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► **To cite this version:**

Abderrahmane Aamiri, Nor-Eddine Rezzoum, Yasmine Touhamia, Hakima Zidane, Lhoucine Benhassan, et al.. Seasonal variation in biochemical and metal content of the invasive seaweed *Sargassum muticum* along Moroccan Atlantic Coast. *Journal of Applied Phycology*, In press, 10.1007/s10811-024-03240-y . hal-04578532

HAL Id: hal-04578532

<https://hal.science/hal-04578532>

Submitted on 17 May 2024

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Seasonal variation in biochemical and metal content of the invasive seaweed *Sargassum muticum* along Moroccan Atlantic Coast

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Abstract

Since 2012 Moroccan Atlantic coast have witnessed the introduction of the brown alien seaweed *Sargassum muticum* with a high invasive potential presenting environmental and economic risks. This study evaluates, for the first time, the effect of seasons and location on biochemical composition of *S. muticum*. Proteins, carbohydrates, lipids, pigments, minerals, and heavy metals were studied during four seasons from spring 2019 to autumn 2020 in four different sites along the Moroccan Atlantic coast (El Jadida, Saada (SA), Sidi Bouzid (SB), Moulay Abdallah (MA) and Jorf Lasfar (JL)). MA present highest proteins content (12.5% dw) during spring and highest levels of lipids and carbohydrates content during summer (1.8% dw and 41.5% dw respectively). Highest chlorophyll a content was found in JL and MA site with (21.6 and 21.45 mg ml⁻¹ respectively) during winter. Maximum chlorophyll c and carotenoids was found at SA with (2.27 and 9.71 mg ml⁻¹ respectively) during winter. In all locations and during all seasons, the mineral profile was rich in essential minerals such as Magnesium (Mg), Manganese (Mn), Zinc (Zn), Iron (Fe), Selenium (Se), Lithium (Li). Levels of toxic metals Al and Pb were lower than in previous studies whereas Cd and As turn to be slightly higher than the legislations limits for dry seaweeds. Therefore, in this context, some strategies of *S. muticum* valorisation should be favoured such as bioenergy, bioplastic or biostimulant over others such as human food and animal feed.

Key words: *Sargassum muticum*. Alien seaweed. Moroccan Atlantic coast. Seasonal variation. Biochemical composition. Heavy metals. Valorisation.

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Introduction

Sargassum muticum (Yendo) Fensholt (Yendo 1907) is one of the marine macroalgae with the

highest invasive potential (Boudouresque and Verlaque 2002) due to its large size, high percentage of reproductive branch biomass and a long fertile season (Engelen et al. 2015). This Japanese seaweed was accidentally introduced with the intentional transfer of the Pacific oyster *Crassostrea gigas* (Haroun and Izquierdo 1992; Rueness 1989). This brown macroalgae was introduced in the Eastern Pacific North America in the 1940s and it gradually extends its distribution to be located from south-eastern Alaska to Baja California (Wonham and Carlton 2006). In Europe, introduction of the species was first recorded as drift on Belgian coasts in 1972 (Belsher et al. 1985) and established populations were observed on the Isle of Wight (Southern England) in 1973 (Aguilar-Rosas and Galindo 1990). It was observed in numerous locations around the Channel region and spread during the next 30 years along European shores from Scandinavia to Portugal (Karlsson and Loo 1999; Monteiro et al. 2009) and in Mediterranean coastal lagoons: Thau (France) and Venice (Italy) (Curiel et al. 1998). In 2012, this species was first recorded in Africa along Atlantic coasts of Morocco (Sabour et al. 2013).

Invasive species are considered as one of the five major threats to aquatic biodiversity worldwide (Ashbrook et al. 1975). The effect of invasive species on marine biodiversity has been largely reported (Sala et al. 2000; Kettunen et al. 2009; Havel et al. 2011). Negative impacts of invasive aquatic species in Europe are high and have been estimated to cost at least 2.2 billion euro per year (Keller et al. 2011).

Seaweeds are one of the most important commercially renewable marine resources of the world with long exploitation history. They have been used since ancient times as food, fodder, biofertilizer and raw material for many industries and as a source of medicinal drugs (Chanda et al. 2010). Moreover, macroalgae are well known for having a wealth of nutritional benefits. A wide range of studies reported the high potential of using algae as a source of prebiotics (Charoensiddhi et al. 2020; Wang et al. 2006; Kuda et al. 2005; Gudiel-Urbano et al. 2002). Moreover, animal models and different experimental approaches reveals potential anti-obesity effect of algae diet as some macroalgae revealed beneficial effects on body weight management and energy metabolism (Park et al. 2012; Oh et al. 2016; Kim et al. 2019). Marine algae are often termed as super-food because it is seen as a healthy and nutritious (Ahmad et al. 2012). They are very low in fat, although they contain high percentages of mono and polyunsaturated fatty acids and are very rich in carbohydrates (mainly dietary fibres), proteins, containing all the essential amino acids, and vitamins, including vitamins A, C, E, and those of the complex B, which are usually absent in land vegetables (MacArtain et al. 2008).

Several studies have reported that *S. muticum* contains high amount of secondary metabolites, such as phlorotannin, fucoxanthin, fucoidan and meroterpenoids with biological activities (Lim et al. 2002; Costa et al. 2010; Kumar et al. 2008; Vijayabaskar et al. 2012). Previous studies have shown that *S. muticum* has antioxidant, antibacterial, immunomodulatory and anti-inflammatory properties (Gudiel-Urbano et al. 2002; Yoon et al. 2010; Chae et al. 2013). Seaweeds show great variation in their nutrient contents due to several environmental factors such as water temperature, salinity, light, nutrients and biotic factors, (Kamiya et al. 2010; Murakami et al. 2011; Dang et al. 2017). The ecological and economic value of *Sargassum* vary with their biochemical characteristics changing with season (Paiva et al. 2018) and geographical position (Haroon et al. 2000). Evaluating this spatial and temporal variability is particularly important in a context of valorisation of *S. muticum* using components for nutraceutical applications.

Despite the nutritional properties associated with algae, they have the capacity to accumulate not only essential elements but also toxic compounds such as heavy metals (Wallenstein et al. 2009; Bauddh et al. 2022), it is well documented that brown algae have the capacity to accumulate metals making them reliable bioindicators of metallic contamination (Caliceti et al. 2002; Al-Masri et al. 2003; Lozano et al. 2003; Abdallah et al. 2005). Holopelagic *Sargassum spp.* are indeed known to present high levels of arsenic (Dassi'e et al. 2021; Devault et al. 2021; Cipolloni et al. 2022). The high metal absorption capacity of *Sargassum* is due to alginate present in cell walls with a high metallic affinity (Davis et al. 1999; Vieira et al. 2000). Thus, the quantification of toxic elements in seaweed are necessary before their valorisation. Nevertheless, the exploitation of the seaweed sector in Morocco is limited, and it is mainly based on the exploitation of crud material (Zaim et al. 2020). Contamination of *S. muticum* depends on the bioavailability of metal potentially changing according to spatial and temporal scales. This contamination can also variate according to biological status of algae.

In this context, the present paper evaluates potential valorisation of *S. muticum* harvested from Moroccan Atlantic Coast measuring their temporal and spatial variations in their *i*) nutritional properties (proteins, carbohydrates, lipids, pigments and minerals) and *ii*) contamination (heavy metals).

Materials and methods

Sampling and Preparation of vegetal materiel

Sargassum muticum is a brown seaweed (Phylum Ochrophyta, Class Phaeophyceae, Order Fucales, Family Sargassaceae and Fucaceae) sampled between April 2019 and Mars 2020

from four sites of the Moroccan Atlantic coast from El Jadida, Saada (SA) (33°13'02.55", 08°33'51.41"), Sidi Bouzid (SB) (33° 13' 52", 8° 32' 51"), Moulay Abdallah (MA) (33° 11' 52", 8° 35' 18") to Jorf Lasfar (JL) (33° 07' 36", 8° 37' 13"). In all sites the alien seaweed *S. muticum* is frequently present forming an extensive canopy. Thalli were handpicked and immediately thoroughly washed with seawater (previously collected from local beach) to remove impurities then kept in icebox and transported to the laboratory. Once at the laboratory the leaves epiphytes were removed with paper towel without damaging the organs. Then the samples were rinsed carefully with tap water and dried at ambient temperature. Finally, the dried seaweed was cut with scissor into small pieces of a few centimetres then grounded to powder using electrical grinder and stored at -20C° for further use.

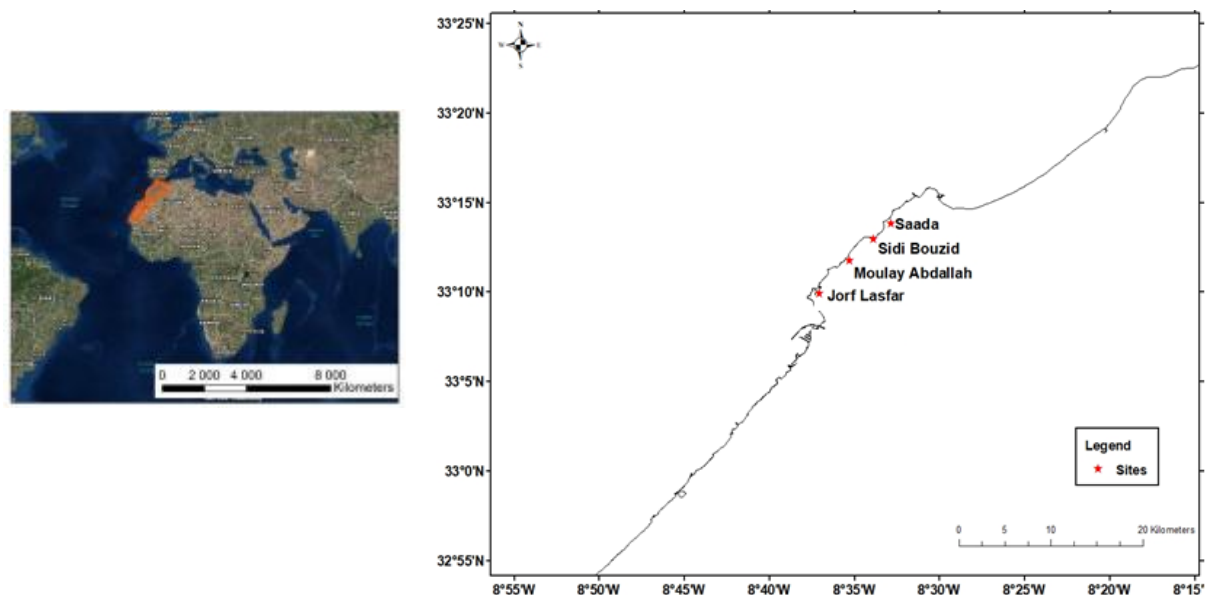


Fig. 1 Location of the four sampling sites of *Sargassum muticum* along the Atlantic coast of Morocco using Arc GIS10.8.

Biochemical analyzes of algal biomass.

Determination of biochemical compositions.

Grounded *S. muticum* samples were used to measure total protein content using spectrophotometer (Bradford 1976), total carbohydrate using method of Dubois et al. (1956) and the lipid content chloroform-methanol mixture as described by Folch et al. (1975).

Determination of chlorophyll a, c and carotenoids.

1g of algal biomass was sonicated for 15 min (130 W, 20 kHz) in 10 ml of acetone and stored

overnight at 4°C. Afterwards, the sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and the absorbance (A) at 664, 648 and 480 nm was measured in a UV-Vis spectrophotometer. Acetone was used as a white. The pigment content (chlorophyll a, chlorophyll c and total carotenoids) is calculated respectively using the equations described by Jeffrey and Humphrey (1975) and Lichtenthaler (1987).

$$\text{Chlorophyll a (Chl a)} = 13.36 \times A_{664} - 5.19 A_{648}$$

$$\text{Chlorophyll c (Chl c)} = 24.36 \times A_{648} - 3.73 A_{664}$$

$$\text{Carotenoids} = \frac{(1000 \times A_{480} - 1.63 \times \text{Chl a} - 104.96 \times \text{Chl c})}{221}$$

221

Minerals and heavy metals

All samples were analyzed using an optical emission spectrometer, THERMO Scientific series iCAP 6200, driven by iTEVA software. For each sample, a fixed amount of algal powder (1g) was placed in a polytetrafluoroethylene tube with a mixture of concentrated nitric acid (HNO₃) and concentrated hydrochloric acid (HCl) in a 3:1 ratio was added. The powder sample was then mineralized for 10 min at 170°C and a pressure of 40 bar using a microwave (BERGHOF) (NF EN 16174). After mineralization, deionized water was added to each sample. With the same process, we analyzed the certified reference materials. Concentrations of 17 elements (Mg, Sr, Al, Fe, As, Mn, Zn, Ba, Cd, Cr, Ni, Se, Li, Co, Cu, Mo, and Pb) were determined following the norms of NF EN 16170, and heavy metal concentrations were expressed as mg of metal per kilogram of algal dry weight (mg Kg⁻¹ d.w.). To prevent contamination, all bottles were immersed in the acid. The quality controls in this work were selected based on the criterion of method accuracy. Each analytical batch included triplicate samples, standard reference samples, and blanks. Analytical accuracy was verified through the analysis of certified reference material. These controls were established using multi-element standards prepared from single-element standard solutions of the CPAchem brand, certified to ISO 17034. The quantification limits (LOQ) of values for Mg, Sr, Al, Fe, As, Mn, Zn, Ba, Cd, Cr, Ni, Se, Li, Co, Cu, Mo, and Pb were 50, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5, 0.25, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5, 2.5 mg Kg⁻¹ d.w., respectively. The limit of detection (LOD) of each element was computed from its calibration plot. The LOD values for Mg, Sr, Al, Fe, As, Mn, Zn, Ba, Cd, Cr, Ni, Se, Li, Co, Cu, Mo, and Pb were 0.001702, 0.000222, 0.016877, 0.004762, 0.003421, 0.000079, 0.000179, 0.000609, 0.000119, 0.000939,

0.000800, 0.003356, 0.006377, 0.000403, 0.000654, 0.000852, 0.001849. mg Kg⁻¹ d.w., respectively.

Statistical analysis

To determine the potential relationships between the studied parameters in *S. muticum* collected at the different sites and seasons, the statistical analysis was executed on RStudio (R Version 4.0.5), Primer/ Permanova and XLSTAT. The normality of distribution data in *S. muticum* samples for all studied sites was checked based on the Shapiro-Wilk's test. Correlation matrix was used to determine the relation among all the studied parameters using Person (parametric) or spearman (nonparametric) rank order correlations. Principal Component Analysis (PCA) was performed to identify data clusters among the stations, seasons and studied parameters. The differences between the amount of the studied parameters in *S. muticum* within sites and seasons were determined by analysis of variance (ANOVA) or Permanova tests. The p value ≤ 0.05 was identified as the significance level.

Results

Biochemical composition in *Sargassum muticum*

Biochemical compositions of *S. muticum* according to site and seasons are presented in table 1.

Table 1 Mean concentrations (% Dry Weight) of Proteins, carbohydrates and lipids content and pigments (mg ml⁻¹) in *S. muticum* collected during each of the four seasons (autumn 2019 - summer 2020) in each the four locations.

biochemical parameters		Proteins	Carbohydrate	Lipids	Chl a	Chl c	Carotenoides
Locations	Seasons						
SA	Autumn	6.4±3.19	27.6±5.34	0.50±0.29	13.45±3.78	4.44±2.33	1.5±0.59
	Winter	7.3±0.43	19.3±7.13	0.6±0.50	15.19±4.29	9.71±2.5	2.27±0.74
	Spring	12±1.19	22.2±6.89	1.1±0.73	9.83±5.69	7.78±3.43	1.11±0.65
	Summer	8.4±2.50	29.8±7.79	1.6±0.53	6.64±6.29	1.9±2.07	0.16±0.59
SB	Autumn	8.5±0.76	17.7±8.86	0.2±0.51	9.51±4.79	3.08±2.10	0.95±0.62
	Winter	6.1±1.1	35.4±6.19	0.7±0.49	15.05±3.99	5.23±2.03	1.47±0.72
	Spring	8.6±2.19	21.1±7.33	0.7±0.3	16.45±4.1	4.81±2.63	1.02±0.58
	Summer	5.3±4.34	36±7.90	1.8±0.58	5.07±4.2	2.54±2.9	0.26±0.66
MA	Autumn	6.5±3.29	37±9.19	0.3±0.44	14.36±4.76	3.59±1.93	0.93±0.47
	Winter	6±1.01	19.6±6.79	0.7±0.5	21.45±6.29	5.59±2.69	1.54±0.60
	Spring	12.5±1.25	36.8±9.99	0.5±0.63	17.33±5.89	5.26±1.53	0.34±0.79
	Summer	9.6±2.03	41.5±7.14	1.8±0.55	13.84±3.01	0.14±2.03	0.21±0.59
J	Autumn	9.1±1.59	30±8.19	0.6±0.78	16.87±6.0	4.1±3.43	1.26±0.62

Winter	8.1±0.69	32±10.27	0.9±0.49	21.6±8.17	7.71±2.43	2.17±0.67
Spring	5.7±1.87	28.1±6.74	1.3±0.42	17.27±3.29	3.27±3.14	0.52±0.71
Summer	10.6±3.09	30.4±10.29	1.6±0.39	7.31±5.05	2.42±2.37	0.46±0.68
Mean ± SD	8.1±2.19	29.03±7.32	0.9±0.53	13.8±4.9	4.4±2.4	1±0.66
Max	12.5±1.25	41.5±7.14	1.8±0.55	21.6±8.17	9.71±2.5	2.27±0.74
Min	5.3±4.34	17.7±8.86	0.2±0.51	5.07±4.2	0.14±2.03	0.16±0.59

As shown in Table 1 the proteins content ranges from 5.3 to 12.5% dw and was always higher during spring in the four sites. In the present study the protein content does not present significant seasonal variation $p>0.05$ (ANOVA).

Regarding the lipid content varied clearly with season $p<0.05$ (ANOVA) ranging from 0.2% to 1.8% dw. Thus, total lipid content was maximum in summer followed by spring then winter and autumn respectively. However, during summer MA site recorded the highest lipid content with 1.8% and minimum in SB site during autumn 0.2%.

The results show that the Carbohydrate content of *S. muticum* is collected from four sites content was relatively constant ranging from 17.7 to 41.5% dw throughout the year except Summer it peaks, hence changes in carbohydrate content were not observed throughout the study period according to ANOVA $p>0.05$.

Briefly maximum carbohydrate content recorded in MA site during summer with 41.5% and the minimum in SB site during autumn with 17.7%.

Regarding the proximate composition of the apolar pigment content a significant seasonal variation $p<0.05$ (ANOVA) was observed. Thus, the highest chlorophyll a content was found in JL and MA sites (21.6 and 21.45 mg ml⁻¹ respectively) during winter, regarding carotenoids and chlorophyll c maximum was found at SA site with 2.27 and 9.71 mg ml⁻¹ respectively during the same season.

Minerals and heavy metals in *Sargassum muticum*

The seasonal variation within sites and season of 17 analysed elements in *S. muticum* reported in Table 2 indicating that the variation in the mean concentrations of minerals and heavy metals in *S. muticum* showed a decrease in the following order: Mg > Sr > Al > Fe > As > Mn > Zn > Ba > Cd > Cr > Ni > Se > Li while Co, Cu, Mo and Pb found to be less than 2.5 mg Kg⁻¹. Hence, statistical analysis using ANOVA revealed no significant sampling site effect on the minerals and heavy metal composition in *S. muticum*. However, for the seasonal effect ANOVA or Premanova tests, showed that the seasonal variation of the following elements Mg, Cr, Sr, Li and As were significant $p<0.05$. In contrast, there is no significant variation in

season for the rest of studied elements $p > 0.05$.

Table 2 Mean concentrations (mg Kg⁻¹ Dry Weight) of Mg, Sr, Al, Fe, As, Mn, Zn, Ba, Cd, Cr, Ni, Se, Li, Co, Cu, Mo and Pb in *Sargassum muticum* collected during autumn 2019 - summer 2020 along the studied sites.

heavy metals	Cu	Mg	fe	Zn	Mn	Co	Se	Cr	Mo	Li	Ni	Al	Ba	Sr	Cd	As	Pb	
SITE	Season																	
SA	Autumn	<2,5	8660	420	9,24	16,8	<2,5	1,39	2,45	<2,5	1,63	<2,5	279	9,62	857	2,09	17,1	<2,5
	Winter	<2,5	12690	204	7,81	7,04	<2,5	1,42	0,96	<2,5	1,08	<2,5	140	10,6	759	3,4	10,4	<2,5
	Spring	<2,5	8704	372	12,7	12,5	<2,5	2,09	1,9	<2,5	1,3	<2,5	292	14,9	998	2,71	32,5	<2,5
	Summer	<2,5	8389	914	15,4	56,7	<2,5	1,84	3,72	<2,5	2,29	3,29	704	10,1	822	2,26	40,8	<2,5
SB	Autumn	<2,5	7459	823	14,7	24	<2,5	3,38	3,54	<2,5	1,92	3,54	926	13,7	1168	3,56	26	<2,5
	Winter	<2,5	10640	207	7,31	8,9	<2,5	2,64	0,98	<2,5	1,18	<2,5	146	9,88	871	3,53	12,1	<2,5
	Spring	<2,5	8852	210	10	8,94	<2,5	3,12	1,15	<2,5	1,1	<2,5	149	10,3	1017	2,18	28,9	<2,5
	Summer	<2,5	8910	325	7,6	13,4	<2,5	2,63	1,32	<2,5	1,45	2,56	251	8,77	994	2,08	59,7	<2,5
MA	Autumn	<2,5	8307	494	12,7	53	<2,5	2,53	3,72	<2,5	1,82	3,3	428	12,1	1055	4,66	26,8	<2,5
	Winter	<2,5	11940	297	14,4	10,5	<2,5	2,1	2,09	<2,5	1,72	<2,5	233	14,6	747	4,42	7,9	<2,5
	Spring	<2,5	8435	292	13,4	9,87	<2,5	1,81	1,47	<2,5	1,04	<2,5	268	16,4	1040	3,91	17,8	<2,5
	Summer	<2,5	9339	504	12,7	23,9	<2,5	2,56	1,57	<2,5	1,23	2,69	236	10,4	992	5,21	53,4	<2,5
J	Autumn	<2,5	10510	498	17,9	17,5	<2,5	2,21	3,12	<2,5	1,74	2,75	540	13,5	992	6,58	7,62	<2,5
	Winter	<2,5	10160	301	13,2	8,82	<2,5	2,4	<2,5	<2,5	1,45	<2,5	253	12,8	894	5,49	10,3	<2,5
	Spring	<2,5	8784	206	10,3	7,88	<2,5	2,83	<2,5	<2,5	1,09	<2,5	209	16,6	1033	4,67	13,7	<2,5
	Summer	<2,5	8546	502	11	15,1	<2,5	2,77	2,5	<2,5	1,47	2,53	431	11,6	1043	3,04	22,1	<2,5
mean	<2,5±0	9395,3±1419	410,3±211	11,8±3,03	18,4±15,1	<2,5±0	2,3±0,56	2,1±1,01	<2,5±0	1,4±0,36	2,9±0,41	342,7±217,8	12,2±2,4	955±117,27	3,7±1,3	24,1±15,8	<2,5±0	
max	<2,5	12690	913,9	17,9	56,74	<2,5	3,38	3,72	<2,5	2,29	3,54	926,3	16,58	1168	6,58	59,74	<2,5	
min	<2,5	7459	204,1	7,314	7,036	<2,5	1,392	0,96	<2,5	1,041	2,525	140,4	8,772	746,5	2,077	7,615	<2,5	

As shown on the Table above, the highest Sr, Al, Ni and Se concentration in *S. muticum* was detected in autumn for SB site (1168, 926, 3.54 and 3.38 mg Kg⁻¹, respectively), on the other hand, the highest levels of As (59,7 mg Kg⁻¹) were observed for samples collected from the same station during summer.

SA site recorded the highest concentration of Fe, Mn, Cr and Li for *S. muticum* in summer with (914, 56.7, 3.72 and 2.29 mg Kg⁻¹ respectively), while the highest Mg concentration (12690 mg Kg⁻¹) recorded in winter in the same site.

The brown alga *S. muticum* collected from JL site recorded the highest concentration of Zn and Cd (17.9 and 6.58 mg Kg⁻¹) in summer and Ba (16.6 mg Kg⁻¹) during spring.

These findings pointed out that oligo-elements and heavy metals distributed differently among the four seasons within sites. Toxic metals (Cd, Al) and essential metals (Zn, Mn, Cr, Li, Mg) show higher concentrations during cold seasons (winter and autumn), on the other hand toxic metalloid As and other essential metals namely (Fe, Se, Sr, Ba) show higher concentrations in warm seasons (spring and summer).

Spatial and temporal variations in Biochemical and heavy metals composition of *Sargassum muticum*

We used Principal Component Analysis (PCA) to determine the influence of site/seasons on the production of metabolites in *S. muticum* harvested from four different sites along Moroccan Atlantic coast.

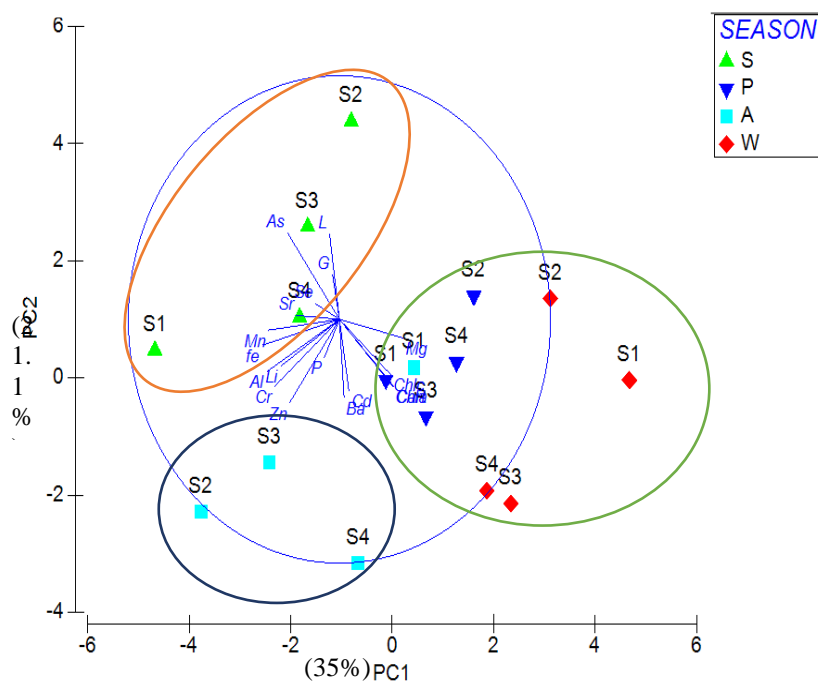


Fig. 2 Principal component analysis scatter plot representing the scores for the biochemical data (proteins, carbohydrate, lipids, pigments, minerals, and heavy metals) in *Sargassum muticum* and their variation within site and seasons. Abbreviations in the figure are as follows: P (Proteins), L (Lipids), G (carbohydrate), Chla (Chlorophyll a), Chlc (Chlorophyll c), Carot (Carotenoids), S1 (Saada site), S2 (Sidi Bouzid site), S3 (Moulay Abdellah site), S4 (Jorf lasfar site), S (Summer), P (Spring), A (Autumn) and W (Winter).

By applying the principal component analysis (PCA) on the biochemical and heavy metals composition, two principal components have been extracted by covering 56.1% of the cumulative variance (figure 2). We focused on the first two dimensions of PCA for the interpretation. The first principal component (PC1) revealed 35% of the total variance and the second, 21.1% (PC2) of the total variance. The PCA analysis of the variation in *S. muticum* biochemical composition by the sites showed an absence of any tendency for spatial preference, as sites are grouped independently of their geographical origins. Whereas seasons are separated from one to other hence it is more influenced on the biochemical composition of *S. muticum*. Thus, the PCA analysis showed that the summer, characterized by high concentrations of nutrients (lipids and carbohydrate) minerals (Sr and Se) and the toxic heavy metal As, whereas autumn was more characterized by higher proteins and minerals (Zn, Mn, Li, Cr, Al and Fe) concentrations. In contrast spring and winter were characterized by high concentrations of pigments (Chl a, Chl c and Carotenoids), minerals (Ba and Mg) and the toxic heavy metal Cd, whereas the dominant season is spring over winter for this cluster.

Discussion

Biochemical composition in *Sargassum muticum*

Proteins contents observed in the present study (5.3 to 12.5% dw) are in the range of values previously observed in *S. muticum* from the Atlantic in France with 8.4 % (Jard et al. 2013) and 3.2-29% (Hardouin et al. 2014) and Spain with 7.0-11 % (Balboa et al. 2015), from Indian Ocean with 19.62% and red sea with 5.31% (Ahmed et al. 2015) and 3.2 % (Fouda et al. 2019). Other studies recorded that the proteins content in seaweeds ranged from 5 to 24% of dry Weight (Azzazy et al. 2019). The elements content in seaweeds may be dependent on various environmental factors, including concentrations of elements in water, interactions between elements, salinity, pH and light intensity (Dhargalkar et al. 1980; Andrade et al. 2004). According to Żbikowski, protein content differs between seaweed species and, in general, red (Rhodophyta) and green (Chlorophyta) algae species are characterized by higher protein content in comparison to brown algae species (Phaeophyceae) (Żbikowski et al. 2006).

Some authors prove that the protein content of seaweeds can vary according to species, seasonal period and geographic area (Chakraborty et al. 2012; Ibañez et al. 2013; Fleurence et al. 1999). The levels of proteins are steady during all seasons with slight increase during the spring could be explained from the suggestion that the nitrogen amount is stable in all studied site, since there is a positive correlation between protein and nitrogen content has previously been reported in brown seaweeds (Kim et al. 2013). With no significant statistical differences between seasons in *S. muticum* collected from Moroccan Atlantic coast. The seasonal variation in the protein content might be mainly due to the locations and prevailing environmental parameters such as temperature, salinity, and nutrient contents carbon, nitrogen nitrate phosphate and water motion (Roleda et al. 2019).

Holdt et al. (2011) and Haroon et al. (2000) highlighted that seaweeds are rich sources of the acidic amino acids namely aspartic acid and glutamic acid; whereas lysine, tryptophan, histidine, threonine, sulfur amino acids (cysteine and methionine) have been perceived as limiting amino acids in algal proteins, whilst their levels are higher than those found in terrestrial plants.

The lipid content (0.2% to 1.8% dw) reported in the present study coincided with the ones recorded for Fouda et al. (2019) found 0.12% from *S. muticum* collected from red sea, moreover Balboa et al. (2015) in Spain showed that in fact, *S. muticum* lipid content varies throughout seasons, reaching its maximum 3.2% during the spring and its minimum 1.6% in the winter. Similar values were described also by others, studying the amount of lipid in *S.*

muticum from different localization in Portugal 1.45 ± 0.07 (Rodrigues et al. 2015) and during spring in the North Brittany (France) with 1.0 ± 0.1 (Hardouin et al. 2014). The seaweed lipid content varies by geographical location, season, temperature, salinity, and light intensity as well as interactions among these factors (Miyashita et al. 2013).

According to Manivannan et al. 2008, seaweeds are known for their low-fat content, which varies significantly throughout the year. It is well known that total lipids content and fatty acids composition varies seasonally in brown seaweeds. Hence, the seasonal changes of lipid composition have been determined in *S. schimperi* (peaks in summer season) (Polat et al. 2013), *Stephanocystis hakodatensis* (peaks in winter season) (Nomura et al. 2013), *S. horneri* increased from mid-autumn and reached a maximum in midwinter and then decreased thereafter (Nomura et al. 2013) and *S. oligocystum* (peaks spring season) (Praiboon et al. 2018).

Lipids are essential in nutrition, even if lipids are considered minor components in brown algae for their low content, most brown seaweed lipids have higher contents of omega-3 polyunsaturated fatty acids than omega-6 PUFAs. Because of this high proportion of omega-3 PUFAs, the omega-6/omega-3 ratio of food lipids could be modified by incorporating brown seaweeds (Prabhasankar et al. 2009). (Rocha et al. 2021) found that among several species collected from Portuguese seashores *S. muticum* presenting the highest value, with the contribution of height unsaturated fatty acids for the total fatty acids content being particularly high (of 28% of total fatty acid).

The present values of carbohydrate (17.7 to 41.5% dw) are in the range reported for *S. muticum* collected from other localizations, such as Spain in June with 26-46 % (Kim et al. 2007), Portugal (Rodrigues et al. 2017) in April with 49 %, while in Isle of Wight (England) in April 1977 and June 1978 with 35.1 % (Gorham et al. 1984). Whereas at red sea in Egypt during summer and spring (35.37- 24.79 % respectively) (Fouda et al. 2019; Azzazy et al. 2019). Highest carbohydrate amount for *S. muticum* collected from Brittany (France) between July 2010 and June 2012 (16.6 and 9.8 % respectively). Moreover, Rodrigues et al. (2017) documented that in *S. muticum* total sugars content 49.3%. Thus, the active period of carbohydrate synthesis coincides with the decrease in protein concentration in seaweed and vice-versa. However, some authors found that the amounts of carbohydrates reached it maximum during the summer months in other brown algae, e.g., *Laminaria* spp. (Lee et al. 2017; Black et al. 1948).

According to Devault et al. (2021) the main carbohydrate families found in *Sargassum* spp. are alginates, fucose-containing sulphated polysaccharides (usually described as fucoidans),

laminarin and mannitol, as well as cellulose, which, in most studies, is not quantified. Moreover, the carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes (Devault et al. 2021).

Polysaccharides are the main constituents of brown algae namely (Fucoïdians, Laminarin and Alginate) their diverse structures allow many unique physical and chemical properties that help to moderate a wide range of biological activities, including immunomodulation, antibacterial, antioxidant, prebiotic, antihypertensive, antidiabetic, antitumor, and anticoagulant activities (Li et al. 2021).

Pigments content of the investigated algae did not varied among sites while, it was fluctuated between seasons. The contents of chlorophyll a, chlorophyll c and the carotenoids decreased dramatically during summer in all studied sites. Therefore, there may be photodegradation of the pigments during the summer days. Thus, our results lined with other researchers found that the content of chlorophylls was higher than the carotenoids content (Hidayati et al. 2019; Yalçın et al. 2021). In the same context Yuan et al. (2014), documented that the temperature affects chlorophyll and carotenoids contents of *S. muticum*, whereas the temperature reached 25 °C, the chlorophyll a and carotenoid content was the highest, when the temperature reached 30 °C, chlorophyll a and carotenoid content was the lowest (Photobleached), thus pigment composition it could be affected elevated temperature for this reason the pigment amount increase in cold weather and decreased in hot weather in the present study.

By monitoring pigment composition and photosynthesis of *S. muticum* every month, Gorham and Lewey (1984) found that the faster the growth, the lower the pigment concentration. Suggesting that it might be the generation speed of pigment was lower than algae growth speed, resulting in pigment reduction because of dilution. Fucoxanthin represents the main pigment responsible for the colour of brown macroalgae. According to Cikoš et al. 2022, the presence of fucoxanthin overlaps the presence of other pigments in a macroscopic point of view even though the chlorophyll content is sometimes higher in brown macroalgal species. Other than fucoxanthin, brown macroalgae contain Chl a, Chl c and β -caroten (Cikoš et al. 2022). The changes in pigment composition of *S. muticum* reported here are also substantial; the pigment composition of *S. muticum*, of which chlorophyll a, chlorophyll c and carotenoids are the major components. In fact, brown algae have been receiving more and more attention in the scientific community due to their richness in this pigment, which has documented biological properties (Osório et al. 2020). Therefore, there in an enormous interest in exploiting these pigments in several applications, algal pigments have great commercial value as natural colorants in nutraceutical, cosmetics, and pharmaceutical industry, besides their

health benefits (Prasanna et al. 2007).

Seasonal variation pattern of pigments is not the same in all species. Authors reports that pigmentation of *Fucus serratus* from the Trondheim fjord (Norway) was found to be relatively constant, as was that of *Ascophyllum nodosum* except for a maximum in early spring (Ramus et al. 1977). In contrast, the pigment content of *Pelvetia canaliculata* showed a pronounced seasonal variation, with maximum values in winter and pronounced minima in September for all the pigments (Hupel et al. 2011 and Zavodnik (1973)) has also reported a winter peak of pigmentation in *F. virsoides*, which confirmed our results.

The present study shows that *S. muticum* harvested from the Moroccan Atlantic coast have a good potential for further valorisation given their good nutrient profiles as a source of nutritional carbohydrates, pigments and lipids. They possess higher nutritional qualities than other *S. muticum* collected from different localisations in terms of pigments and lipids during winter and summer season respectively. In comparison proteins and carbohydrate amount is found to be steady during the seasons with slight increase in spring. Spring would consequently be an adapted period for harvesting this alien seaweed in terms of economic aspects. Nevertheless, the different collection sites did not significantly change the biochemical composition of *S. muticum*.

Minerals and heavy metals in *Sargassum muticum*

The capacity of algae to accumulate metals depends on a variety of factors, the two most relevant ones being the bioavailability of metals in the surrounding water and the uptake capacity of the algae (Sanchez et al. 2001). Moreover, it has been documented that physiological changes and growth can affect concentrations of metals in the macroalgal tissue (Huerta et al. 2007). The concentrations are generally low in summer when growth rates are high and the accumulated metals are diluted, and high in winter when the metabolic processes slowdown (Brix et al. 1983; Hou et al. 1998; Zbikowski et al. 2006). Moreover, the concentrations found at these sites, lead us to assume the presence of one or several sources of pollution near the studied sites. As is a metalloid present naturally in the open and coastal marine environment, at a concentration level of 15 and 25 nM as hydrogen arsenate. Moreover, only certain forms of As can be toxic, as inorganic forms present higher toxicity than the organic ones (trivalent state As III and the pentavalent state As V) (Fattorini et al. 2006; Circuncisao et al. 2018). Traditional processes of soaking and boiling applied to *S. fusiforme* present the advantage of significantly reducing their arsenic total content 30 to 60% (Hanaoka et al. 2001). Nonetheless, it was also shown that cooking can decrease total arsenic

content by up to 90% depending on temperature and pH. Nevertheless, arsenic concentrations in the seaweed after processing can still exceed regulatory levels (Katayama et al. 2015).

For most countries, there is no regulation on the maximum levels of heavy metals in seaweeds. Surprisingly, only a few countries have actually established specific regulations for the use of seaweeds in human food including limit values for relevant toxic elements. There is little legislation on seaweeds in the European Union, so little control is exercised on potentially harmful metals. France was the first European country to set up regulations on the use of seaweeds for human consumption as non-traditional foods (Burtin 2003). Regarding the concentrations of total As, Cd and Pb, in *S. muticum* detected in this study was above the standard limits established for human consumption and animal feed consisting exclusively or mainly of seaweed except for lead, whereas these concentrations were below the standard limits for agriculture as biostimulant (table 3).

Table 3 Maximum levels of heavy metals in algae authorized for human consumption, animal feed and agriculture (mg kg⁻¹)

Countries	Human consumption					Animal feed					Agriculture				
	TolAs	iAs	Cd	Hg	Pb	TolAs	iAs	Cd	Hg	Pb	TolAs	iAs	Cd	Hg	Pb
French	–	3	0.5	0,1	5	40	2	1	0,1	10	18	–	3	2	180
Europe	40	3	1	–	–	40	3	1	0,1	10	30-50	40	1,5	1	120
China	–	3	1	–	–	40	3	1	–	–	–	–	–	–	–
USA	–	3	–	–	10	–	3	–	–	10	–	41	39	17	300
Mexico	–	–	–	–	–	–	–	–	–	–	22-75	–	–	–	–
References	AFSSA (2009) , Regulation (EU) 2015/1933,Food Chemical Codex 3rd Ed seaweeds,(Cherry et al. 2019),Regulation (EC) 396-2005,Standards NF U 42-001, NF U,Regulation (EU)2019/1009,(US Composting Council) Based on 40 CFR 503, norma oficial mexicana nom-147-semarnat/ssa1-2004														

Nevertheless, the levels of toxic metals Pb in all the studied sites were lower in the current study compared with previous reports and the standard limit legislation, while Cd and As turn to be slightly higher than French, Europe, USA and China legislations for dry seaweeds for food and feed. Worthy of note is the fact that in this feed study, As was measured as total As.

Our results discussed based on comparison of these heavy metals levels in *S. muticum* from other different locations in the world. Therefore, the comparison of our data with those previous studies shows that the heavy metal concentrations reported in this paper were generally lower while oligo-elements were higher (Table 3 and 4). Thus, as previously reported by many other researchers (Phillips et al. 1994; Akcali et al. 2011; Haritonidis et al. 1999), there may be a number of reasons for the seasonal differences found, including environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH etc., metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors. These data might help us to understand the effect of seasonality on the importance of

minerals for harvesting *S. muticum*. The richness of these minerals such as Mg, Fe, Zn and Mn also makes *S. muticum* important source for valorisation due to its enhanced nutritional properties.

Table 4 Mean metal concentrations (mg Kg⁻¹ dry weight) in *Sargassum muticum* from different localization worldwide.

Metals	Italy (Curiel et al. 1998)	Italy Favero et al. 2002)	Italy (Marzocchi et al. 2016)	Egypt (Fouda et al. 2019)	Egypt (Azzazy et al. 2019)	Australia (Chakraborty et al. 2014)	Present study Morocco 2020
As			17.2 ± 3.1			78.8	7.61 - 59.71
Cd	1.55±0.19	0.26 ± 0.01	0.65 ± 0.13	0.246	< 0.001	0.07	2.07- 6.57
Pb	16.45±0.36	1.1±0.01	5.5 ± 3.1	1.49	0.001	0.9	<2,5
Al	550±64		500 ± 420		0.366		140.40- 926
Mg			12,600 ± 1400		6.777		7459-12690
Mn	188 ±7.86	270±8.78	230 ± 140	1.833	0.025	14.8	7.03 - 56.74
Fe	447±23	44.45 ±4.32	500 ± 380		0.537	224	204 - 914
Cu	44.45±4.32	0.96 ±0.15	25 ± 46	0.971	0.003		<2,5
Zn	311±11.6	311 ±11.6	111 ± 41	0.48	0.128	23.2	7.31 - 17.90
Co	1.18 ±0.05		2.1 ± 1.4		< 0.001		<2,5
Ni	1.99±0.23		9 ± 33	0.657	0.003	0.4	<2,5

Conclusion

In this study a green strategy is proposed to turn a threat into an opportunity of exploitation of the invasive brown seaweed *S. muticum*. Such exploitation would reduce *S. muticum* pressure on coastal areas, and limit health issues and economic problems.

Hence, seasonal characterization constitutes a preliminary step for future valorisation of *S. muticum* biomass. A significant correlation between measured variables suggests temporal changes on biosynthetic pathways of seaweeds. Such results allow an optimization of harvesting strategy.

According to the legislations reference for Pb, Cd and As limits in seaweeds for human consumption and livestock feed, the present results pointed out that Cd and As content was on average above the limits recommended, whereas the Pb contents were much lower than the same legislations limits. Due to this content in Cd and As, the possible use of *S. muticum* in some strategies of valorisation such as bioenergy, bioplastic and biostimulant, should be favoured over human dietary and livestock diets.

Declarations

Acknowledgments

The authors would like to thank Water resources division, Public Laboratory for Tests and Studies (LPEE), Center for Studies and Research on the Environment and Pollution, (CEREP), Casablanca, Morocco for their laboratory analysis of minerals and heavy metals, their collaboration was crucial to obtain precise and complete results. Special thanks are also extended to Pr. Selfati Mohamed for his invaluable contribution in carrying out the statistical analysis of our data.

Competing interests

The authors declare that they have no competing interests of a financial or personal nature which could have influenced the design, conduct, analysis or interpretation of this scientific study.

Authors' contributions

A.A., N.R and T.O.B wrote the manuscript. They contributed equally to the development of the research concept, data collection, analysis, and interpretation of results. L.B conducted the analysis of heavy metals and minerals. Y.T provided help in the biochemical analysis. P.Y.P provided valuable feedback on the manuscript and helped to deepen our understanding of the data. His insightful suggestions and critical review contributed to the overall clarity and coherence of the manuscript.

Funding

None

Data availability

The data presented in this study is contained within the article.

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