FIRST STUDY OF THE ECOLOGICAL STATUS IN THE ATLANTIC COAST OF MOROCCO USING THE BROWN SEAWEED *CYSTOSEIRA TAMARISCIFOLIA*

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Abstract. This study is a first attempt to evaluate the toxic effects of prominent aquatic pollutants (nutrients and toxic metals) on the brown seaweed *Bushy Rainbow Wrack (Cystoseira tamariscifolia)* physiology along the Atlantic coast of Morocco. The physicochemistry (nutrients) of seawater, toxic metals (Chromium, Lead, Copper and Cadmium) and physiological parameters (Chlorophyll contents, Proline, Glycinebetaine and Total Phenolic Compounds) of the brown macroalgae *Bushy Rainbow Wrack* were studied in order to assess the pollution degree of 8 coastal areas. The results show that the toxic metal contents of *Bushy Rainbow Wrack* (especially Cadmium) and the concentration of phosphorus are correlated with stress physiological parameters, and inversely correlated with pigment contents. It shows that while these brown algae exist in the less polluted areas, their physiology is significantly affected. However, in the highly polluted areas, this brown seaweed disappears. Thus, this specie could be used for monitoring the pollution degree in coastal areas.
Keywords: *environmental pollution, heavy metals, physicochemical parameters, algal physiology*

Introduction

Cystoseira is a genus of brown macroalgae. Most of the species of this genus are very sensitive to pollution and to other anthropogenic pressures, and their numbers have diminished considerably during the last decades (Cormaci et al., 1999; Thibaut et al., 2005). With the rapid industrialization and economic development of coastal regions, heavy metals continue to be introduced into coastal zones, our country's coast must be in such a state that local organisms can live, develop and reproduce without hindrance. To achieve this goal, it is essential that pollutant inputs to water be reduced or even avoided, regardless of their origin: domestic, artisanal, industrial, agricultural or other sources (Blinda et al., 2013). Seaweeds are widely recognized as autogenic “ecosystem

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engineers” (Jones et al., 1994) or “foundation species” (Dayton, 1975). In general, the direct causes of change in marine biodiversity loss and coastal ecosystems, are: pollution, habitat destruction, increases in sedimentation, overexploitation of resources, climate change, and invasive species (Walker and Kendrick, 1998; Claudet and Fraschetti, 2010; Munday et al., 2013). The aim of this study is to portray the decrease of *Bushy Rainbow Wrack* (*Cystoseira tamariscifolia* (Hudson) Papenfuss) and to clarify the source and the conceivable reasons of its decrease in the Atlantic coast of Morocco.

Materials and Methods

Location

Eight (8) stations located on rocky substrates along the Atlantic coast of Morocco were chosen:

- Eljadida city with 2 stations: Sidi Bouzid coast: 33°13’N-8°55’W as a control area (S1). According to Moroccan beaches position, this station is ranked each year among the beaches called "Blue Flag". The Mohammed VI Foundation for the Protection of the Environment awards this distinction each year to beaches that fit the international standard norms of cleanliness (F.M.6, 2018). Jorf Lasfar: 33°07’N -8°37’W (S2) known as a polluted one. This area is characterized by the presence of multiple industrial units including a phosphate complex and a power thermal plant (Essedaoui et al., 2001; Kaimoussi et al., 2001; Ferssiwi et al., 2004).
- Safi city with 3 stations: Beddouza: 32°54’N-9°27’W (S3), less polluted station located 34 km from the industrial city of Safi (Goumri et al., 2018); Industrial Area: 32°28’N-9°24’W (S4) and Phosphate Area: 32°18’N-9°26’W (S5).
- Essaouira city where 3 stations were selected: Moulay Bouzerktoun (31°63’N-9°67’W) (S6); located approximately 15 km from the enclosure of the city, it is less affected by anthropogenic activities, just an ephemeral tourism activity during the summer. Bab Doukala: 31°51’N-9°76’W (S7) and the port: 31°51’N-9°77’W (S8), receive domestic and some industrial releases (Sabri et al., 2017; Cherifi et al., 2018). Among them, 3 stations less polluted and 5 more polluted near to industrial factories (*Fig. 1*).

Sampling

Algal Samples were collected during Autumn 2017 to Summer 2018 at 0-5 meter depth depending on the geomorphology of the stations (*Fig. 2*) and following the principle of the quadrats. Then, washed in seawater, put in plastic packs and transported to the research center in a cooler for metal analysis. The samples expected for identification are preserved into alcohol at 10% while samples for physiology analysis are kept dry. Those of ocean water were collected during the same period, placed in a cooler and were conveyed fresh to the research center.

Physicochemical parameters

Concentration of inorganic nutrients of seawater (Phosphorus and Nitrogenous compounds) was measured according to AFNOR norms: T90-012 for Nitrate, T90-015 for Ammonium, T90-061 for Total nitrogen, T90-022 for Orthophosphate and T90-023 for Total phosphorus. Metal Concentrations of Cd, Cu, Pb and Cr were measured in seawater and the dried *Bushy Rainbow Wrack* according to Blinda et al (2013) and

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Topcuoglu et al (2003) methods, respectively. The samples were digested with concentrated nitric acid and analyzed using Atomic Absorption Spectrophotometer.

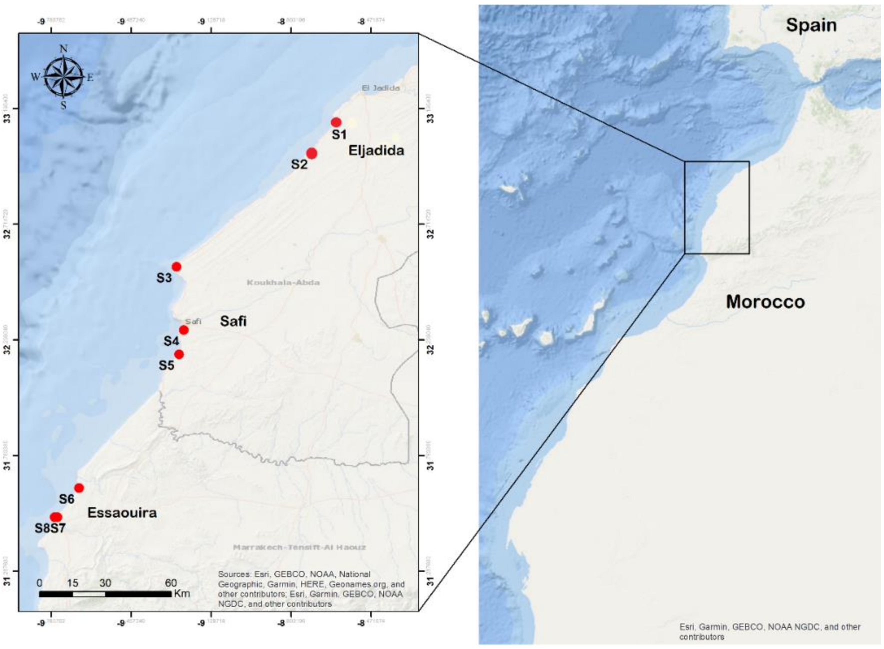

Figure 1. *The location of the sampling points using ArcGIS, version 10.7. (S1) Sidi Bouzid: 33°13'N-8°55'W, (S2) Jorf Lasfar: 33°07'N-8°37'W, (S3) Beddouza: 32°54'N-9°27'W, (S4) Industrial Area: 32°28'N-9°24'W, (S5) Phosphate Area: 32°18'N-9°26'W, (S6) Moulay Bouzerktoun: 31°63'N-9°67'W, (S7) Bab Doukala: 31°51'N-9°76'W, (S8) The port: 31°51'N-9°77'W*

Figure 2. *Bushy Rainbow Wrack captured with underwater camera from (S1) Sidi Bouzid coast near Eljadida city at 5m depth. Picture: Younes Boundir © June 2018*

Biochemical and physiological parameters

Chlorophyll

The contents of chlorophyll a, c were estimated using the method of Jeffrey and Humphrey (1975). Pigment concentrations are expressed as µg/g FW (Fresh Weight) and calculated according to the following equations:

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$$\text{Chlorophyll a} = 11.47 \times \text{O.D.}664 - 0.40 \times \text{O.D.}630 \quad \text{(Eq.1)}$$

$$\text{Chlorophyll c} = 24.36 \times \text{O.D.}630 - 3.73 \times \text{O.D.}664 \quad \text{(Eq.2)}$$

The pigments were extracted from 1 g seaweed *Bushy Rainbow Wrack* with 10 mL of acetone in the presence of calcium carbonate. Then put in 4°C for 24 hours in the dark. The chlorophyll content was determined in triplicate.

Total carotenoids were calculated according to the equation of Lichtenthaler (1987):

$$\text{Carotenoid} = \frac{1000 \times \text{O.D.}470 - 1.9 \times \text{Cha} - 63.14\,\text{Chb}}{214} \quad \text{(Eq.3)}$$

Glycinebetaine (GB)

Analysis of GB was performed according to the method of Grieve and Grattan (1986). 0.5 g DW (dry weight) of the seaweed *Bushy Rainbow Wrack* prepared in 20 mL test tubes was mechanically shaken with 20 mL of deionized H_2O at 25°C for 24 h. The extracts then were diluted 1:1 with 2N H_2SO_4. 0.5 ml of this solution were measured into heavy walled glass centrifuge tubes and cooled in ice water for about 1 hour. A preparation of 0.2 ml KI-I2 was made by dissolving 20 g of KI and 15.7 g of iodine in 100 ml. Then, water was added and the reactants were gently stirred with a vortex mixer. The tubes were stored for 16h at 0-4°C and then centrifuged at 10000 rpm at 0°C for 15 min. The supernatant was aspirated and dissolved in 9 ml of 1,2-dichloroethane. Vortex mixing was made to effect complete solution in the developing solvent. The absorbance was measured after 2 h at 365 nm. The concentration was estimated by using a standard curve developed with different concentration of GB in triplicate.

Proline

The method used is the one of Monneveux and Nemmar (1986). 100 mg of the seaweed *Bushy Rainbow Wrack* are directly weighed and then placed in a test tube. 2 ml of methanol 40% is added to the tubes and placed in a water bath at 85°C for about 1 hour. 1 ml of the solution is added to 25 mg ninhydrin after cooling, 1 ml of acetic acid and 1 ml of the mixture distilled water-acetic, acid-acetic, acid-acid orthophosphoric, of density 1.7 (120, 300, 80: v / v / v). The solution is brought again for 30 minutes in a water bath at 100°C, then cooled and added to 5 ml of toluene. After agitation, a pinch of Na_2SO_4 is added to each tube. Absorbance was measured at 528 nm.

Total phenolic compounds (TPC)

TPC of the extract was estimated by the method of Taga et al. (1984). Dry weight samples of the seaweed *Bushy Rainbow Wrack* were prepared in 60:40 (0.3% HCl) acidified methanol/water. Solutions of 100 µL were added to 2 mL of Na_2CO_3 (2%). 100 µL of Folin-Ciocalteau (50%) reagent were added after 2 min. Absorbance was measured after 2 hours at 750 nm. Gallic acid standards was prepared with a range of concentrations of 10 mg/mL to 200 mg/mL. The phenolic concentrations were determined by comparison with the standard calibration curve. Phenolic content was expressed as gallic acid equivalent (GAE).

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Statistical analysis

All the analyzed parameters were established in triplicate and gave mean values and standard deviation. The values were tested for normality and homogeneity of variance, as well as for significance between parameters using a one-way and two-way analysis of variance with Excel 2016 and SPSS (IBM, USA), version 22. Correlation matrix was used to determine the relation between the physiological parameters, Heavy metals and the physicochemical compounds studied. Principal Component Analysis (PCA) was performed using SPSS (IBM, USA), version 22.

Results

Distribution of Bushy Rainbow Wrack

The preliminary study shows that there is a first presence of Bushy Rainbow Wrack in the control station (S1) at Eljadida coast during the 4 seasons. Essaouira city comes in the second range where this specie is more present in (S5). Safi coast comes in the thirth place where the Bushy Rainbow Wrack is abondant in unpolluted station (S3) (*Table 1*).

Table 1. *Bushy Rainbow Wrack inventory in the studied areas during autumn-summer 2018*

City	Station	Autumn	Winter	Spring	Summer
Eljadida	**S1**	A	A	A	A
	S2	-	-	-	-
Safi	**S3**	R	R	R	R
	S4	-	-	-	-
	S5	-	-	-	-
Essaouira	**S6**	R	R	F	A
	S7	-	-	R	F
	S8	-	-	R	F

-: Absence of Bushy Rainbow Wrack. A: Abundant, F: Frequent, R: Rare

Physicochemical parameters: nitrogenous and phosphorus compounds

Statistical analysis of nitrogenous compounds didn't show significant difference between control and polluted stations ($F= 11.29$; $p<0.01$). Thus, it could not explain the degradation of the species studied. Total nitrogenous concentrations are ranged between 2.52±0.30 and 4.70±0.16 mg/L and those of nitrate between 1.30±0.56 and 2.56±0.46 mg/L (*Table 2*).

Statistical analyses of phosphorus compounds, however, have showed a highly significant difference between the areas studied ($F= 1.65$; $p<<0.01$). The maximum total phosphate concentration was recorded in the Phosphate industrial area (S5), at Safi coast with 2.00±0.13 mg/L and the minimum in the control station (S1), in Sidi Bouzid coast with only 0.04±0.01 mg/L (*Table 2*).

Heavy metals analysis

Heavy metals analysis in seawater

The analysis of metals shows that the mean metal levels in seawater decreased in the following order: Pb > Cd > Cr > Cu (*Table 3*). The results obtained are discussed on the basis of the Moroccan standards for direct discharges of heavy metals into aquatic

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environments (surface water). The maximum concentrations required by the above standard are: 0.5 mg/l for Pb, 2 mg/l for Cr, 0.5 mg/l for Cu and 0.2 mg/l for Cd (FAO, 2006). The highest concentrations of toxic metals were recorded in two polluted stations near to Safi coast: a fish industrial discharges (Cd: 1.12±0.15 µg/L; Pb: 2.563±0.73 µg/L) and phosphate discharge area (Cd: 1.15±0.88 µg/L; Pb: 1.53±0.55 µg/L).

Table 2. *Variation of Phosphorus and Nitrogenous compounds in different stations*

Station	Season	TP	DP	PO_4^{3-}	TN	DN	NO_3^-	NH_4^+
S1	Autumn	0.06±0.03^{C*}	0.05±0.02^{C}	0.02±0.01^{C}	4.03±0.14^{A}	3.74±0.11^{A}	2.14±0.20^{A}	0.89±0.09^{BC}
	Winter	0.07±0.02^{C}	0.08±0.03^{BC}	0.05±0.02^{C}	4.40±0.13^{A}	4.10±0.14^{A}	2.40±0.16^{A}	1.14±0.04^{A}
	Spring	0.06±0.03^{C}	0.07±0.02^{B}	0.04±0.01^{C}	4.31±0.14^{A}	3.65±0.13^{A}	2.33±0.14^{A}	0.90±0.05^{BC}
	Summer	0.05±0.02^{C}	0.05±0.03^{C}	0.03±0.01^{C}	4.12±0.15^{A}	3.21±0.12^{A}	2.20±0.12^{A}	1.11±0.12^{A}
S2	Autumn	0.04±0.01^{C}	0.05±0.02^{C}	0.05±0.02^{C}	2.15±0.12^{C}	1.23±0.21^{B}	1.30±0.56^{B}	0.74±0.16^{AB}
	Winter	0.09±0.05^{C}	0.08±0.01^{BC}	0.07±0.03^{BC}	2.40±0.14^{C}	2.30±0.23^{B}	1.50±0.20^{B}	1.12±0.13^{A}
	Spring	0.08±0.03^{C}	0.07±0.03^{B}	0.06±0.02^{BC}	2.32±0.15^{C}	1.96±0.17^{B}	1.45±0.23^{B}	1.10±0.16^{A}
	Summer	0.05±0.02^{C}	0.04±0.01^{C}	0.06±0.01^{BC}	2.30±0.13^{C}	1.50±0.12^{B}	1.33±0.12^{B}	0.88±0.18^{A}
S3	Autumn	0.26±0.03^{B}	0.06±0.03^{C}	0.03±0.01^{C}	2.65±0.15^{C}	1.65±0.18^{B}	1.70±0.13^{AB}	1.15±0.13^{A}
	Winter	0.38±0.02^{B}	0.08±0.03^{BC}	0.05±0.02^{C}	2.90±0.12^{BC}	2.40±0.17^{B}	1.90±0.16^{AB}	1.21±0.18^{A}
	Spring	0.25±0.03^{B}	0.05±0.02^{C}	0.04±0.01^{C}	2.85±0.15^{BC}	1.96±0.13^{B}	1.88±0.18^{AB}	1.12±0.22^{A}
	Summer	0.26±0.03^{B}	0.06±0.03^{C}	0.03±0.01^{C}	2.74±0.14^{BC}	1.80±0.12^{B}	1.65±0.14^{B}	1.10±0.17^{A}
S4	Autumn	0.14±0.04^{B}	0.06±0.01^{C}	0.05±0.02^{C}	4.33±0.18^{A}	1.78±0.13^{B}	2.22±0.25^{A}	0.54±0.18^{B}
	Winter	0.29±0.02^{B}	0.10±0.03^{AB}	0.07±0.02^{BC}	4.70±0.16^{A}	2.60±0.14^{B}	2.40±0.24^{A}	0.99±0.17^{AB}
	Spring	0.13±0.03^{B}	0.10±0.02^{AB}	0.06±0.01^{BC}	4.62±0.12^{A}	2.20±0.14^{B}	2.33±0.22^{A}	0.85±0.18^{AB}
	Summer	0.15±0.02^{BC}	0.05±0.02^{C}	0.06±0.02^{BC}	4.56±0.13^{A}	1.96±0.15^{B}	2.10±0.14^{A}	1.23±0.13^{A}
S5	Autumn	1.55±0.08^{A}	0.95±0.04^{AB}	0.11±0.03^{A}	2.33±0.11^{C}	2.90±0.14^{AB}	2.30±0.33^{A}	0.64±0.13^{B}
	Winter	2.00±0.13^{A}	1.58±0.06^{A}	0.11±0.02^{A}	3.10±0.14^{B}	3.00±0.17^{A}	2.60±0.45^{A}	0.65±0.17^{B}
	Spring	1.22±0.12^{A}	1.15±0.08^{A}	0.11±0.03^{A}	2.63±0.17^{B}	2.80±0.20^{A}	2.56±0.46^{A}	0.24±0.18^{C}
	Summer	1.69±1.13^{A}	1.22±0.07^{A}	0.09±0.02^{B}	2.54±0.15^{B}	2.55±0.13^{A}	2.41±0.34^{A}	0.32±0.13^{C}
S6	Autumn	0.25±0.09^{B}	0.03±0.01^{C}	0.03±0.01^{C}	3.21±0.13^{AB}	1.80±0.12^{AB}	1.46±0.21^{B}	0.54±0.21^{C}
	Winter	0.48±0.07^{AB}	0.05±0.02^{C}	0.05±0.02^{C}	4.20±0.15^{A}	2.00±0.16^{B}	1.90±0.12^{B}	1.21±0.12^{A}
	Spring	0.35±0.05^{B}	0.01±0.01^{C}	0.04±0.01^{C}	4.10±0.13^{A}	1.55±0.13^{B}	1.56±0.11^{B}	0.74±0.16^{AB}
	Summer	0.35±0.06^{B}	0.02±0.01^{C}	0.04±0.02^{C}	3.32±0.12^{B}	1.69±0.17^{B}	1.70±0.10^{B}	0.64±0.17^{AB}
S7	Autumn	0.24±0.02^{B}	0.09±0.02^{C}	0.05±0.01^{C}	2.12±0.11^{C}	2.54±0.12^{A}	2.22±0.22^{A}	0.45±0.15^{B}
	Winter	0.30±0.01^{B}	0.17±0.03^{B}	0.07±0.02^{B}	2.90±0.15^{AB}	2.60±0.13^{A}	2.50±0.14^{A}	0.87±0.12^{AB}
	Spring	0.25±0.01^{B}	0.12±0.04^{B}	0.07±0.01^{B}	2.55±0.13^{B}	2.33±0.14^{A}	2.45±0.11^{A}	0.32±0.14^{C}
	Summer	0.23±0.02^{B}	0.14±0.06^{B}	0.06±0.02^{BC}	2.31±0.12^{C}	2.30±0.18^{A}	2.30±0.24^{A}	0.35±0.12^{C}
S8	Autumn	0.26±0.03^{B}	0.21±0.02^{B}	0.11±0.01^{A}	3.31±0.16^{B}	2.50±0.12^{A}	1.30±0.12^{BC}	0.58±0.13^{BC}
	Winter	0.36±0.06^{B}	0.23±0.03^{B}	0.11±0.02^{A}	4.30±0.14^{A}	2.80±0.21^{A}	1.50±0.13^{B}	0.65±0.13^{BC}
	Spring	0.26±0.03^{B}	0.16±0.02^{B}	0.11±0.01^{A}	4.23±0.16^{A}	2.66±0.23^{A}	1.45±0.23^{AB}	0.67±0.16^{B}
	Summer	0.25±0.05^{B}	0.19±0.01^{B}	0.11±0.02^{A}	3.56±0.12^{A}	2.12±0.18^{A}	1.36±0.14^{AB}	0.54±0.11^{B}

*The different upper-case letters in the same row indicate the differences between the studied stations and seasons at the level ($p<0.05$). TP: Total Phosphorus; DP: Dissolved Phosphorus; PO_4^{3-}: Orthophosphate; TN: Total Nitrogenous; DN: Dissolved Nitrogenous; NO_3^-: Nitrate; NH_4^+: Ammonium

Heavy metals analysis in Bushy Rainbow Wrack

The analysis of heavy metals shows that the mean levels in seaweed *Bushy Rainbow Wrack* decreased in the following order: Cu > Pb > Cr > Cd. The results obtained are discussed on the basis of comparaison of these heavy metals levels in seaweed *Cystoseira* sp. from other different locations in the world. The highest concentrations of all heavy metal were recorded in the polluted area (S8), the port station at Essaouira coast (Cd: 2.60±0.15 µg/g during spring; Pb: 4.43±0.73 µg/g during autumn, Cu: 10.60±1.14 µg/g during summer and Cr: 2.90±0.23 µg/g during autumn) (*Table 4*).

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Table 3. *Heavy metals mean concentrations of Cd, Pb, Cu, and Cr in seawater along the studied stations*

Station	Cd	Pb	Cu	Cr
S1	0.09±0.02^{C*}	0.93±0.28^{B}	0.08±0.02^{C}	0.07±0.02^{C}
S2	0.11±0.04^{BC}	0.98±0.27^{B}	0.12±0.07^{C}	0.09±0.03^{C}
S3	0.17±0.03^{B}	-	0.19±0.06^{C}	0.25±0.06^{BC}
S4	1.12±0.15^{A}	2.56±0.73^{A}	0.20±0.04^{BC}	0.98±0.17^{A}
S5	1.15±0.88^{A}	1.53±0.55^{A}	0.60±0.17^{A}	0.84±0.07^{A}
S6	0.12±0.07^{B}	1.12±0.24^{AB}	0.09±0.04^{C}	0.03±0.01^{C}
S7	0.15±0.09^{B}	0.97±0.26^{B}	0.16±0.08^{C}	0.04±0.02^{C}
S8	0.17±0.06^{B}	0.87±0.19^{B}	0.13±0.03^{C}	0.05±0.01^{C}

*The different upper-case letters in the same row indicate the differences between the studied stations and seasons at the level ($p<0.05$)

Table 4. *Heavy metal mean concentrations (µg/g Dry Weight) of Cd, Pb, Cu, and Cr in Bushy Rainbow Wrack collected during autumn - summer 2018 along the stations studied*

Station	Season	Cd	Pb	Cu	Cr
S1	Autumn	0.10±0.02^{C*}	2.30±0.32^{AB}	0.21±0.05^{C}	0.11±0.02^{C}
	Winter	0.22±0.05^{BC}	1.20±0.08^{C}	3.07±0.19^{BC}	0.20±0.05^{C}
	Spring	0.39±0.09^{BC}	1.30±0.05^{C}	0.36±0.05^{C}	0.36±0.03^{C}
	Summer	0.10±0.03^{C}	0.50±0.04^{C}	0.17±0.09^{C}	0.14±0.01^{C}
S2	Autumn	-	-	-	-
	Winter	-	-	-	-
	Spring	-	-	-	-
	Summer	-	-	-	-
S3	Autumn	0.20±0.06^{C}	2.51±0.08^{B}	4.00±0.18^{BC}	0.14±0.04^{C}
	Winter	0.42±0.05^{BC}	2.14±0.04^{BC}	2.72±0.07^{C}	0.25±0.06^{C}
	Spring	0.17±0.04^{BC}	1.25±0.08^{C}	0.45±0.05^{C}	0.24±0.03^{C}
	Summer	0.20±0.02^{C}	1.22±0.06^{C}	1.90±0.09^{C}	0.36±0.02^{C}
S4	Autumn	-	-	-	-
	Winter	-	-	-	-
	Spring	-	-	-	-
	Summer	-	-	-	-
S5	Autumn	-	-	-	-
	Winter	-	-	-	-
	Spring	-	-	-	-
	Summer	-	-	-	-
S6	Autumn	0.50±0.07^{C}	1.20±0.06^{C}	2.10±0.14^{C}	0.28±0.04^{C}
	Winter	0.60±0.05^{C}	2.00±0.05^{BC}	4.55±0.08^{B}	0.58±0.08^{BC}
	Spring	0.20±0.06^{C}	1.13±0.09^{C}	4.10±0.05^{B}	0.26±0.05^{C}
	Summer	0.15±0.04^{C}	1.11±0.06^{C}	6.10±0.10^{B}	0.12±0.07^{C}
S7	Autumn	1.22±0.13^{B}	3.20±0.45^{A}	4.62±0.12^{B}	2.66±0.13^{A}
	Winter	1.21±0.18^{B}	1.05±0.07^{C}	5.78±0.13^{B}	0.98±0.11^{BC}
	Spring	1.90±0.17^{AB}	2.25±0.23^{B}	7.50±0.07^{B}	1.85±0.13^{B}
	Summer	1.70±0.16^{AB}	2.20±0.30^{B}	8.80±0.09^{A}	1.50±0.18^{B}
S8	Autumn	2.50±0.15^{A}	4.43±0.73^{A}	5.60±0.11^{BC}	2.90±0.23^{A}
	Winter	2.26±0.13^{A}	1.90±0.20^{AB}	4.32±1.23^{BC}	1.99±0.16^{A}
	Spring	2.60±0.15^{A}	2.54±0.32^{AB}	10.41±1.56^{A}	2.23±0.17^{A}
	Summer	1.60±0.13^{B}	1.90±0.24^{AB}	10.60±1.14^{A}	2.89±0.18^{A}

-: Absence of Bushy Rainbow Wrack.
*The different upper-case letters in the same row indicate the differences between the studied stations and seasons at the level ($p<0.05$)

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Biochemical and physiological parameters

Chlorophyll contents

Maximum value of chlorophyll a were recorded at (S1) Sidi Bouzid coast near Eljadida city with 586±167 µg/g FW during spring season, and reached its minimum at (S8) The port station in Essaouira city with 59±14 µg/g FW during autumn season. Values of chlorophyll c has its maximum as well at (S1) Sidi Bouzid coast near Eljadida city with 387±93 µg/g FW during spring and summer season, and its minimum also at (S8) The port station in Essaouira city with 34±13 µg/g FW during spring season. The same thing for carotenoid that shown a maximum value at (S1) Sidi Bouzid coast near Eljadida city with 399±175 µg/g FW during summer season and a minimum likewise at (S8) The port station in Essaouira city with 34±0.9 µg/g FW during summer season (*Table 5*).

Table 5. *Concentrations of Chlorophyll a, c, carotenoid and proline (µg/g Fresh Weight), GB and TPC (mg/g Dry Weight) of Bushy Rainbow Wrack in different stations during the 4 seasons of 2018*

Station	Season	Chl a	Chl c	Carotenoid	Proline	GB	TPC
S1	Autumn	475±133^{A*}	267±97^{AB}	255±76^{A}	69.06±24.22^{C}	2.06±0.25^{C}	0.56±0.14^{C}
	Winter	567±128^{A}	267±86^{AB}	276±94^{A}	69.06±13.03^{C}	2.06±0.53^{C}	0.52±0.12^{C}
	Spring	586±167^{A}	387±93^{A}	376±146^{A}	71.94±18.13^{C}	2.15±0.33^{C}	0.56±0.23^{C}
	Summer	489±120^{A}	387±56^{A}	399±175^{A}	112.23±14.50^{BC}	3.35±0.45^{C}	0.73±0.15^{C}
S2	Autumn	-	-	-	-	-	-
	Winter	-	-	-	-	-	-
	Spring	-	-	-	-	-	-
	Summer	-	-	-	-	-	-
S3	Autumn	393±117^{AB}	143±75^{AB}	129±87^{B}	57.55±15.11^{C}	1.72±0.12^{C}	0.72±0.17^{C}
	Winter	398±128^{AB}	176±86^{AB}	175±95^{B}	120.86±45.16^{B}	3.61±0.15^{C}	0.74±0.14^{C}
	Spring	498±127^{A}	198±95^{AB}	176±84^{B}	94.96±34.00^{BC}	2.83±0.45^{C}	0.68±0.12^{C}
	Summer	373±57^{AB}	187±97^{AB}	183±77^{AB}	112.23±43.12^{B}	3.35±0.36^{C}	0.72±0.16^{C}
S4	Autumn	-	-	-	-	-	-
	Winter	-	-	-	-	-	-
	Spring	-	-	-	-	-	-
	Summer	-	-	-	-	-	-
S5	Autumn	-	-	-	-	-	-
	Winter	-	-	-	-	-	-
	Spring	-	-	-	-	-	-
	Summer	-	-	-	-	-	-
S6	Autumn	487±156^{A}	276±74^{AB}	166±83^{B}	92.08±15.00^{BC}	2.752±0.14^{C}	0.65±0.17^{C}
	Winter	476±186^{A}	387±59^{A}	276±75^{A}	129.49±34.65^{B}	3.87±0.56^{C}	0.90±0.27^{BC}
	Spring	492±94^{A}	254±122^{AB}	285±86^{A}	115.10±54.66^{B}	3.44±0.76^{C}	0.72±0.26^{C}
	Summer	530±82^{A}	376±110^{A}	236±93^{A}	109.35±45.56^{B}	3.268±1.78^{BC}	0.58±0.17^{C}
S7	Autumn	183±56^{BC}	45±12^{C}	98±24^{B}	460.43±176.76^{A}	13.76±1.54^{AB}	1.36±0.28^{B}
	Winter	287±86^{B}	98±16^{C}	176±37^{B}	215.82±76.44^{AB}	6.45±1.76^{B}	1.04±0.93^{AB}
	Spring	287±89^{B}	65±18^{C}	111±30^{BC}	330.93±165.66^{AB}	9.89±1.77^{B}	1.11±0.28^{AB}
	Summer	245±112^{B}	57±14^{C}	123±34^{BC}	503.59±164.43^{A}	15.05±2.65^{A}	1.20±0.17^{A}
S8	Autumn	59±14^{C}	35±09^{C}	96±12^{C}	517.98±174.11^{A}	15.48±3.17^{A}	1.54±0.28^{A}
	Winter	165±87^{BC}	76±29^{C}	54±15^{C}	546.76±145.19^{A}	16.34±4.75^{A}	1.10±0.16^{AB}
	Spring	156±75^{BC}	34±13^{C}	87±17^{C}	566.90±175^{A}	16.94±07.56^{A}	1.14±0.27^{A}
	Summer	74±19^{C}	76±33^{C}	34±09^{C}	647.48±198^{A}	19.35±14.97^{A}	1.51±0.37^{A}

-: Absence of Bushy Rainbow Wrack.
*The different upper-case letters in the same row indicate the differences between the studied stations and seasons at the level ($p<0.05$)

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Glycinebetaine (GB), proline and total phenolic compounds (TPC)

GB parameter reaches a high value of 19.35±14.97 mg/g DW at (S8) The port station in Essaouira city during summer season, and achieved its minimum at (S3) Beddouza station near Safi city with 1.72±0.12 mg/g DW during autumn season. In the same way, the proline parameter has the same result as GB. The maximum value is recorded at (S8) The port station in Essaouira city with 647.48±198 µg/g DW during summer season, and a minimum value of 57.55±15.11 µg/g DW at (S3) Beddouza station near Safi city during autumn season. Moreover, TPC parameter has given the same result as GB and proline parameters which we have found a maximum value of 1.54±0.28 mg/g DW during autumn season at (S8) The port station in Essaouira city, as well as a minimum value of 0.52±0.12 mg/g DW at (S1) Sidi Bouzid coast near Eljadida city during winter season (*Table 5*).

Statistical analysis

A tow-way mixed ANOVA was conducted to investigate the impact of seasons, the sampling sites and Heavy metals concentrations in sea water. There was a significant main effect of sampling sites and seasons, $F(1, 66) = 183.397$, $p<0.001$ (*Table 6*). Moreover, there was a significant interaction between Heavy metals, Sampling sites and seasons, $F(1, 66) = 2.197$, $p<0.001$ (*Table 7*).

Table 6. *Tests of Within-Subjects Contrasts*

Source	Seasons_Sites	Type III Sum of Squares	df	Mean Square	F	Sig.
Seasons_Sites	Linear	298.239	1	298.239	183.397	0.000
Seasons_Sites * HM	Linear	316.802	66	4.800	2.952	0.000
Error(Seasons_Sites)	Linear	99.198	61	1.626		

Table 7. *Tests of Between-Subjects Effects*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	2251.892	1	2251.892	1114.998	0.000
HM	292.802	66	4.436	2.197	0.000
Error	123.198	61	2.020		

By performing the Principal Component Analysis (PCA) on the physiological parameters (Chlorophyll a, Chlorophyll c, Carotenoid, Proline, Glycinebetaine (GB) and Total Phenolic Compounds (TPC)) combined with the physicochemical parameters (Phosphorus and Nitrogenous compounds) and heavy metals (Cu, Cd, Pb and Cr) under study, two principal components have been extracted by covering 74.085 % of the cumulative variance (*Table 8*).

Discussion

Based on the online Algaebase data which includes marine algal species, Guiry and Guiry (2019) provided information about *Cystoseira* species diversity and discussed the description of new taxa and distribution. In Morocco, some authors have discussed the

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geographic distribution of *Bushy Rainbow Wrack* in the Mediterranean sea of Morocco (Ribera et al., 1992; Báez et al., 2005; Taskin et al., 2012; Bermejo et al., 2018; Moussa et al., 2018). However, no studies have been made in the Atlantic coast of Morocco concerning these species. Some authors have mentioned their sensitivity (Ballesteros et al., 1984; Thibaut et al., 2005). In contrast, the situation is more critical at Safi coast where only *Bushy Rainbow Wrack* seems to be more tolerant but all *Cystoseira* species have disappeared in 3 polluted stations (S2, S4 and S5). Many authors have showed the sensitivity of these brown seaweeds in polluted coasts (Cormaci et al., 1999; Thibout et al., 2005; Sales and Ballesteros, 2009). Furthermore, *Cystoseira* species are used as indicators of good water quality (Ballesteros et al., 2007).

Table 8. *Varimax with Kaiser Normalization rotated loading for 2 components. converged in 3 iterations with Principal Component Analysis extraction method according to heavy metals, physiological parameters in Bushy Rainbow Wrack and physicochemical compounds for the stations studied*

Parameter	**Component**	
	1	**2**
Cd	0.951	-0.059
Pb	0.645	-0.177
Cu	0.735	-0.327
Cr	0.961	-0.097
TP	0.101	-0.797
DP	0.912	0.093
PO_4^{3-}	0.905	-0.034
TN	-0.113	0.569
DN	0.084	0.965
NO_3^-	-0.215	0.528
NH_4^+	-0.653	0.162
Chl a	-0.940	0.240
Chl c	-0.824	0.279
Carotenoid	-0.753	0.509
Proline	0.967	-0.104
TPC	0.915	-0.220
GB	0.967	-0.104
Variance (%)	57.500	16.858
Cumulative (%)	57.500	74.085

The values of nitrogenous compounds are higher in comparison with the other ocean areas (Sverdrup et al., 1943). It is widely accepted that nitrogen may be an important factor in limiting marine algal productivity. Although the need to examine macroalgal-nitrogen relationships has long been recognized (Haas and Hill, 1933). Efforts with benthic marine algae have not paralleled those with phytoplankton.

The highly concentration of phosphorus was due to the huge amount of phosphogypsum used by the industrial factory. Inorganic nutrients are present in natural environments but their concentration near urban areas is usually enhanced (Nixon, 1995; Scavia and Bricker, 2006). The overloading of inorganic nutrients stimulates algal production and increases turbidity leading to changes in the species composition and the structure of littoral communities (Mcglathery et al., 2007). In addition, increased nutrient concentration in seawater favors opportunistic species, while long-lived species such as seagrasses and perennial macroalgae gradually decline (Munda, 1982; Schramm et al., 1999). Thus. the high phosphorus concentration in (S5) could explain the disappearance

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of *Cystoseira* species. It is known according to Celis-Plá et al. (2014) that nitrate and phosphate represent important macronutrients for macroalgae development and can protect the algae against stress. Moreover, highly concentrations of nutrients in seaweeds can reduce photoinhibition, as it has been observed in *Bushy Rainbow Wrack*. Other observations showed that nutrient enrichment could also have effects on photosynthesis, photo-protection and biochemical responses (Celis-Plá et al., 2016). Nonetheless, stress biology studies on heavy metal in brown seaweeds has demonstrate different results of molecular, biochemical and physiological effects, as it has been studied in *Ascophyllum nodosum* (Connan and Stengel, 2011a,b), *Fucus vesiculosus* (Nielsen and Nielsen, 2010) and *Ectocarpus siliculosus* (Roncarati et al., 2015; Sáez et al., 2015).

The comparison of our data with those previously studied by some authors (*Table 9*) shows that the concentrations of heavy metals: Cd, Pb, Cu and Cr for *Bushy Rainbow Wrack* in the present study recorded higher values in *Cystoseira* sp obtained by Akcali and Kucuksezgin (2011) of the Aegean Sea in Turkey, Al-Masri et al. (2003) at the Syrian Coast, Schintu et al. (2010) at Sardinia coast in Italy and Caliceti et al. (2001) at the Venice lagoon in Italy. Nonetheless, lower level of Pb and Cu than those reported by Schintu et al. (2010) and Caliceti et al. (2001), respectively.

Table 9. *Comparison of heavy metal levels (µg/g dry weight) in seaweed Cystoseira sp. from other different locations in the world*

	Cd	Pb	Cu	Cr	Location	References
***Cystoseira* sp.**	0.18	0.003	6.00	-	Aegean Sea, Turkey	(Akcali and Kucuksezgin, 2011)
	1.72	10.3	1.80	-	Sardinia, Italy	(Schintu et al., 2010)
	0.1-0.5	1.31	7.21	-	Syrian Coast	(Al-Masri et al., 2003)
	0.2	5.6	21	1.5	Venice lagoon, Italy	(Caliceti et al., 2001)
	2.6	4.43	10.6	2.9	Atlantic coast of Morocco	Present study

The relatively high levels of Lead in seawater could be attributed to discharges from industries near the study sites that could increase directly this element (leaching from gas stations), as well as leaching at the dump, traffic and leaching of farmlands, can contribute to the highly lead concentration at these stations. However, the lead concentrations at all stations are below the Moroccan standard 500 µg/l (FAO, 2006). For Cadmium detected in polluted waters, concentrations indicate a non-contamination by this element. Indeed, they are all below the limit value of 200 µg/l (FAO, 2006) (*Table 10*). The concentrations found at these sites, lead us to assume the presence of one or several sources of pollution near the stations above.

Table 10. *Comparison of heavy metal levels in seawater (µg/l) for the studied stations with the Moroccan standards for seawater (FAO, 2006)*

Heavy metals	Sea water	Moroccan norm FAO, 2006
Cd	1.151	200
Pb	2.563	500
Cu	0.604	500
Cr	0.987	2000

The resultant PCA (*Fig. 3*) presenting the loading of the variables on the two principal components demonstrate that Cd, Pb, Cu, Cr, DP, PO_4^{3-}, Proline, TPC and GB were the

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dominant correlated positive variables on the PC1 (0.951; 0.645; 0.735; 0.961; 0.912; 0.905; 0.967; 0.915; 0.967, respectively) while Chla, Chlc and Carotenoid the negatif correlated ones (-0.940; -0.824; -0.753, respectively), in addition to TN, DN and NO_3^-, the dominant variables on the PC2 (0.569; 0.965; 0.528, respectively). Indeed, according to PCA results, an important positive correlation and proportionate augmentation in proline, GB and TPC contents was recorded with increase in concentration of heavy metals and excessive Phosphorus in polluted stations mentioned, in addition to a negative correlation with pigments. It is proved by Alia et al. (1999) that Cadmium is the strongest inducer for proline and GB accumulation. Moreover, TPC can be released in stressful conditions from algal thalli and could react rapidly with carbohydrates and proteins to form UV-absorbing exudates (Koivikko et al., 2005). It has also been shown that proline, GB and TPC are the stress-induced substances in plants and algae under different kind of stress (Anbazhangan et al., 1988; Fadma et al., 2007; Abdel Latef and Sallam, 2015). Proline and GB concentrations in *Bushy Rainbow Wrack* in polluted areas are much higher than those found in other green, brown and red seaweeds (Fleurence, 2004). Furthermore, the maximum value of TPC in this study (1.54 mg/g dry weight during autumn season at (S8) The port station in Essaouira city) is higher than that found by Pereira and Yoneshigue (1999) in brown seaweed *Sargassum furcatum* (0.2-0.5 mg/g DW). According to this author, TPC may also be present in brown seaweed for reasons other than defense, as they have been considered to have a number of physiological and ecological functions.

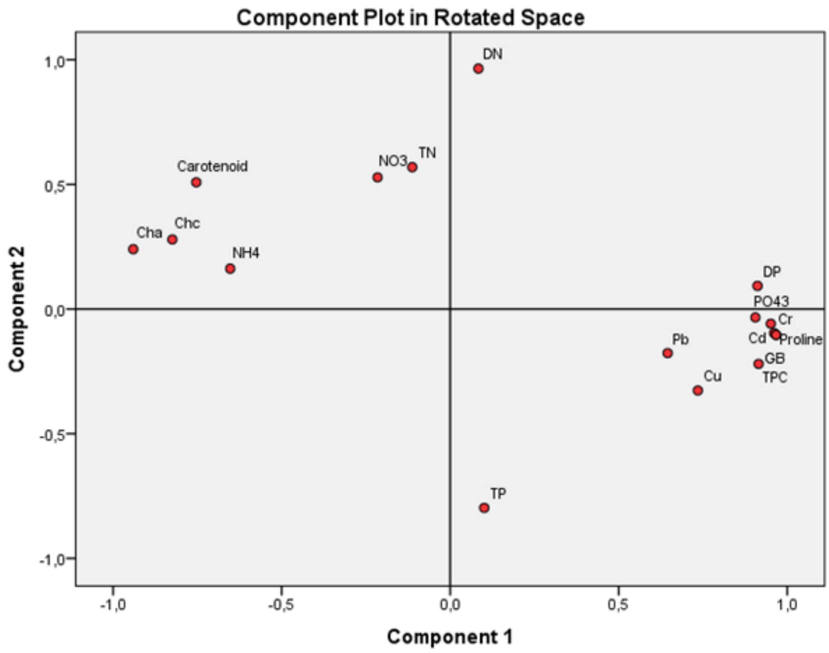

Figure 3. *Component Plot in Rotated Space for the parameters studied*

In the basis of the Conceptual framework of Mineur et al. (2014), anthropogenic local stressors create additional disruption often altering dramatically assemblage's structure. Global stressors are not manageable locally, but have local impacts and may indirectly affect local stressors. All stressors are affecting seaweed diversity (populations and communities), and directly impacting coastal ecosystems (*Fig. 4*) (Mineur et al., 2014).

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At the Industrial area (S4) and Phosphate area (S5) in Safi city and Jorf Lasfar area (S2) near Eljadida city, these stressors are due to increased nutrient inputs, heavy metal pollution and mostly derived from the industrial activity out there. According to Ferreira et al. (2011), marine biota living in coastal waters are under constant threat from exposure to elevated concentrations of pollutants, such as metals and nutrients, mostly derived from domestic, industrial and farming activities.

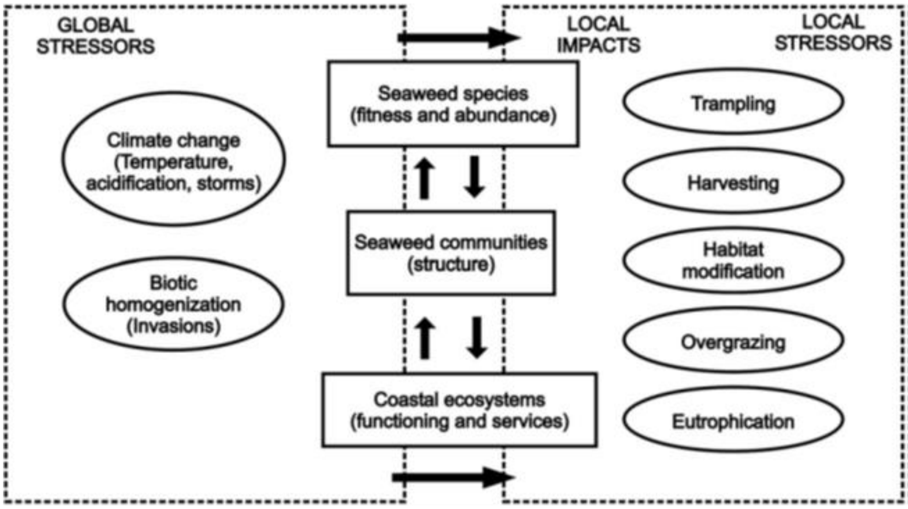

Figure 4. *Conceptual framework of Mineur et al. (2014)*

Changes in the composition of *Bushy Rainbow Wrack* through these stressors in polluted stations (S2, S4 and S5) has resonated through entire coastal ecosystems in this area. Furthermore, anthropogenic activities on these coastal areas, such as industrialization and urbanization create increasing anthropogenic stress. *Cystoseira* and seaweed biodiversity in general of the Moroccan coastlines need more attention to predict how they are affected by human activities.

Conclusion

To conclude, we have found a regular element variety in species lavishness with least qualities in winter and an auxiliary least top in spring, and most extreme qualities in summer. The regularity is for the most part because of the adjustment in biomass of the overwhelming species. Just *Bushy Rainbow Wrack* is by all accounts progressively tolerant to the contamination in polluted stations. Nitrogenous compounds did not clarify the decay of the species around there, yet the highly phosphorus concentration in the phosphate station and highly values of heavy metal concentrations could affect the physiology and clarify the vanishing of *Bushy Rainbow Wrack*, notwithstanding the nearness of dangerous components like Pb, Cd, Cu and Cr.

Thus, the investigation on the combined effects of nutrients, metals and biochemical parameters studied in this brown seaweed would provide relevant information about their capacity to withstand the future pollution scenarios. The interaction between metals and nutrients excess is still not well understood for macroalgae. Further examinations ought to be done to characterize different parameters that lead to *Bushy Rainbow Wrack* debasement.

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For future research, we aim to develop the culture experiments under controlled laboratory conditions on *Bushy Rainbow Wrack* as well as the genetic characterization of this brown seaweed based upon DNA barcoding of the cytochrome oxidase subunit 1 (COI), 23S rDNA (23S), and 23S-tRNAVal intergenic spacer (mt-spacer).

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