4.6, *E* = 0.62) was characterized by the dominance of the following species: *Cystoseira* sp. associated with *Lithophyllum incrustans* and *Jania rubens*. This site was subject to very few sources of pollution. Indeed, according to some authors, the presence of these sensitive species in a coastal area reflects its safety [57,58].

The decrease in the number of species in S2 and S3, located closely to the effluents from household and industrial units is to be linked to the deterioration of the coastal waters due to the discharges. The degree of this deterioration makes it possible to classify these stations in order S1, S2 and S3 according to the decreasing degree of the algal species. The values of *H′* and equitability indexes indicate the same progression of the studied sites in terms of water quality which decreases in the same order (Table 3). It is well known that different kind of pollution affect the algal biodiversity in water bodies since the 1970s [59,60] until now [61,62].

5. Conclusion

The present study reveals a state of stress and indicates instability in the macroalgal ecosystem. The reference station S1, located in an area sheltered from the sources of

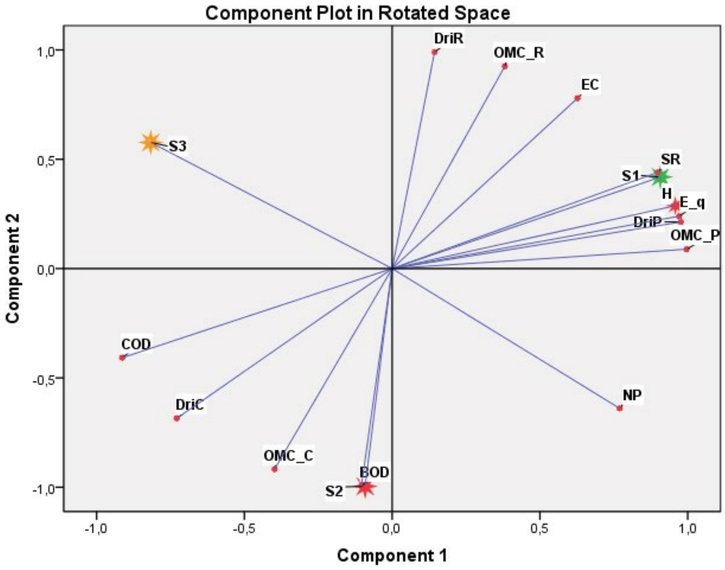

Fig. 7. The principal component analysis (PCA) of the sampling sites and their grouping based on: dissolved oxygen (DO), electrical conductivity (EC), nitrogen–phosphorus quotient (N/P), specific richness (SR), overall mean cover (OMC%) and quantitative dominance (DRi%) of the three groups of algae (*Chlorophyceae*, *Phaeophyceae* and *Rhodophyceae*), BOD: biological oxygen demand; COD: chemical oxygen demand; specific diversity index (H') and equitability (E) in the three sampling stations.

pollution, is characterized by a diversified algal population. The diversity index $H' > 4.5$ confirms the stability of the macroalgal ecosystem in this station and reinforces its ecological status as "reference station". The values of the Shannon-Weaver diversity index (H') and the lowest equitability index (E) observed in S2 (industrial district) and S3 (phosphate complex) show that the two stations are relatively polluted and unstable. The opportunistic species *Ulva lactuca* in S2 and *Cladophora* sp. in S3 establish a dominant structure in the environment and limit the living areas of other algae. This results in a drop in algal specific richness, instability of the study area, and thus an imbalance in the ecosystem. These observations confirm the impact of anthropogenic activity on the studied coastal area and indicate that macroalgae would be excellent bioindicators for determining the ecological status of marine ecosystems. Additionally, the study of physiological parameters of *Ulva lactuca* in the 3 study sites showed that this green algae is more stressed in S2 and S3 according to pigments and proline concentrations.

Only pH, phosphorus, nitogenous and N/P ratio determined the physico-chemical changes between the study sites. It could be due to the anthropogenic pollutants resulting from the intense urban and industrial activities. However, this observation illustrates the interest of phytobenthos in the biomonitoring and bioassessment of coastal marine ecosystems.

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