



## Secondary analysis of mineral proximate data of selected seaweeds collected from Veraval western coast of India.

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### ABSTRACT

Seaweed is known as a rich source of mineral composition and a huge number of nutritive elements. The mineral composition of seaweed is variable because of many exogenous and endogenous aspects besides the large significant differences within the same species. The mineral content of some selected species, belonging to classes, viz. Chlorophyta: *Ulva clathrate*, *Ulva rigida*, *Chamaedoris auriculata*, Rhodophyta: *Gracilaria friifern*, *Griffithsia opuntioides*, and *Jania rubens* Phaeophyta: *Padina tetrastomatica*, *Cystoseria trinodis* were investigated. They were obtained from Veraval coastal region of Junagadh district, India. The aim of the study was to evaluate the water-soluble mineral composition, such as ( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) and bioactive components; namely, protein and carbohydrate of different marine seaweeds (red, green, and brown) with respect to water mineral composition and other water physical parameters. Biological data intertwined with several inclusive analytical methods such as secondary data analysis, combining several data measurements are required for the investigation of entering coalition within and among species. This study described a method of data pre-processing that can be widely integrated, based on a variety of multi measurements of the mineral composition of different seaweed species and the water parameter of the coastal area. This analytical tactic using a variety of statistical tools and graphical representation, such as correlation network analysis, principal component analysis, and piper diagram revealed multiple relationships between mineral composition and different seaweed species and water analysis.

### INTRODUCTION

Seaweeds are biologically and ecologically important constituents in aquatic ecosystems. Marine macroalgae are a primitive type of plant. Seaweeds inhabit every part of the ocean system, floating on the water, attached to rocks in the inter-tidal regions, or rooted in the benthic zones, thus showing a captivating wonder world in the ocean. However, these marine algae have been adapted to a wide range of environmental challenges such as unfavourable tidal turbulence, variations in water temperature, etc. Besides, the wide range of seaweed species offers food and shelter to a variety of sea animals. Mary (2012) postulated that the marine environment is not uniform everywhere. It varies from place to place. The environment near the coast is in complete contrast to that found in deep trenches and canyons. It also varies from the ocean surface to the ocean bottom. The seaweeds adapt

themselves to the specific marine environment. Based on the primary pigments in seaweeds, biologists have classified them into three major groups; namely, Chlorophyta, Phaeophyta, and Rhodophyta (**Satheesh & Wesley, 2012**).

The marine macroalgae consist of various inorganic and organic compounds that are beneficial to human health and mankind. The most common ones are those belonging to the family of Rhodophyta, followed by the Chlorophyta and Phaeophyta. They are characterized as primary producers which grow abundantly in the shallow waters and the intertidal zone of the ocean, estuaries, and other backwaters (**Chapman & Chapman, 1970**). They efficiently take up inorganic compounds from the seawater and convert them into macronutrients, such as carbohydrates, lipids and proteins (**Dangar *et al.*, 2020**). Seaweeds are very low in fat. They contain high percentages of carbohydrates and proteins, which are usually less in land vegetables. Due to their high concentration of carbohydrates and proteins, seaweeds are known as super-food. Marine algae comprise more than 60 trace elements in a concentration much higher than in terrestrial plants (**Gadhvi *et al.*, 2019**). Additionally, the value of edible seaweeds in human nutrition is based on their richness in several minerals, including sodium ( $\text{Na}^+$ ), magnesium ( $\text{Mg}^{+2}$ ), potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{+2}$ ) and chloride ( $\text{Cl}^-$ ), carbohydrate and protein (**Circuncisão *et al.*, 2018**).

The mineral composition of the seaweeds is greatly influenced by various topographical factors, which include the salinity of the water, temperature amount of light, and the availability of nutrients. These environmental conditions show variations based on the season, thereby several physiological and metabolic mechanisms in the seaweeds either stimulating or inhibiting (**Zhang *et al.*, 2020**). The nutritional qualities of the seaweeds are not known, and they are generally evaluated based on their chemical composition. Based on the season, topography, species, as well as temperature in the water, the composition, varies among different seaweeds. **Jose and Xavier (2020)**, their valuable mineral content permits them a great potential for application in the food industry as new ingredients for the development of numerous functional food products. In view of the current increasing demand for seaweed products, the aim of this work was to study the nutritional value of some seaweed (red, green, and brown) from the Veraval coastal area, in order to assess their validity towards the Multivariate analysis was applied to classify the samples according to the type of seaweed and extraction zone of the study area.

## **MATERIALS AND METHODS**

### **1. Study area**

Veraval Coastal region is located at 20.9° N Latitude and 70.37° E Longitude in the Gujarat coastline of Somnath Gir district is about 3.5 km long with rocky substratum, slightly muddy with an abundance of coral species.



## 2. Sample collection

Diverse seaweed species representing Chlorophyta: *Ulva clathrate*, *Ulva rigida*, *Chamaedoris auriculata*, Rhodophyta: *Gracilaria friifern*, *Griffithsia opuntioides*, and *Jania rubens*, Phaeophyta: *padina tetrastomatica*, *Cystoseria trinodis* were collected from Veraval coastal region located at 20.91° N Latitude and 70.34° E Longitude on earth scale of India. All the seaweeds were harvested manually, and samples were washed in the sea and fresh water to remove salt, associated organisms, and other extraneous matters and transferred to the laboratory and free from the dirt and detritus. The cleaned algal biomass was then subjected to be identified taxonomically and classified according to seaweeds of India (**Jha and Reddy, 2009**).

## 3. Sample Analysis

### 3.1 Plant sample preparation

The species were shade dried and grounded into fine powder and stored for further use. The material will be once more dried in an oven, before using for analysis of mineral ions. Samples were oven-dried and weighed. Mineral extraction was carried out by 1 g dry weight of each sample, dry ashing with muffle furnace and treated with HNO<sub>3</sub> and HCL after that the extract was filtered through Whatman filter paper 44, final volume makes up with distilled water up to 250 ml for the further analysis (**Chopra and Kanwar, 1976**).

### 3.2 Mineral analysis

All parameters were as follows, EC (EC meter (Systronics)), Mineral constituents were measured as follows; Ca<sup>2+</sup> and Mg<sup>2+</sup> by EDTA titration (**Vogel, 1978**), Na<sup>+</sup> and K<sup>+</sup> measured by flame photometry (FPM Systronics), chloride according to (Argentometric method), Ash content was obtained by the gravimetric method according to by heating the sample in the crucible till the sample converted into ash (**Horwitz and Albert, 2006**).

### 3.3 Proximate analysis

- **Estimation of carbohydrate**

Total carbohydrate contained in seaweeds was carried out by Enthroned method by referring to standard D- Glucose and results have been expressed as mg/g sugar.

- **Estimation of protein**

The protein presence in seaweeds was determined by the biuret method. Protein was calculated by using BSA as standard and expressed as mg/g protein (Lowry, 1951).

### 4. Water analysis

Marine water samples were collected at four different locations in the Veraval coastal area. The mineral analysis of water and quantifying the concentration of cations such as calcium, magnesium, and sodium-potassium. Similarly, the anions are the most common anions bicarbonate and two strong acids sulfate and chloride. The physicochemical analysis of water quality parameters was carried out by following standard methods by APHA (1992).

### 5. Statistical analysis

The statistical analyses were carried out using the SPSS software. One-way ANOVA was employed to analyze the mean values to identify significant differences between the mineral compositions of the seaweeds. The nutritional content of the sample such as Protein and Carbohydrates was acquired as a set of three replicates  $\pm$  SD. To find out the relation between the different eight selected species of seaweed mineral composition, Pearson's Correlation analysis was performed, which was carried out using IBM SPSS version 10. Water mineral data was plotted in a piper diagram using the Grapher software. Piper graph for water quality analysis by using Grapher software.

## RESULTS AND DISCUSSION

### 1. Principal component analysis

Multivariate characterization (PCA: principal component analysis) was applied using eight different species analyzed in three different groups which belong to Chlorophyta: *Ulva clathrate*, *Ulva rigida*, *Chamaedoris auriculata*, Rhodophyta: *Gracilaria friifern*, *Griffithsia opuntioides* and *Jania rubens* Phaeophyta: *padina tetrastomatica*, *Cystoseria trinodis* in addition to five different mineral composition namely,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Cl}^{-}$  The first three principal components (PC1, PC2, and PC3) were selected for a further interpretation of the results (Table 1).

The weights for eight original variables along the first three principal components are shown in table no 1 according to the results of the PCA ordination, the eigenvalue of the first three ordination axes was greater than 1, which can together explain 61.30% of the total variance in those properties. Specifically, the first principal axis (PC1) accounted for 61.30% of the total variance and was strongly and positively related to *U. rigida* which is from group

Chlorophyta with *G. opuntiodes* and *J. rubers lamourox* both from group Rhodophyta. Similarly, in the first PCA Chlorophyta, and Phaeophyta groups had a negatively strong relation except for *U. rigida*. The second principal component (PC2) explained 21.00% of the total variation. *U. clathrate*, *C. trinodis*, *G. foliferm*, and *G. opuntiodes* had a positive correlation. The third principal component (PC3) accounted for 5.54% of the total variation. Specifically, *U. clathrate* and *J. rubers lamourox* had shown a positive correlation.

Table 1: PCA: principal component analysis of minerals composition of selected seaweeds.

Group of species	Name of species	PC1	PC2	PC3
Chlorophyta	<i>C. auriculata</i>	-0.18313	-0.32223	-0.21772
	<i>U. rigida</i>	0.4304	-0.61509	-0.34808
	<i>U. clathrate</i>	-0.19393	0.43272	0.38513
Phaeophyta	<i>P.tetrastomatica</i>	-0.5935	-0.66458	-0.13676
	<i>C. trinodis</i>	-1.165	0.38745	-0.32171
	<i>G. foliferm</i>	-1.2247	0.80271	0.31078
Rhodophyta	<i>G. opuntiodes</i>	2.0824	1.0911	-0.13684
	<i>J. rubers lamourox</i>	0.84746	-1.112	0.4652
<b>Eigen value</b>		1.21719	0.618946	0.110116
<b>Proportion of variance (%)</b>		61.30	31.17	5.54
<b>Cumulative variance (%)</b>		61.30	93.04	98.04

(Ca<sup>+2</sup>, Mg<sup>+2</sup>, Na<sup>+</sup>, K<sup>+</sup>, (meq.g<sup>-1</sup>))

The mineral concentrations can vary extensively among the different families, genera, and species of seaweed, even under similar environmental conditions, geographical origin, and harvesting time. This could be explained due to differences in the biosorption of minerals as a consequence of differences in the amount of salinity of water pH, the presence of other living organism and their metabolic activity, and the stress physiology of respected plants to accumulate the surrounding mineral uptake.

## 2. Mineral ion correlation of selected species

Table 2: Pearson's correlation analysis of selected seaweeds.

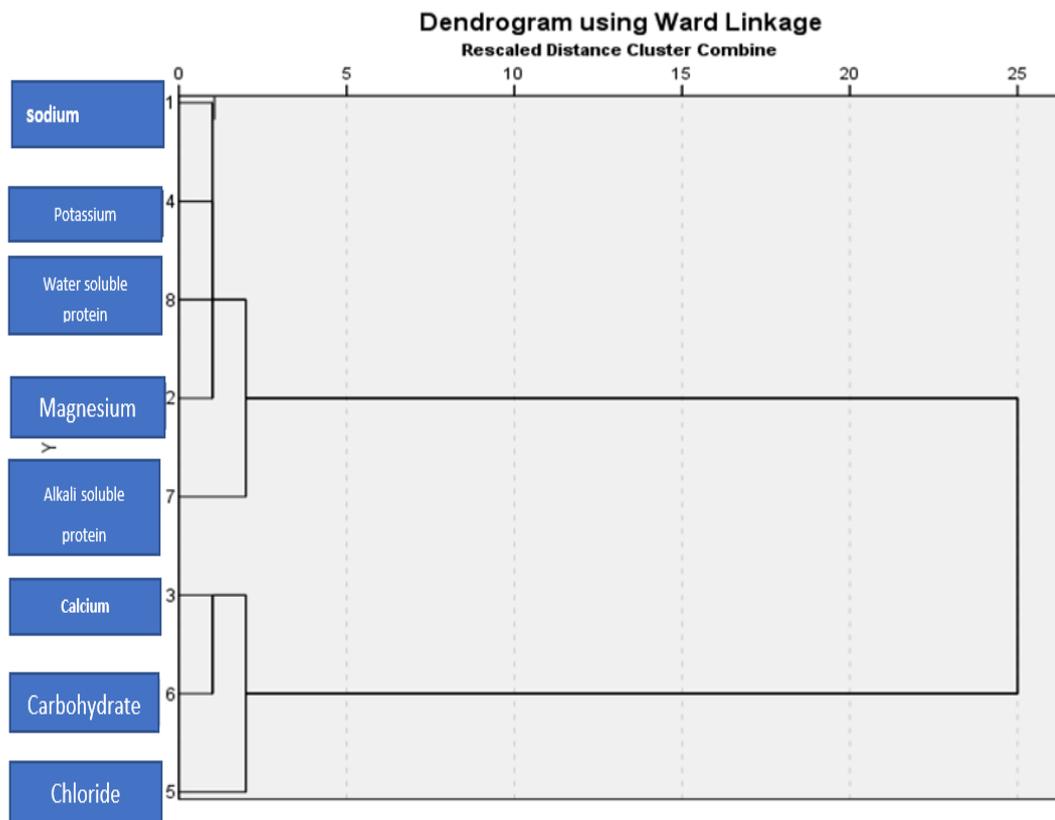
Parameters	Ca <sup>+2</sup>	Mg <sup>+2</sup>	K <sup>+</sup>	Na <sup>+</sup>	Cl <sup>-</sup>
Ca <sup>+2</sup>	1				
Mg <sup>+2</sup>	0.016	1			
K <sup>+</sup>	-0.65**	-0.55	1		
Na <sup>+</sup>	-0.048	.922**	-0.392	1	
Cl <sup>-</sup>	0.557	-0.324	.872**	-0.164	1

Pearson's correlation analysis reveals the pairwise associations for a set of variables (mineral composition of seaweeds) and determines their relativity among the different classes of seaweed species. The positive significant correlation ( $r = .922^{**}$   $P \leq 0.01$ ) between sodium and magnesium. Similarly, Calcium and chloride had a positively significant correlation ( $r =$

872\*\*  $P \leq 0.01$ ) (Table 2). Specifically, calcium and potassium had a negatively significant correlation ( $r = -0.65$ \*\*  $P \leq 0.01$ ). Furthermore, magnesium and chloride showed a positive correlation with calcium and specifically, sodium showed a negative correlation with sodium. However, magnesium showed a negative correlation with potassium and chloride. Potassium showed a negative correlation with sodium. Whenever chloride showed a negative correlation between sodium and magnesium and a positive correlation with calcium.

### 3. Cluster analysis

The dendrogram (Fig.1) represented visually displays a particular cluster configuration. Rows that are close together (have small dissimilarity) will be linked near the right side of the plot. One had five branched namely sodium, potassium, magnesium, and protein. Similarly, another one had three-branched namely calcium, chloride, and carbohydrate. In graphical presentation clearly showed that cations and proteins had a very strong connection, Similarly, the second cluster showed that calcium and carbohydrate had a strong connection with a very short distance as compared to calcium and chloride, which indicates that carbohydrate concentrations of seaweeds affected by the calcium content.



( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Cl}^{-}$ ; ( $\text{meq.g}^{-1}$ )) (The value indicates the mean of the triplicate sample, here values of mean  $\pm$  SE)

Fig. 1: Dendrogram represented the Pearson correlation between the actual distances and the predicted distances based on this particular hierarchical configuration.

#### 4. Box plot chart

The data shown in (Fig. 2) is the transactions between eight different seaweed species collected from the Veraval coastal region during the month of October 2019. The horizontal axis represents the electrical conductivity and the vertical axis trades the total ash content. Comparing the eight different slops of pH in the central axis shown the chat` no 3. This diagram representation showed the relationship between two variables with reference to the dependent factor electrical conductivity. Checking the sequence number in the initial data of pH, the EC, more acidic pH, and the low electrical conductivity recorded in selected species. Similarly, with increasing the value there was EC value of species also increasing order. While the basic pH, had high EC detected in selected seaweeds. The box plot interpreted with respect to the pH and total ash of seaweeds, clearly indicated that both variable pH and ash content of seaweeds showed the inverse relation (with an increase of pH the ash content decreased), except for the initial one. Acidic conditions fevered electric conductivity.

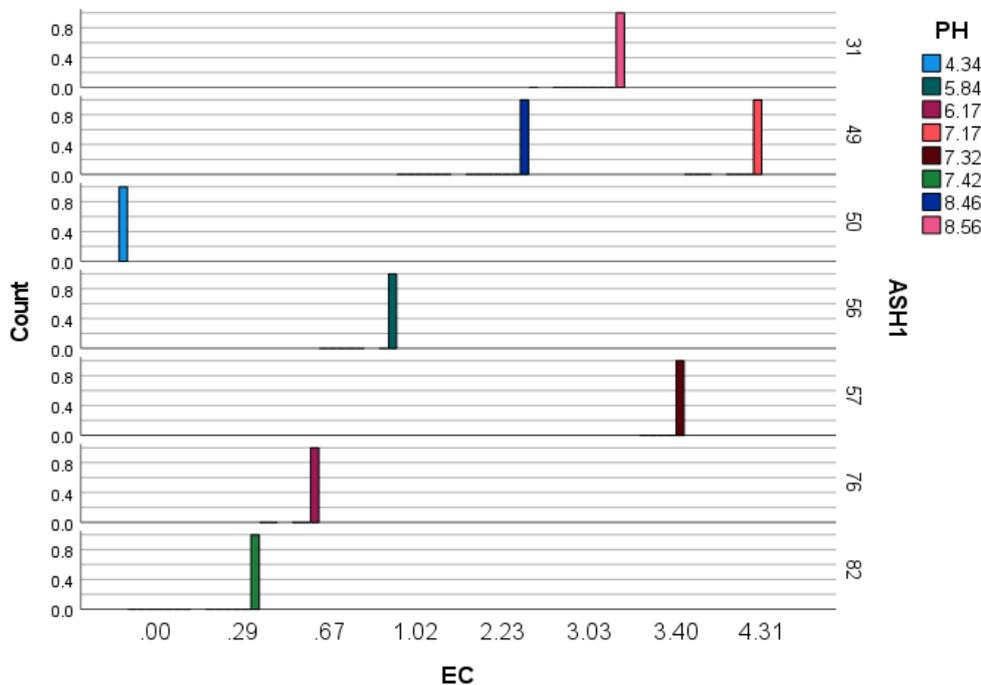


Fig. 2: There were three indicators, electrical conductivity (EC), pH and Total ash content of different seaweeds plotted in the graph using SPSS software.

#### 5. Piper plot

The interpretation of distinct facies from the 0 to 10% and 90 to 100% domains on the diamond-shaped cation to anion graph (Fig. 3). It clearly explains the variation or domination of cation and anion concentrations during each season.  $\text{Na}^+$ -type of water predominated with 75 to 80% in all the four-water samples it simply means that water was alkali rich. Sodium was the key element in all the samples. However, location four water samples were slightly chloride type with 40%, and other domains showed the empty space hence it was clearly shown that the effect of carbonate was totally negatable. Demonstrating the dominance of alkali over alkaline earth. (viz.,  $\text{Na}^+ + \text{K}^+ > \text{Ca}^{+2} + \text{Mg}^{+2}$ ) and strong acidic anions over weak

acidic anions (i.e.,  $\text{Cl}^- + \text{SO}_4^{2-} > \text{HCO}_3^-$ ). None of the samples represented fields of sodium bicarbonate type, magnesium bicarbonate, and calcium chloride type water.

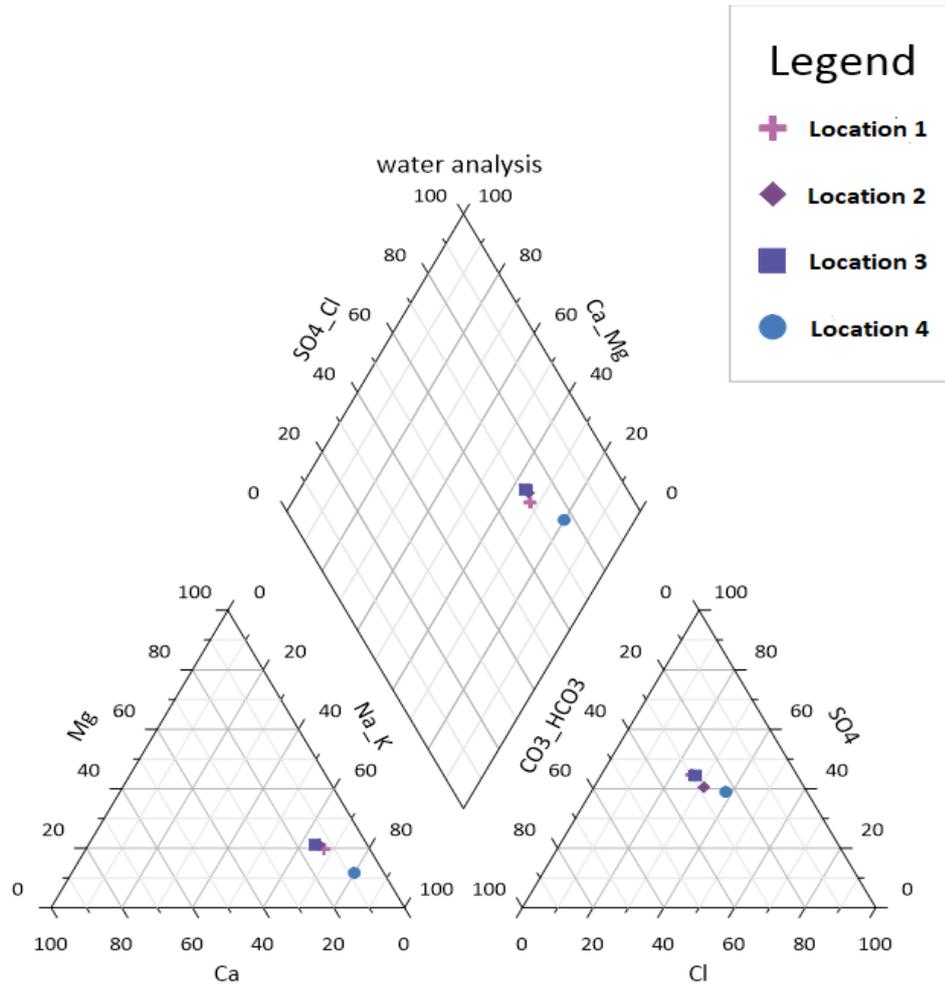


Fig. 3: shows the water analysis of four different locations in the study area.

## CONCLUSION

Seaweed is considered a significant source of many nutritional factors such as proteins, carbohydrates and minerals. According to the recent research that always discovers and explores seaweed benefits, this study is concerned with the validity of different seaweed species collected from the Veraval coastal area. The elemental ( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}$ ), protein and carbohydrate composition in different seaweeds (red, green, and brown) were determined. The variations in the electrical conductivity, ash, pH, etc., in all the eight species, have been studied and their significance in the particular zone of study area discussed. It was observed that almost all the elements estimated vary not only with species but with different geographical zone and different growth stages of the plants. Water analysis of all three zones was carried out, particularly to analyze a complete account of the mineral constituents of the seaweeds and their relationship with the organic constituents on the ionic exchanges taking place between the algae and the surrounding water. The data on the water-soluble

constituents indicate that selective absorption of particular elements takes place in the algae resulting in the accumulation of that element. There is evidence that some of the elements like sodium and chloride absorbed by the algae remain almost completely in the inorganic form.

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