CHAPTER – V
SUMMARY AND CONCLUSION

The experiment entitled “metabolomics and morphological characterization of macro algae” was conducted at Department of Biochemistry and Biotechnology, Junagadh Agricultural University, Junagadh during 2017-18. The results were summarized and concluded in this chapter. The experimental material was comprised of 15 algal species for Morphological, molecular and biochemical analysis.

India has a 7500 km long costal stretch. Indian costline with macroalgae growth with high species diversity notably in Gujarat, Maharashtra, Andaman and nicobar group of islands. Gujarat has 1600 km of costline with rich diversity of macroalgae. Based on relative abundance and diversity stations, Okha station has important genera of macroalgae. The samples were collected from Okha region of Gujarat. The present experiment was preplanned and conducted with four main objectives as given below.

1. To characterize macroalgae based on physical and elemental composition through SEM-EDAX (energy dispersive analysis of X-rays).
2. To study the molecular characterization of macroalgae through RAPD molecular marker.
3. To assess biochemical difference among species through proximate compositions.
4. To find out metabolites difference among various species of macroalgae.

Scanning electron microscopy coupled with EDAX shows that morphological changes in surface and elements present in that surface area. Morphological changes may vary due to change in the structural pattern of algal species. Elemental composition varied from 3 to 10, Boodlea composita and Scinaia complanata respectively. Boodlea composita followed by Padina tetrastromatica, Sciana fasciculari, and Padina boryana in case of number of elements present in surface of algal species.

Algal species exhibited 12 mineral elements; out of them eight were found to be macro elements viz., C, O, Ca, Na, K, Cl, Mg, and S. Iron was found to be micro essential element, and non-essential elements like Si, Al. Major Elements were contained in higher quantity than that of minor and non-essential elements. Atomic massb% and Atomic weight % of algal species varies from species to species and elements to elements.
Summary and Conclusion

Total genomic DNA was isolated from the fresh thallus of algal species. DNA extraction was carried out by CTAB method as with minor modifications. In order to perform PCR based analysis, the DNA was quantified spectrophotometrically to determine DNA concentration. The quality measured by calculating the absorbance ratio of DNA at A260/A280 which ranged from 1.65 to 1.99 and quantity was ranged from 193.2 to 690.2 ng/µl. In present investigation, RAPD markers were used to obtain highly diversified map of 15 algal species and identification of specific markers.

Total 13 RAPD primers were used to amplify genomic DNA and generate the fragments in which 75 bands were polymorphic with 55 shared and 17 unique bands having an average of 5.53 bands per primer. The Polymorphism Information Content (PIC) values for RAPD marker were ranged from 0.33 (OPN-09) to 0.85 (OPP-03) with an average value of 0.67 per primer and RAPD primer index (RPI) differed from 0.67 (OPN-09) to 6.81 (OPP-03) with an average value of 5.53. The RAPD primer OPN-09 showed lowest PIC and RPI among all the primers used. Out of 13 RAPD primers OPN-03, OPN-10, OPP-04, OPO-06, OPM-03, OPO-04, OPM-01, OPQ-04, OPQ-10 and OPQ-03, 10 primers were able to produce species specific unique bands and identified ten algal species. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 30 to 75%.

The proximate composition of fifteen algal species was carried out for the moisture, Ash, carbohydrate, protein and lipid content. In the present study, the moisture content of the fresh macroalgae samples ranged between 71.03 to 79.23 %. Ash% was varied between 4.77 to 12.39 for the Padina boryana and Cheatomorpha crassa respectively. For total carbohydrate% content ranged from 2.58 (Boodlea composita) to 6.93 (Padina boryana). The quantitative analysis of protein content ranged between 1.06% to 7.32% in Sargassum prismaticum and Padina tetrastromatica, respectively. Furthermore, Chaetomorpha crassa (9.76%) shown to have highest percent of oil content followed by Caulerpa taxifolia (8.61%). In most of the algal species found higher oil content as compared to carbohydrates content as well as protein content. However, there were no perfect correlation was observed between the Oil content and number of fatty acid found in algal species studied. Finally, the fatty acid composition ranged as follows 58.33-75.86% Saturated Fatty
Acids, 24.14-42.31% Unsaturated Fatty Acids, 13.64-42.86% Mono Unsaturated Fatty Acids, and 13.33-44.44% Poly Unsaturated Fatty Acids.

Among the different algal species, total chlorophyll content were found between 0.14 mg.g⁻¹ to 0.88 mg.g⁻¹ (dry weight basis) for species *Sciania fascicularis* and *Enteromorpha flexuosa* respectively. Chlorophyll a content was observed between 0.04 to 0.67 mg.g⁻¹. Chlorophyll b content was observed between 0.05 to 0.37 mg.g⁻¹. Among the different algal species, total phenol content varied between 0.07 to 0.99 mg.g⁻¹ (dry weight). Species difference for total phenol content was also found significant. The highest value was recorded for total phenol content from *Ulva rigida* (0.99 mg.g⁻¹) and the lowest value was found for algal species *Padina boergesenii* (0.07 mg.g⁻¹).

Untargeted metabolites profiling results showed metabolites of known structure comprising sugar, fatty acids, organic acids, phenols, sterols were identified. GC-MS analysis revealed that total 628 metabolites were found out of them 305 were found to be unique. The highest numbers of metabolites were recorded in *Chaetomorpha crassa* species. The lowest numbers of metabolites were noticed in *Sargassum cinctum*. In case of highest number of unique metabolites were found in *Chaetomorpha crassa* followed by *Boodlea composita* and *Caulerpa racemosa*.

Metabolites found in algal species contains mass/charge ratio was ranges from 52 to 319.2 and retention time was ranges from 8.23 to 45.80. Mass/charge ration varies due to molecular weight of compound. Retention time was varied due to different compound eluted at different time interval.

**CONCLUSION**

SEM-EDAX images showed the variation in surface as well as elemental composition. Morphological variation due to difference in the surface pattern of algal species and Elemental variation was due to accumulation and changes in the environmental condition.

Based on the molecular and biochemical markers associated with algal species, it was indicated that RAPD primers OPP-03, OPP-07, OPN-03, OPN-10, OPS-08, OPP-04, OPO-06, OPM-03, OPO-04, OPO-07, OPM-01, OPN-09, OPQ-04, OPQ-10 and OPQ-03 were informative to distinguish algal species.
Summary and Conclusion

The Biochemical parameters, Chlorophyll a, Chlorophyll b, Total Chlorophyll varies due to environmental conditions and seasonal variations. Concerning the fatty acid profile, the dominance of SFAs and MUFAs reported in this study varied among macroalgae. Saturated fatty acids were higher in Enteromorpha flexuosa 75.86% than in rest of fifteen algal species, whereas the, PUFAs contents were higher in Boodlea composita (44.44%) were observed as major fatty acid in the present study. In general, variations in fatty acid contents might be attributed to both environmental (location, water temperature, light, concentrations of nitrogen and other compounds in the water) and genetic differences between species.

Gas-chromatography mass spectrometry results showed the variation in different metabolites due to variation in the cellular macromolecules amino acid, sugar, lipid constitutes, sterols.