Assessment of light intensity and salinity regimes on the element levels of brown macroalgae, *Treptacantha barbata*: Application of response surface methodology (RSM)

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Abstract

In this research, the effect of light intensity and salinity regimes on the element levels of *Treptacantha barbata* (formerly *Cystoseira barbata*) was studied, and the elemental compositions of this brown alga collected from wild stocks also compared with cultured ones. In culture trials, 11 different experiments that have ranges of light intensity as 50 to 150 µmol photon m^{-2}s^{-1} and salinity as 24 ‰ to 42 ‰ were designed according to response surface methodology (RSM). Our results show that the element accumulation with changes of light intensity and salinity on the *T. barbata* was modeled. Most of the elements were affected by the salinity instead of light intensity. All macro and microelements were detected within the recommended dosages and exposure limits. In toxic elements, the least accumulations of Al, As, Cd, and Pb were observed in low light and salinity. Also, the levels of all toxic elements, including trace elements that exceed limits, can be reduced with using these models. The most effective experiment was found as 52.0001 µmol photon m^{-2}s^{-1} light and 24.086 ‰ salinity for minimized toxic element accumulation on *T. barbata* with 0.869 desirability.

Keywords: Brown algae; salinity; light intensity; seaweed; *Cystoseira barbata*.

Practical Application: Control of elemental composition using response surface methodology.

1 Introduction

Macroalgae have been used as food, feed, and fertilizers for centuries, although there are differences in regional or agricultural habits (Duinker et al., 2016). While aquatic species such as macroalgae are considered as alternative food sources, the first issue that appears is to ensure food safety (Duinker et al., 2016). Algae take up elements directly from seawater (Sun et al., 2019), and they are the first target affected by elemental pollution in the marine environment (Pfeiffer et al., 2017). Elemental pollution caused by rapid industrialization and an increasing population is one of the most critical factors that directly affect seafood safety (Stankovic et al., 2014). Seaweeds are an excellent source of minerals needed for human metabolism due to their exceptional mineral accumulating capacity (Circuncisão et al., 2018). However, some beneficial trace elements that they have may exceed legal limits defined by authorities with the aforementioned anthropogenic activity (Coenen, 2013). Adequate trace elements monitoring programs of the marine environment should be a priority for the development of better environmental policies for evaluating such risks (Bonanno et al., 2020). With this regard, the Marine Strategy Framework Directive (MSFD) of the European Commission was aimed to get Good Environmental Status (GES) in all marine environments until 2020. According to MSFD, concerning contaminants on the marine environment and seafood, contaminants should below the maximum levels set for the humans, and contaminant levels should be declining over the years even if they were below the legal limits (Law et al., 2010;

Swartenbroux et al., 2010). That is why the elemental composition of marine food sources should be continuously monitored.

Several studies stated that there are various techniques based upon biosorption that aims to remove toxic elements from waters with using biological materials such as algae, bacteria, fungi, and plant residues (Deniz & Ersanli, 2018). As a new approach, response surface methodology (RSM) having a distinctive feature as less-consuming time compared to other techniques required to optimize any biochemical process (Tabaraki et al., 2014), need sufficient attention for modeling element accumulation in multi-metal systems (Kumar et al., 2016). RSM designed for model building and calculating statistical relationships between the data obtained from experiments and independent variables directly effects the results of the experiments (Alvarez, 2000). RSM applications are already using for the heavy metal removal from wastewaters (Esfandiar et al., 2014; Tajernia et al., 2014) or determining biosorption of some elements in brown algae like *Cystoseira indica* (Keshtkar et al., 2019), *Cystoseira myricaas* (Zarei & Niad, 2017), and *Cystoseira trinodis* (Salehi et al., 2014). Considering such information, RSM can be used for the reduction of toxic elements along with enhancing beneficial ones in marine algae by manipulating this biochemical process vice versa. *Treptacantha barbata* is one of the most critical macroalgae species for both the marine environment and the human diet as a promising novel food source. It is an indicator species for

elemental pollution (Aydın-Önen & Öztürk, 2017) conjunction with wide range distribution of the species from Eurasia to Africa (Guiry & Guiry, 2020), the monitoring of this species becomes particularly important. The environmental conditions limit independent variables that can affect biosorption. Among them, salinity and light intensity are the most significant factors for the distribution of this algae. By interfering with the levels of salinity and light intensity on the prevailing culture procedure, it is possible that monitoring the elemental composition of this brown seaweed.

In this study, the effects of light intensity and salinity regimes on the element accumulation in *Treptacantha barbata* were studied. The Box-Behnken design of response surface methodology (RSM) was used to determine experimental trials. The accumulation of elements of *T. barbata* was formulated with RSM application. Also, the elemental composition of wild stock was determined and modeled along with the effects of light intensity and salinity.

2 Material and methods

2.1 Study material

Brown Alga *Treptacantha barbata* (Stackhouse) Orellana & Sansón, 2019 (formerly known as *Cystoseira barbata*) was used as the main material. Thallus of *T. barbata* were collected from the shores of the Dardanos district in Çanakkale, Turkey (40°4'38.47"N; 26°21'35.08"E). Samples were stored in sea water-filled bottles and transferred immediately to the laboratory. After the epiphytes and the other organisms were cleaned, thallus were divided into three different groups and had been adapted to the salinity conditions for one week. The thallus of *T. barbata* has used stock cultures, which were grown in 50 L plexiglas tanks having UV sterilized seawater at 20 °C, 100 µmol photon m^{-2} s^{-1}. Experiments were conducted at 3 L cylindrical glass bottles (15 cm in diameter). Artificial seawater, which is a culture medium in all groups, was prepared according to literature (Guillard, 1975). In experiments, cultures were aerated by bubbling air. The temperature and pH values of the cultures were monitored with pH meter (Hanna, HI8314). The light intensity was measured by LI-250 light meter (LiCOR, USA).

2.2 Experimental design

In this study, the effects of two independent factors, just as light intensity and salinity on the element accumulation in *Treptacantha barbata,* were evaluated. The Box-Behnken design of response surface methodology (RSM) was used to determine experimental trials. The maximum and minimum values of the factors mentioned above were specified according to optimum growth ranges, and environmental conditions of the *T. barbata*. The upper or lower values of these factors cause stress on algae and inhibit growth. The light intensity was selected as ranging from 50 to 150 µmol photons m^{-2} s^{-1} and salinity was selected as ranging from 24 ‰ to 42 ‰. Eleven experiments having differences in the combination of these factors and suggested by the Box-Behnken design were run. All experiments were replicated three times (n=3). At the end of the experiments, the elemental compositions of experimental groups were determined.

2.3 Element analysis

The element compositions of *T. barbata* were determined according to the method of the Nordic Committee on Food Analysis (method 18) (Nordisk Metodikkomité for Næringsmidler, 2007). To avoid secondary contamination and ensure standardization between element analyses, the procedures recommended by the 8th and 9th task group of Marine Strategy Framework have been carried out (Law et al., 2010; Swartenbroux et al., 2010). Samples were analyzed via ICP-AES (Varian Liberty AX Sequential ICP-AES). The analyzed elements were silver (Ag), aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), and zinc (Zn). Finally, results were compared with the standards (VH6 lab single element atomic absorption CRM) and calculated as parts per million (ppm). All analyses were carried out as three parallel and three replicates.

2.4 Data evaluation

Differences between elemental compositions of the trials were evaluated by one-way analysis of variance (ANOVA) after the normality and homogeneity were specified with Anderson–Darling test and Levene's test, respectively. In the evaluation of the effects of Box-Behnken design, the obtained data were written in response columns in the design matrix and evaluated with Design Expert 7.15 software. All models were formulated individually, and surface response plots were given. Formulations 1 and 2 were given below:

$$y = \beta_0 + \sum \beta_i X_i \, (Linear \, model) \tag{1}$$

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j \, (2FI \, model) \tag{2}$$

Where y is the response; β_0 is the regression intercept; X symbols are independent variables; β_i is coefficient of the linear parameters; and β_{ij} is coefficient of interaction between factors (Davarnejad et al., 2018).

Coded values were used in equation terms. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. High levels of the factors are coded as +1, and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Optimized values were suggested to get minimal values of toxic elements. In optimization, Al, As, Cd, and Pb were selected for getting minimum along with other elements selected as in the range, except for elements having insignificant accumulation models. Data on the insignificant models were not used in optimization.

3 Results and discussion

According to our results, sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and iron (Fe) were detected as highest in *T. barbata* collected from

Çanakkale strait, respectively. It is a known fact that macroalgae species are abundant in terms of Ca, Mg, Na, P, and K (Rodrigues et al., 2015). Macroalgae species are good sources of iron (García-Casal et al., 2007). Likewise, in our research, iron was detected in high amounts and evaluated within the macro group for better display on the optimization process. Similar elemental compositions were found in Sargassaceae family (Kravtsova et al., 2014; Vizetto-Duarte et al., 2016), and the different algae species which were collected from nearby locations (Berik & Çankırılıgil, 2019).

T. barbata is also a good source of trace elements (Bonanno & Orlando-Bonaca, 2018; Vizetto-Duarte et al., 2016), which are needed for human metabolism within optimum values (Circuncisão et al., 2018). According to the results; boron (B), and manganese (Mn), were found the highest trace elements and followed by chromium (Cr), tin (Sn), zinc (Zn), nickel (Ni), copper (Cu), and molybdenum (Mo), respectively. Aydın-Önen & Öztürk (2017) stated that *T. barbata* collected from the Aegean Sea shows high bioconcentration factor for manganese and zinc. Similarly, chromium was found high in *T. barbata*. Several studies stated that this seaweed could absorb a high amount of chromium and nickel from the seawater (Niemiec et al., 2015; Simeonova & Petkova, 2007). Although tin known as an essential trace element for some animals, the necessity for the human is still unclear (Tomza-Marciniak et al., 2019). Tin in the marine environment is usually found in the form of organic tributyl-tin (TBT) complex (Feldstein et al., 2003), which is mostly used for in paints for ships due to their antifouling feature (Takahashi et al., 2000). Considering the sampling area in the Çanakkale Strait, which is amongst the most important trade routes (Başar, 2010), it can be said that this activity may cause the tin content of the marine alga. Low copper concentration in the *T. barbata* may be a reason for the low molybdenum level as well. In living organisms, the biologically active form of molybdenum is found as the molybdenum cofactor (Moco), and the biosynthesis of this cofactor requires proteins, iron, ATP, and copper (Mendel, 2013). Moreover, cobalt (Co), selenium (Se), silver (Ag), and antimony (Sb) were found just slightly higher than the detection limits, respectively. Cobalt found very low concentrations in the Mediterranean Sea due to the marine redox process (Swanner et al., 2014), thereby it found very low in the algae as well. Fish, shellfish, and other aquatic animals are rich in terms of selenium (Calatayud et al., 2012; Liu et al., 1987; Wang et al., 2019), whereas most of the plants are selenium sensitive and non-accumulators (Wang et al., 2019). Besides, Liu et al. (1987) stated that algae species have little selenium concentrations compared to other aquatic species. Silver and antimony, which are occurring natural metalloids, can be found in small amounts in the environment, but the amount of those elements can rise with the industrial activities (Hiriart-Baer et al., 2006; Ungureanu et al., 2018). In this research, levels of silver and antimony in the *T. barbata* were found very low.

Mercury concentration of the *T. barbata* found very low due to algae are primary producers, and mercury risk exists in higher predators. Similarly, cadmium and lead were found below the legal limits described by the authorities (Food and Agriculture Organization of the United Nations, 2007, 2011).

Although there was no limitation described to aluminum contaminations on the fish and other seafood, the European Food Safety Authority (2013) stated that aluminum exposure should not exceed 1 mg kg^{-1} weekly for humans. The toxicological and biochemical activity of the arsenic depends on its chemical structure (Mushtaque & Chowdhury, 2004). Organic arsenic compounds are accepted as non-toxic for the living organisms (Oya-Ohta et al., 1996; Pergantis et al., 2000). Ma et al. (2018) were investigated 282 macroalgae species and stated that brown algae have the highest arsenic, which is consisted of both organic and inorganic forms.

It is a clear fact that *T. barbata* collected from Çanakkale Strait rich in terms of macro and microelements. This elemental richness is related to algae's capacity to retain inorganic compounds accounting for up to 36 % of dry matter in some species (Lordan et al., 2011). Although, the high presence of minerals on the algae is an essential advantage for human nutrition and human health (Mišurcová et al., 2011), excessive trace elements (or microelements) concentrations are harmful to all organisms. Thus, research about on elemental composition of the macroalgae can be show alterations even if they carried out on the same species. In our study, all elements were detected within the recommended dosage stated by the Reference Nutrient Intake (British Nutrition Foundation, 2016; Mišurcová et al., 2011). However, these values can be exceeded limits, especially in some aquaculture implementations. Therefore, we also evaluated the element accumulation on *T. barbata* in our study. According to results, levels of Ag, Al, B, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, and Zn were detected as the lowest in the wild compared to culture trials. In culture experiments, an artificial seawater medium was used to provide nutrients such as macronutrients, trace metals, anhydrous, and hydrous salts needed for the species' growth. Thus, the elemental composition of the *T. barbata* obtained from culture trials were differ by wild ones in parallel with element supplementation. Only elements that were found highest in the wild specimens were determined as Ca, Cr, Sn, and toxic ones such as As, Cd, and Hg. All of those elements were not added with artificial seawater medium, and that is why their levels reduced in culture trials, except Ca, which is one of the components of the artificial seawater. Despite calcium supplementation, the relatively low amount of calcium detected in the samples can be explained by photosynthesis. In the algal growth, algae specimens use saturated CO_2 from the environment as well as light to produce carbohydrates needed for the growing, and they release O_2 into the water in consequence of the photosynthesis process (Beer & Koch, 1996; Sant & Ballesteros, 2020). In the meantime, some amounts of CO_2 is also released to the environment due to respiration (Raven & Beardall, 2003). If there is an adequate light source, a balance can be achieved between the amount of oxygen and carbon dioxide in the water, but the CO_2 may increase when the light is cut off (Larkum & Wood, 1993; Zou et al., 2011). Excessive CO_2 in the water can be precipitated as calcium carbonate ($CaCO_3$) by binding with Ca (Raven & Beardall, 2003). This biochemical process can be the reason to low Ca levels of the cultured specimens compared to wild algae. When we examine levels of elements according to culture trials, it is shown that the level of each element was changed at different rates by salinity and light intensity.

Table 1. Elemental composition of *Treptacantha barbata* obtained from wild and culture trials.

Elements (ppm)	Wild	T1	T2	T3	T4	T5*
Ca	19810.41 ± 15.78[a]	17247.29 ± 15.85[f]	17963.89 ± 13.55[b]	17636.49 ± 5.64[d]	17710.22 ± 11.69[c]	17419.28 ± 9.67[e]
Fe	116.86 ± 1.42[e]	323.82 ± 1.21[a]	310.64 ± 1.14[b]	227.98 ± 1.14[c]	225.32 ± 1.08[c]	177.33 ± 1.10[d]
K	12025.00 ± 16.64[f]	55494.33 ± 17.45[b]	56244.37 ± 19.50[a]	51455.64 ± 14.96[e]	52132.44 ± 18.53[c]	52025.12 ± 14.80[d]
Mg	5587.75 ± 9.84[f]	7609.25 ± 7.32[a]	7575.33 ± 6.08[b]	7428.15 ± 7.32[c]	7312.80 ± 5.21[d]	7136.10 ± 4.12[e]
Na	20638.00 ± 28.56[f]	24563.33 ± 26.56[d]	23104.90 ± 27.29[e]	31107.50 ± 25.21[a]	30156.63 ± 25.13[b]	24625.17 ± 27.11[c]
P	194.19 ± 1.64[d]	248.01 ± 1.87[ab]	250.26 ± 1.01[a]	198.76 ± 1.32[c]	196.35 ± 1.48[cd]	224.25 ± 1.65[b]
Ag	0.03 ± 0.01[b]	0.07 ± 0.01[a]	0.07 ± 0.01[a]	0.07 ± 0.01[a]	0.08 ± 0.01[a]	0.07 ± 0.01[a]
B	19.68 ± 0.38[f]	40.80 ± 0.22[b]	42.56 ± 0.13[a]	33.68 ± 0.13[d]	35.12 ± 0.13[c]	33.06 ± 0.14[e]
Co	0.09 ± 0.01[d]	0.19 ± 0.01[b]	0.17 ± 0.02[b]	0.22 ± 0.02[ab]	0.25 ± 0.01[a]	0.12 ± 0.01[c]
Cr	2.60 ± 0.02[a]	1.63 ± 0.02[b]	1.18 ± 0.02[c]	0.99 ± 0.03[e]	1.02 ± 0.01[e]	1.10 ± 0.01[d]
Cu	0.43 ± 0.09[d]	0.89 ± 0.02[a]	0.78 ± 0.01[b]	0.69 ± 0.02[c]	0.72 ± 0.01[c]	0.47 ± 0.03[d]
Mn	5.26 ± 0.09[f]	12.60 ± 0.07[a]	11.16 ± 0.05[b]	10.52 ± 0.06[c]	9.63 ± 0.03[d]	9.06 ± 0.04[e]
Mo	0.18 ± 0.03[f]	1.15 ± 0.02[c]	1.02 ± 0.02[d]	1.64 ± 0.02[a]	1.54 ± 0.02[b]	0.34 ± 0.03[e]
Ni	0.75 ± 0.04[d]	0.85 ± 0.02[c]	0.86 ± 0.02[c]	0.93 ± 0.02[b]	0.94 ± 0.02[b]	0.99 ± 0.02[a]
Sb	0.03 ± 0.01[b]	0.03 ± 0.01[b]	0.05 ± 0.01[a]	0.04 ± 0.01[ab]	0.04 ± 0.01[ab]	0.03 ± 0.01[b]
Se	0.04 ± 0.01[e]	0.10 ± 0.01[b]	0.15 ± 0.01[a]	0.07 ± 0.01[cd]	0.08 ± 0.01[c]	0.06 ± 0.01[d]
Sn	0.80 ± 0.09[a]	0.52 ± 0.02[b]	0.50 ± 0.04[b]	0.28 ± 0.01[c]	0.28 ± 0.01[c]	0.55 ± 0.03[b]
Zn	0.79 ± 0.08[d]	1.58 ± 0.05[a]	1.55 ± 0.04[a]	1.31 ± 0.04[b]	1.32 ± 0.02[b]	1.24 ± 0.03[c]
Al	238.59 ± 1.59[c]	240.35 ± 1.68[c]	239.56 ± 1.62[c]	245.19 ± 1.79[b]	250.12 ± 1.73[a]	250.51 ± 1.86[a]
As	110.91 ± 1.82[a]	88.40 ± 1.21[d]	88.56 ± 1.23[d]	98.87 ± 1.01[bc]	99.56 ± 0.94[b]	97.28 ± 1.07[c]
Cd	1.45 ± 0.26[a]	0.08 ± 0.01[c]	0.08 ± 0.01[c]	0.14 ± 0.01[b]	0.13 ± 0.01[b]	0.05 ± 0.01[d]
Hg	0.03 ± 0.01[a]	0.01 ± 0.01[b]	0.01 ± 0.01[b]	0.01 ± 0.01[b]	0.01 ± 0.01[b]	0.01 ± 0.01[b]
Pb	0.50 ± 0.02[b]	0.54 ± 0.02[a]	0.55 ± 0.02[a]	0.52 ± 0.02[ab]	0.50 ± 0.02[b]	0.45 ± 0.01[c]

*T1: Trial 1, T2: Trial 2, T3: Trial 3, T4: Trial 4, T5: Trial 5. Data were expressed as Mean ± SD (n = 3). Means with different superscript letters in a line are significantly different ($p < 0.05$).

The elemental composition of the specimens of culture trials was shown in Table 1.

To better understand the accumulation of each element in *T. barbata* by salinity and light intensity, the obtained results modeled considering applied levels of the independent variables. Alternative model types such as linear, two-factor interaction (2FI), quadratic, and cubic models were analyzed automatically with Design-Expert software to achieve an insignificant lack of fit and maximizing adjusted R-squared (R^2) values, thereby the most significant model suggested by the software was applied to each element. According to statistical analysis, while the linear model was applied for Al, As, B, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Se, Sn and Zn, 2FI model was applied for Ag, Ca, Cr, P, Pb, and Sb. Analyses of variance for element accumulation models of *T. barbata* shown in Table 2. The F-value of the model implies the model is significant or not, which is decided by the software considering the F-value and Prob > F value together. Prob > F is the p-value for the whole model test, and it shows the probability that F-value is caused by noise. Considering these values of models (Table 2); while models of Al, As, B, Ca, Cd, Cr, Fe, K, Mn, Na, Ni, P, Pb, Sb, Se, Sn, and Zn were found significant, Ag, Co, Cu, Hg, Mg, and Mo models were found as not significant. The insignificant models have 11.56 %, 10.31 %, 25.12 %, 12.85 %, 6.93 %, and 17.85 % chance that an F-value this large could occur due to noise for the Ag, Co, Cu, Hg, Mg, and Mo respectively. Values of "Prob > F" less than 0,0500 indicate model terms just as light intensity (A), salinity (B),

and interaction of light intensity and salinity (AB) accepted as significant. According to our results, light intensity (A) and salinity (B) detected as significant in Cr, Mn Pb, and Se models. Interaction of light intensity and salinity (AB), which exists only 2FI models, were also found significant in the Cr model, while it was found not significant in the Pb model.

Salinity (B) was found significant, whereas light intensity (A) was found insignificant in the models of Al, As, B, Cd, Fe, K, Na, Ni, Sn, and Zn. In the P model, the significance of the terms was found similar to the element, as mentioned earlier models. In addition to those, the interaction of the terms (AB) was specified as significant instead of insignificant light (A). On the contrary, only insignificant salinity (B) model terms were observed in Ca and Se models among all models. They also have significant light intensity (A) and interaction of light intensity and salinity (AB) terms (Table 2).

The results were evaluated in the numerical optimization option in Design Expert 7.15 software. The sodium model was not used in the optimization procedure, although it was found a significant model as expected due to sodium addition to adjusting salinity in experiments. So, to get more concise results, sodium was not evaluated with other significant models just as insignificant ones. In optimization, significant element models such as Al, As, B, Ca, Cd, Cr, Fe, K, Mn, Ni, P, Pb, Sb, Se, Sn, and Zn were selected as "in the range" choice except for Na, whereas all significant toxic elements models. Al, As, Cd

Table 2. Analyses of variance for element accumulation models of *Treptacantha barbata*.

Yi*	Type	SS	MS	F-value	P-value (Prob > F)				SGNF
					Model	A:Light	B:Salinity	A×B	
Ag	2FI	0.0001	0.0001	2.48	0.1156	0.1447	0.1832	0.1135	NS
Al	Linear	190.6101	95.3126	8.62	0.0048	0.3015	0.0017	-	S
As	Linear	346.2502	173.1302	73.05	<0.0001	0.6437	<0.0001	-	S
B	Linear	166.56	83.2804	16.74	0.0003	0.2368	0.0001	-	S
Ca	2FI	792218	264100	24.11	<0.0001	<0.0001	0.2861	<0.0001	S
Cd	Linear	0.0101	0.0051	5.87	0.0167	0.7953	<0.0001	-	S
Co	Linear	0.0081	0.0043	2.76	0.1031	0.8859	0.0371	-	NS
Cr	2FI	0.7902	0.2616	51.91	<0.0001	0.0002	<0.0001	<0.0001	S
Cu	Linear	0.0601	0.0317	1.55	0.2515	0.6126	0.1179	-	NS
Fe	Linear	24804.1	12402.1	6.91	0.0101	0.7519	0.0030	-	S
Hg	Linear	0.0001	0.0001	24.45	0.1285	0.7375	0.0494	-	NS
K	Linear	$5135×10^4$	$2568×10^4$	39.27	<0.0001	0.1524	<0.0001	-	S
Mg	Linear	164305	82159.1	3.36	0.0693	0.4244	0.0302	-	NS
Mn	Linear	13.8203	6.9111	9.11	0.0039	0.0392	0.0037	-	S
Mo	Linear	0.8114	0.4012	2.01	0.1785	0.6685	0.0750	-	NS
Na	Linear	$143×10^6$	$7149×10^4$	51.90	<0.0001	0.1008	<0.0001	-	S
Ni	Linear	0.0172	0.0085	4.06	0.0450	0.7584	0.0151	-	S
P	2FI	8102.99	2701.01	16209	<0.0001	0.0965	<0.0001	<0.0001	S
Pb	2FI	0.0143	0.0046	21.91	<0.0001	0.0051	<0.0001	0.0513	S
Sb	2FI	0.0008	0.0003	10.49	0.0015	0.0040	0.4131	0.0015	S
Se	Linear	0.0096	0.0048	12.02	0.0014	0.0396	0.0010	-	S
Sn	Linear	0.1711	0.0831	17.45	0.0003	0.8272	<0.0001	-	S
Zn	Linear	0.1912	0.0932	11.25	0.0018	0.8276	0.0005	-	S

*Yi: Response; SS: Sum of squares; MS: Mean squares; SGNF: Significance; S: Significant; NS: Not significant; F-value: Variance between the means; P-value: The probability of obtaining results.

and Pb were selected as "minimum" choice in the numerical optimization section on the Design Expert 7.15 to get minimum toxic element accumulation along with moderate trace ones. All insignificant models of Ag, Co, Cu, Hg, Mg, and Mo were not taken into account in optimization as well as Na model. According to optimizations, amongst 12 solutions offered by Design Expert, the most effective one having the highest desirability (0.869) is determined as 52.0001 µmol photon m^{-2} s^{-1} light and 24.086 ‰ salinity for minimized toxic element accumulation on *T. barbata*. Finally, 3d response surface plots of significant models were shown as macro, micro, and toxic elements in Figure 1.

In macroalgae, the elemental composition of the species is directly related to species and the environment (Bonanno & Orlando-Bonaca, 2018). Our study shows that it is possible to manipulate the element composition of algae by light and salinity, considering the accumulation pattern of selected elements. With RSM application, algae can be transformed into more nutritious compared to wild forms. According to the results of our study, *T. barbata* has calcium, sodium potassium, magnesium, iron, and some trace elements in high amounts such as boron, zinc, manganese. Calcium was the most crucial element (MacArtain et al., 2007), and brown seaweeds accumulated tend to accumulate higher concentrations of sodium and potassium than green seaweeds (Circuncisão et al., 2018). Iron is an essential element for humans because it participates in fundamental cell functions (Mišurcová et al., 2011). Iron deficiency is a

nutritional disorder that can be affected in the development of the neuropsychomotor systems (Rodrigues et al., 2015). Boron is needed to bone growth, protecting the nervous system, hormone secretion, and it is associated with reducing cardiac diseases and some cancer types (Nielsen, 2014). With such benefits, boron abounds in marine algae (Dembitsky et al., 2002). *T. barbata* also has manganese and zinc, which are essential trace elements for human metabolism as well as boron. Manganese is an essential trace element which is involved in the metabolism of protein, lipid, and carbohydrate, and performs as various enzymes cofactors (Mišurcová et al., 2011). Zinc is an antioxidant, regulates immune response, and it is necessary for growth and development (Salgueiro et al., 2000).

Despite such benefits of rich element composition, macroalgae can also accumulate some toxic elements such as arsenic (As), aluminum (Al), cadmium (Cd), lead (Pb), and mercury (Hg) which can represent a health risk for the people (Circuncisão et al., 2018). Mercury is accumulating in the marine food chain and leading to an elevated concentration in predator species (Spada et al., 2012). Cadmium and lead contaminations are causing long term toxic effects with accumulating in organisms (Baloch et al., 2020). Aluminum, which is a neurodegenerative element causes serious brain diseases such as Parkinson and Alzheimer's disease (Iglesias-González et al., 2017; Yokel et al., 1994). In view of the results, it is possible to avoid such risks by reducing these toxic elements with RSM application.
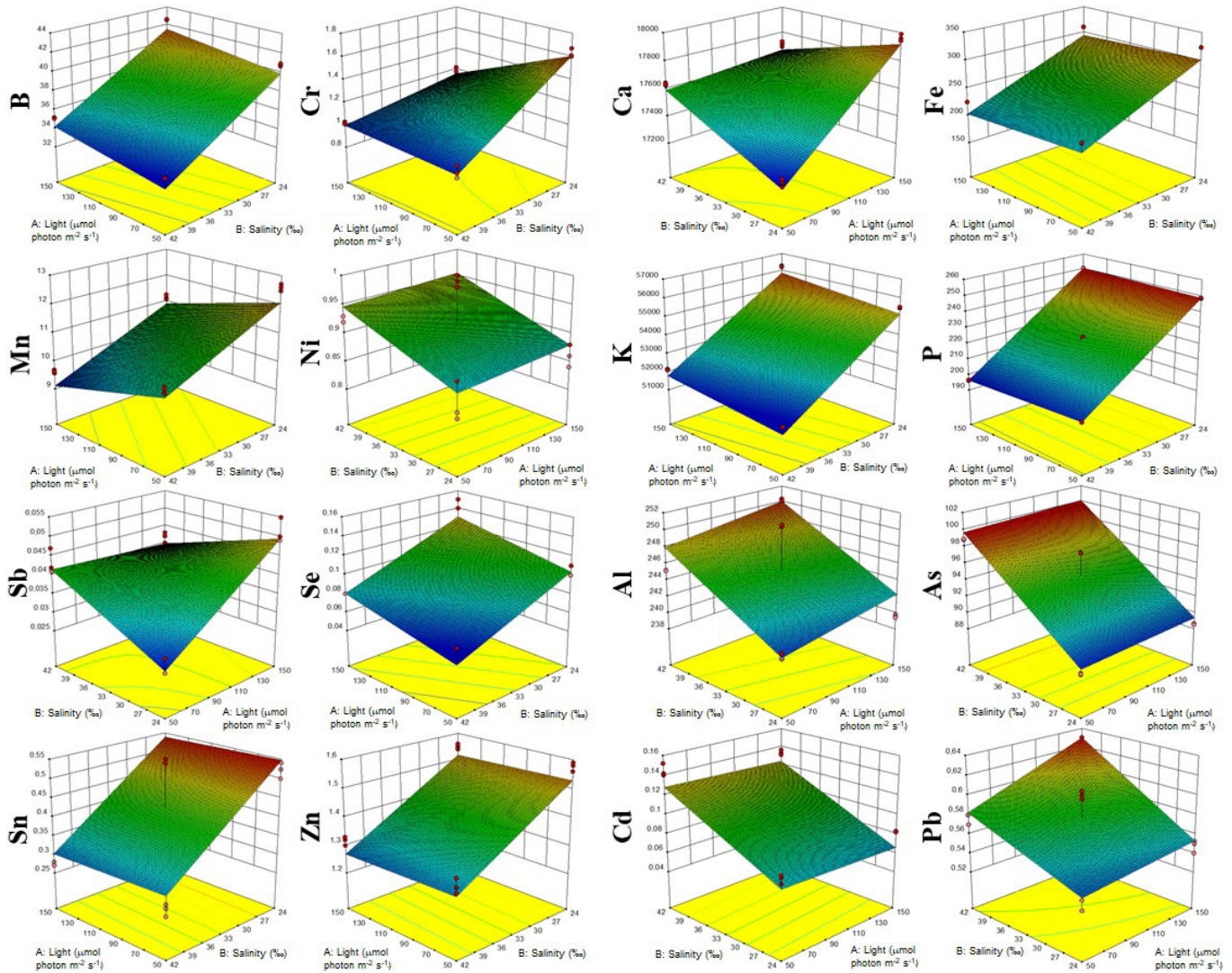
Figure 1. Response surface plots for the accumulation of elements in *Treptacantha barbata*.

4 Conclusion

Seaweeds are one of the most crucial vegetable sources of minerals. According to our results, *T. barbata* can be used as mineral sources with rich macro and microelements as well as low content of toxic elements. Besides, the influence of light intensity and salinity on the elemental composition of *T. barbata* culture was determined. According to element accumulation models, most of the elements were affected by the salinity instead of light intensity. These preliminary results will shed light on further studies, especially on the manipulation of element accumulations of macroalgae. The ability of element manipulation on macroalgae species is vital for both environmental studies and seafood science. Macroalgae species are already using as heavy metal removals in polluted water sources. With RSM application, they can be used as metal adsorbents more effectively considering different accumulation patterns of each element on the *T. barbata,* which is determined in this study. Inversely, macroalgae species can be transformed into more nutritious food sources with RSM methodology. It is possible to obtain macroalgae that are rich in

terms of beneficial trace elements, whereas containing less toxic elements. All macro and microelements were detected within the recommended dosages and exposure limits stated by authorities. As the element analysis was done with ICP, only the amount of the elements in the algae was determined. Even if these elements are detected below the limit values, few compounds which are toxic forms of some trace elements can be dangerous. The levels of all toxic elements, including trace elements that exceed limits, can be reduced by using these models. Moreover, *T. barbata* has high amounts of arsenic compounds which caused by organic arsenoriboses. However, the mechanism of the arsenoribose accumulation on the brown macroalgae is still unknown. Future elemental studies should focus on understanding this mechanism, and the RSM application can help achieve this goal.

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