PHYCOCHEMICAL DISTINCTIVENESS OF SELECTED MARINE MACROPHYTES OF KERALA COAST

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CERTIFICATE

This is to certify that the thesis entitled "Phycochemical Distinctiveness of Selected Marine Macrophytes of Kerala Coast" is an authentic record of the research work carried out by Sri. Kalesh. N. S. under my supervision and guidance in the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the Ph.D. degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any University.

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PREFACE

The plant life in the sea is extremely rich and some exploitation of these resources has been taken place over hundreds of years. At the present time, when the increasing world population imposes greater pressures upon the available land resources, man is increasingly turning his attention to the oceans as a major source of food and industrial chemicals and in this context, the marine plants especially the macroscopic marine algae are becoming of great importance. Marine macroalgae, which are found attached to rocks, corals and other submerged solid substrata in the intertidal and shallow subtidal zones of the sea, constitute an important renewable marine resource. They have been consumed by man as staple food or food-supplements for centuries in many parts of the world particularly in the southeast Asian countries. They have also been used as animal fodder and agricultural fertilizer. Algae serve as the only source for the production of commercially important hydrocolloids such as agars, alginates and carrageenans. Products such as mannitol, laminaran and fucoidan are also obtained from marine macroalgae. Attempts are now being made for screening bioactive compounds from algae and also for the production of fuels like methane.

According to FAO, the total world marine macroalgal production now exceeds 7 million tonnes (wet weight) annually. Of this total, about half of the quantity is utilised for human consumption and the rest for the production of industrially and pharmaceutically important compounds.

Asian countries account for about 90% of the total world production of marine macroalgae. Japan, China, South Korea, Philippines and Indonesia are the major macroalgae producing countries. India produces over 70,000 tonnes (wet weight) of macroalgae annually which are mainly used for the production of agars and alginates.

The various uses of marine macroalgae depend mainly on their richness of biochemical constituents such as proteins, lipids, carbohydrates, amino acids, fatty acids, vitamins, macrominerals, trace elements, bioactive compounds etc. Thus, a proper utilization of available natural resources of macroalgae requires detailed information on the biochemical composition of algae. The present study involves the biochemical investigation of some selected species of marine macroalgae from rather unexplored coasts of Kerala, southwest coast of India. The different biochemical parameters studied include proteins, lipids, carbohydrates, fibres, amino acids, caloric content, ash content, halides and trace metals. The study provides information on interspecies, interclass, spatial and temporal variations in these biochemical constituents. It also incorporates qualitative and quantitative evaluation of agar isolated from the red alga of the genus *Gracilaria* from northern Kerala coast.

The results of the investigation are presented and discussed in seven chapters. Chapter 1 gives an introduction about the marine macroalgae and their importance. Chapter 2 gives a description of the sampling sites, the algal species selected and the methods adopted for the study. Chapter 3 focuses on the major biochemical constituents of macroalgae such as proteins, carbohydrates, lipids and fibre. It also gives variations in the calorific value (energy content) of algae. Chapter 4 describes the trace mineral content of algae, which includes trace metals (both essential and toxic), halides and total inorganic minerals (ash). Chapter 5 describes the amino acid composition of algae. Chapter 6 is agars, which focuses on the quality and quantity of the agar extracted from the red alga *Gracilaria corticata*. Chapter 7 is conclusion based on the findings of the present study.

The references are incorporated at the end of each chapter.

CONTENTS

Chapter 1

INTRODUCTION

1.1 Introduction

Life on the earth originated in the sea and until about 450 million years ago, the only plants on earth were marine algae. The next 400 million years witnessed the evolution of the land flora including bryophytes, pteridophytes, gymnosperms and angiosperms. During the evolutionary process, most of these plants lost their ability to live in seawater and throughout the modern bryophytes, pteridophytes and gymnosperms, there is not a single marine species. In the angiosperms, there is only one small group of seagrasses, which can be described truly marine. Thus, the sea still remains, as it was in the pre-Devonian times, the province of algae, and today about 90% of all the species of marine plants belong to one or other groups of algae (Dring, 1982).

Algae are defined as the photosynthetic nonvascular plants that contain chlorophyll-a and accessory pigments and have simple reproductive structures (Dawes, 1981). They differ from the higher plants in that they do not possess true roots, stems or leaves. However, some of the larger species possess attachment organs called holdfasts that have appearance of roots and there may also be a stem-like portion called a stipe, which flattens out into a broad leaf-like portion called lamina. Some species consists simply of a flat plate of tissue while in others, the plant body, or thallus, is composed of a narrow, compressed or tubular axis with similar branches arising from it. Smaller species are mainly filamentous (Chapman and Chapman, 1980). Algae vary in size from microscopic unicellular forms (phytoplankton) to the giant benthic macrophytes (e.g., macrocystis), which are attached to solid substrata such as rocks or boulders (Dawes, 1981). The phytoplankton dominates the water column, while the rocky shores are abundantly covered almost exclusively with macroalgae. Muddy and sandy areas have fewer macroalgae because most species cannot anchor there.

Algae are located at the base of the food chain for marine organisms. Since the oceans occupy about 71% of the earth's surface area, the role of algae in supporting aquatic life is essential. All marine organisms ultimately depend up on algae for their existence. In addition to directly and indirectly supplying organic molecules for different organisms, algae produce oxygen as a by-product of photosynthesis. They may supply about 30 – 50 % of the net global oxygen. Algae function as chemical modulators in marine ecosystems and also provide habitats for many marine organisms such as snails, limpets, seaurchins etc. (Anon, 2000).

The marine macroalgae, commonly known as seaweeds, constitute an important renewable marine resource with several commercial applications. Most of these macroalgae belong to the three traditional divisions of algae – chlorophyta (green algae), rhodophyta (red algae) and phaeophyta (brown algae). These groups of algae find wide applications in food, pharmaceutical and various other industries. The uses of these algae as food, fodder and fertilizer have been well known in many countries for centuries. The marine macroalgae are often called 'sea vegetables' since they contain sufficient amounts of diverse macro as well as micronutrients of human food value. They contain substantial amounts of proteins, carbohydrates, lipids, vitamins, amino acids, fatty acids, trace elements and minerals. The trace metal content of macroalgae is higher than that of terrestrial plants. They also contain substances of stimulatory and antibiotic nature. Marine macroalgae are the only source of the hydrocolloids such as agars, carrageenans and alginates, which are extensively used in various industries as gelling, thickening and stabilizing agents. Attempts are being made for screening pharmaceutically active compounds from marine macroalgae. With the energy crisis, they are also being evaluated as sources of methane fuel (Chapman and Chapman, 1980).

Macroalgae as Food

Marine macroalgae have been consumed by humans both in raw and in cooked form for centuries. There are historical references to such uses as early as 600 – 800 B.C. and they were undoubtedly used in prehistoric times. Today, as processed and unprocessed food, algae have a commercial value of several billion dollars annually. Approximately 500 species are eaten by humans and some 160 are commercially important (Anon, 2000). In addition to the use of algal extracts in prepared foods, algae are eaten directly in many parts of the world. They are eaten as staple items of diet in Japan, China, Korea and many other Asian countries. The principal users of algae as food are the Japanese who have been using algae in everyday cookery since eighth century. At present, algae account for about 10% of Japanese diet (Ninawe, 2003). People from Hawaiian Islands, Scandinavian and a few other European countries use marine macroalgae and their products as food additives and supplements. In western countries, algae are largely regarded as health food and there has been an upsurge interest in algae as food. In India, marine algae such as *Gracilaria* have been used by some coastal populations for making gruel (Chennubhotla *et al.*, 1987). Some of the edible marine algae occurring along the Indian coast are species of *Ulva, Enteromorpha, Chaetomorpha, Caulerpa, Codium, Gracilaria, Grateloupia, Centroceras, Laurencia, Hypnea, Acanthophora, Dictyota, Padina, Porphyra* and *Sargassum.*

The food value of marine macroalgae lies in their richness of various macro and micro- nutrients including proteins, carbohydrates, lipids, amino acids, fatty acids, vitamins and trace minerals especially iodine. The pattern of free and combined amino acids of many algae are more or less similar to that of vegetables. Some of the algal species contain a high amount of the basic amino acid arginine that in general is abundant in animal protein. The lipid contents of algae are low in general, but contain higher percentage of unsaturated fatty acids, which are more than 10% of the total fatty acid content. Eicosapentanoic acid (EPA) constitutes about 50% of these unsaturated fatty acids. These fatty acids have gained prominence in recent years due to their effectiveness in preventing arteriosclerosis, cancer, coronary heart disease, ageing etc. It has also been reported that derivatives of EPA are beneficial to the human body because they act as local hormones and helps in human metabolism. Like vegetables, marine macroalgae contain all types of vitamins, antioxidants and ascorbic acid. They are rich in group-B vitamins particularly B_{12} than their vegetable counterpart. Vitamin A content of many algae amounts to half of the same spinach contents. Marine algae are rich sources of iodine, which serves as a precursor of thyroxine. They are also rich in essential trace elements (Chapman and Chapman, 1980; Chennubhotla *et al.*, 1987; Anon, 2000; Ninawe, 2003).

Macroalgae as Fodder

Marine macroalgae constitute one of the best renewable resources for livestock feed. The importance of algae as fodder has been recognised since a long time. Historical accounts from 46–43 B.C. report such uses of algae (Waaland, 1981). As animal feed, algae are used to supplement protein intake. They are also valued as a source of trace minerals and vitamins (Metting *et al.*, 1990). In number of countries, animals are regularly fed upon fresh algae or are given a prepared algal meal. Algae are utilised in many countries as feed for cattle, pigs and poultry. They are also used as feed for fish and other aquaculture species. It has been found that the milk produced by the cows that feed on algal meals is richer in fat content than that produced by cows fed on conventional fodder. Likewise, hens fed with algal meals produce eggs rich in iodine (Chapman and Chapman, 1980).

Macroalgae as Fertilizer

The application of marine macroalgae as agricultural fertilizer is a common and widespread practice in coastal areas throughout the world. In India, it is used for coconut plantations especially in coastal areas of Kerala and Tamil Nadu (Chennubhotla *et al.*, 1987). It has been found that algal fertilizer is superior to the conventional organic fertilizer. Its effects on plants include higher yields, increased nutrient uptake, changes in plant tissue composition, increased resistance to frost, fungal diseases and insect attack, longer shelf life of fruits, and better seed germination (Metting *et al.*, 1990).

Marine algae contain all major and minor plant nutrients and trace elements. These can be readily absorbed by plants and this helps in controlling many deficiency diseases in plants and enhancing crop yield. Algae also contain a wide range of amino acids and vitamins, which might be utilised by plants. It has been suggested that many of the crop responses to algal fertilizer are primarily due to the presence of growth substances such as cytokinins, auxin and gibberellin. Algal fertilizer also function as a soil conditioner. It improves aeration and aggregates stability. The carbohydrates and other organic matter present in algae alter the nature of the soil and improve its moisture retaining capacity. The high amount of organic nitrogen present in marine algae helps in maintaining a high level of available nitrogen in the soil (Chennubhotla *et al.*, 1987).

Macroalgae in Medicines

Marine macroalgae have been used as medicines or drug sources for a great many years, stretching back to the era of folk medicines. The Chinese, Koreans and Japanese recognized the pharmaceutical values of many types of marine algae for centuries. Algae have been extensively used in the traditional medicines of maritime nations for treatment of goitre, cancer, hypertension, cough and other diseases. They are used as vermifuges and in the treatment of many viral diseases (Chapman and Chapman, 1980). Most of the marine algae contain sterols and related compounds, which are antagonistic to cholesterol in mammalian systems and could reduce elevated blood pressure. The vitamin and mineral contents of algae are potentially important in the prevention of dietary insufficiency diseases. Algal extracts such as agars and alginates are widely used for medical and dental purposes. In dentistry, they are used as intra – oral impression materials. Agar is used as a laxative and as an anticoagulant for blood. It is useful in the preparation of food prescribed for diabetic patients and promotion of tissue growth. The phycocolloid carrageenan is used in the treatment of peptic ulcer in humans.

The ability of carrageenan to form metal salts indicate an important use as non-toxic chelating agent in the treatment of heavy metal poisoning. Many bioactive compounds and polysaccharides of marine algae have been identified for use in medicines. Sulphated polysaccharides from macroalgae have been reported to inhibit the activities of viruses especially the herpes and HIV. These carbohydrates have shown to strengthen the immune system of human and animals. Algae also possess active principles that control and prevent various microbial infections (Ninawe, 2003).

The numerous food and medicinal values of marine macroalgae suggest that they can function as a unique therapeutic super food for human beings and they are being considered as the 'medical food of 21st century'.

Industrial Applications of Macroalgae

Marine macroalgae constitute the basis for a multimillion-dollar industry, in which billions of dollars worth agar, carrageenan, alginates and other valuable minerals and chemicals are extracted annually. Many of these products are extensively used in industries such as food, confectionery, dairy, pharmaceuticals, textiles, paper etc. all over the world. The phyco-colloids such as agar, carrageenan and alginates are used as thickening, gelling, emulsifying and stabilizing agents in various industries. Agar extracted from red algae is used as a solidifying agent in bacteriological culture media. It is also used in processing canned meat, fish and poultry. It is used for stabilizing bakery icings and clarifying wines, juices and vinegar. Agar is used as a stiffening agent in a number of food products. It is used in the manufacture of various pharmaceutical preparations, photographic film coatings and paints. Alginates obtained from brown algae are extensively used in food, pharmaceutical, cosmetic, textile, paper, dairy, paint and various other industries. Carrageenans isolated from red algae are used in dairy products, imitation creams, puddings, syrups and canned foods (Chennubhotla *et al.*, 1987).

Other Applications

The potential role of microscopic algae in sewage treatment has long been recognised. However, there is as yet no cheap and efficient means of harvesting such algae on a commercial scale (Dring, 1982). Consequently, macroalgae, which are much easier to harvest are now being considered for this purpose. Algae are also used to recover heavy metal ions from industrial effluents or process waters (Greene and Bedell, 1990).

Marine macroalgae have frequently been used as indicators of trace metal pollution in coastal waters. They concentrate metal ions from sea-water and the variations in the concentrations of metals in algal thalli often are taken to reflect the metal concentrations in the surrounding seawater. Algae accumulate only those metals that are biologically available (Lobban and Harrison, 1997).

One of the major new uses of marine macroalgae is their use as a biomass source for the production of fuels such as methane and alcohol by bacterial fermentation, thus providing a renewable replacement for the dwindling supplies of natural fuels from fossil sources (Dring, 1982). They are also being considered for the photobiological production of hydrogen, which is recognised as an ideal energy carrier that does not contribute to environmental pollution or global warming (Melis, 2002).

1.2 The Status of Marine Macroalgae in India

India, with a long coastline of about 7500 km including those of Island territories, possesses great potential of marine algal resources. The littoral and sublittoral rocky areas of Indian coast support a good growth of different marine algae including agarophytes (agar producing algae), alginophytes (algin producing algae), carrageenophytes (carrageenan producing algae) and edible algae. Out of about 20,000 marine algal species distributed throughout the world, 844 species have been reported from Indian coast (Oza and Zaidi, 2001). This includes 434 species of red, 216 species of green and 191 species of brown algae. Of these, 18 species of red algae are agarophytes, 13 species are carrageenophytes and 54 species of brown algae are potential alginophytes. The estimated total standing stock of marine algae on Indian coasts is about 70,000 - 80,000 tonnes wet weight (Zaidi *et al.*, 1999). Among the maritime states of India, Tamil Nadu on the southeast coast occupies the prime position in algal availability (22,000 tonnes wet weight). The richest resources are found in the area from Mandapam to Kanyakumari. Gujarat on the west coast of India has an algal resource of about 20,000 tonnes wet weight. The important places of algal interest on this coast are Okha, Dwaraka, Adatra and Veraval. The island territories like Andaman and Nicobar Islands in the Bay of Bengal and Lakshadweep group of Islands in the Arabian Sea have been found to harbour a variety of marine algae in good quantities. Fairly rich algal beds are present along the coasts of Maharashtra, Karnataka, Kerala, Goa and Andhra Pradesh. Along the coast of Kerala, approximately 1000 tonnes of marine algae are produced annually and the important places of algal interest on this coast are Kovalam, Vizhinjam, Varkala and Kannur.

Although India has a good wealth of naturally occurring marine algae, they are exploited commercially only for the manufacture of phycocolloids such as agar and alginates. The algal industry in India produces about 60 tonnes of agar and 500 tonnes of alginates annually. The annual demand of raw materials is 2000 tonnes of agarophytes and 13,000 tonnes of alginophytes. Since the indigenous production of phycocolloids is unable to meet the increasing demand, India imports about 10 – 12 tonnes of agar, 35 tonnes of alginates and 140 tonnes of carrageenan annually costing foreign exchange of about Rs. 10 crores (Zaidi *et al.*, 1999). The cultivation of commercially important marine algae is being attempted in India to meet the increasing demand of raw materials for algae based industries.

The use of marine macroalgae as an alternate staple food or food-supplement has not caught any appreciable level in India. However, as the alarming growth of population has resulted in an increased demand for non-conventional food sources, marine macroalgae, which form an annually renewable resource, are becoming increasingly important. In India, where the coastal area is very long and where more than half the population is vegetarians, marine algae have a great potential as human food. The utilization of algae as food and food supplements could help in meeting the food and nutritional security and health measures of Indian population.

In this context, many studies have been done on the nutritional and biochemical aspects of Indian marine macroalgae. However, most of these studies are confined to a few coastal regions like Gujarat, Maharashtra, Goa and Tamil Nadu. In Kerala, most of the studies are confined to the southern coastal region. No detailed biochemical investigation has been carried out so far on the marine algae from central and northern coast of Kerala. Hence, the present study has been undertaken to investigate the biochemical composition of marine macroalgae from two localities of Kerala coast – one from central and the other from northern Kerala coast.

1.3 Area of the Present Study

Kerala, situated on the southwest part of India, lies between north latitudes 5^0 15′ and 12^0 85′ and east longitudes 74^0 55′ and 77^0 05′ and covers 38.864 sq. km. It has a coastline of about 580 km, which is about 8% of the total coast length of India. The coasts north of Kozhikkode and south of Kollam are mainly rocky, while the central part is mainly sandy. Sea erosion on the coastal tract is a frequent feature of Kerala where groins and seawalls have been constructed as a protective measure. The tides are semidiurnal type and the mean tidal ranges vary from 0.9 m in the south to 1.8 m in the north. The coastline is very low and coastal areas are flooded by storm tides in many sections during the southwest monsoon season. The climate is typical of tropical features. The annual rainfall is high ranging from 200 – 300 cm, most of which falls during the southwest monsoon season. During the northeast monsoon season, rainfall is negligible. Based on the temperature and rainfall, the seasons of the year are designated (Menon and Rajan, 1989) as:

i. Wet summer monsoon season (southwest monsoon) – June to September
ii. Retreating monsoon season (withdrawal of southwest monsoon) – October to November
iii. Dry winter monsoon season (northwest monsoon) – December to February
iv. Hot season (Transitional period between NW monsoon and SW monsoon) – March to May

Ettikkulam along the northern coast and Narakkal along the central coast of Kerala were the sites chosen for the present study. The former is a rocky beach while the latter, a sandy beach. Algal samples were collected during the period August 1999 – April 2001.

1.4 Scope and Objectives of the Present Study

In the context of use of marine macroalgae as an ideal health food for humans, information on their biochemical composition is of immense value since the nutritive value of algae comes from various biochemical constituents such as proteins, carbohydrates, lipids, amino acids, vitamins, trace elements, iodine etc. Also, knowledge of the mineral constituents of marine algae is important in their use as livestock feed and fertilizer. Information on the toxic heavy metal content of marine algae is of utmost importance from the point of view of using them as food and fodder. The trace metal composition of marine algae also provides information on the pollution status of coastal environments. Likewise, an assessment of the available natural resources of agarophytes for their agar content and its quality is necessary in view of the increasing demand of raw materials for agar industry in recent years. Since marine algae exhibit specieswise, classwise, seasonal and geographical variations in their biochemical composition, a thorough biochemical investigation is essential for the proper utilisation of natural beds of marine algal resources and for the cultivation of economically and nutritionally important species of marine algae.

The objectives of the present study can be summarized as:

- Evaluation of the nutritional values of some selected species of marine macroalgae from central and northern Kerala coast by identifying their biochemical compositions.
- Determination of the interspecies, interclass, spatial and temporal variations in the biochemical compositions and nutritional values of macroalgae.
- Screening of macroalgae for their wide variety of amino acids.
- Estimation of the trace mineral content of marine algae including toxic metals.
- Qualitative and quantitative evaluation of agar extracted from agarophytes.

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Chapter 2

MATERIALS AND METHODS

2.1 Algal Materials

Six species of marine macroalgae belonging to two different systematic groups (three each) were chosen for the present study (Fig. 2.1). Taxonomy of these species is given in Table 2.1. The selection was made considering the abundance of algae at the sampling sites and the easiness for recognition and collection.

2.2 Sampling Technique

Sample collection was done during the period of low tides. The algae were hand picked from their natural habitats, sorted out and washed thoroughly in seawater followed by rinsing with tap water and then with de-ionised water (milli-Q grade). After air-drying, they were dried to constant weight at 60 ^{0}C in an air oven. They were powdered and stored in sealed polythene bags for further analysis.

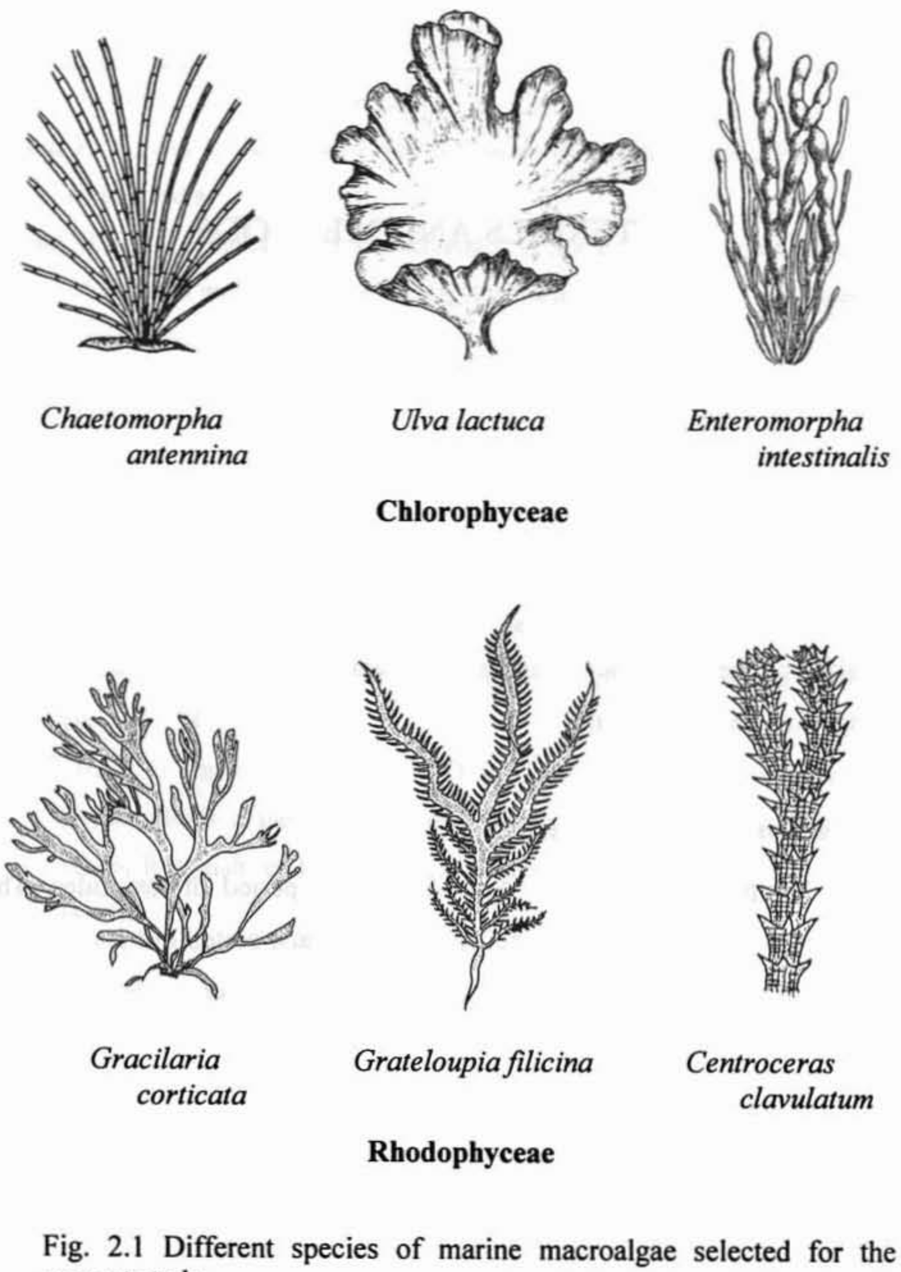

Fig. 2.1 Different species of marine macroalgae selected for the present study.

between the slope of the hills and the beach is under marginal cultivation. There is the influence of back-land drainage in the area.

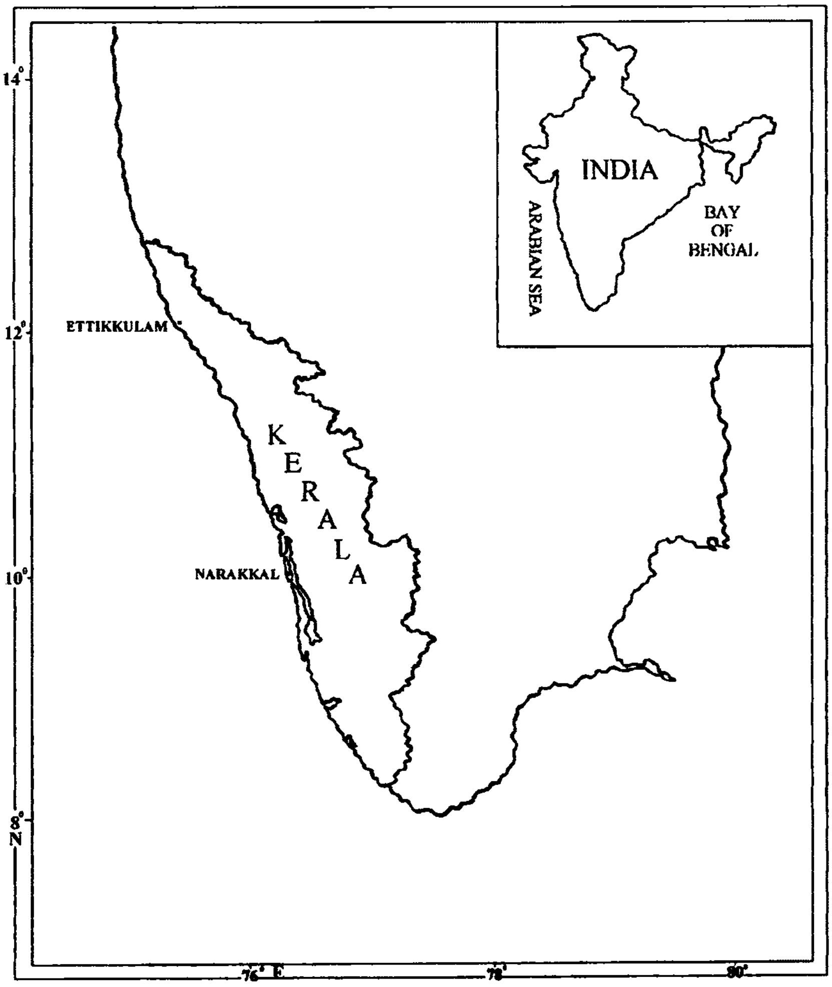

Fig. 2.2 The location map of sampling sites.

Plate 1 Macroalgal growth at Ettikkulam

Plate 2 *Chaetomorpha antennina* and other macroalgae growing at Ettikkulam

Plate 5 Macroalgal growth at Narakkal

Plate 6 *Chaetomorpha antennina* and *Centroceras clavulatum* growing at Narakkal

Narakkal, about 10 km north of Kochi is a sandy beach. Here, the substrata for macroalgae are the artificial dykes of rubbles constructed as a preventive measure against coastal erosion during monsoon months. Being constructed on a sandy base, part of the material have sunk into the sand and drifted seawards due to the action of waves. This site is proximate to the Cochin barmouth through which the Cochin backwaters open out into the Arabian Sea. This backwater is intensely polluted due to the inputs from several major and minor industries situated in the upstream region. Several rivers, irrigation channels and sewers open into this backwater making it polluted. The harbour activities and oil jetty operations add to the pollution. The organic and inorganic substances delivered to the coastal waters through barmouth may have some influence on the marine algae present at Narakkal.

2.4 Sampling period

Sample collections were made during the period from August 1999 to April 2001. A total of six collections were made from each location: August and December 1999, May and October 2000 and January and April 2001. The collection dates fall in different seasons of the year as follows: August – summer monsoon season, December – early winter monsoon season, May – late hot season, October – retreating monsoon season, January – mid winter monsoon season and April – mid hot season.

2.5 Analytical Methods

2.5.1 General

All the chemicals used were of analytical grade and reagents were prepared using milli-Q water. Quality control was carried out by parallel analysis of standard reference material (CRM 279 *Ulva lactuca*) following the same procedure as that used for samples. The results were largely in agreement with certified values. In the case of non-certified parameters, standard addition method was used to identify the quality of the data. In all cases, recovery was above 90%. The precision of analysis was ascertained by triplicate analyses and the results were reported as mean values.

2.5.2 Proteins

Total protein contents of algae were determined colourimetrically by the method of Lowry *et al.* (1951) after the extraction of proteins with dilute alkali.

To 10 mg of dried algal powder taken in a test tube, 5ml of 1N NaOH was added and allowed to stand for 24 hrs at room temperature. 0.5 ml of the extract was pipetted into a separate test tube and 5 ml of freshly prepared alkaline copper tartrate reagent was added followed by 0.5ml of 1N Folin – Ciocalteu phenol reagent. The contents were mixed thoroughly and allowed to stand for 20 min for colour development. The absorbance was then read at 750 nm against a reagent blank using Spectronic Genesys 10 UV-Visible spectrophotometer. Bovine serum albumin was used as standard and the results are expressed as percentage of dry weight of algae.

2.5.3 Carbohydrates

Total Carbohydrates

Total carbohydrate contents of algae were determined colourimetrically by phenol – H_2SO_4 method (Dubois *et al.*, 1956) after extraction into 5% trichloroacetic acid.

To 5 mg of dried algal powder taken in a test tube, 10 ml of 5% trichloroacetic acid was added and heated in a water bath at 80 – 90 ^{0}C for 3 hrs. After cooling to room temperature, the volume was made up to 10 ml with milli-Q water. 0.2 ml of the extract was pipetted into a separate test tube and 1 ml of 5% phenol was added followed by rapid addition of 5 ml of conc. H_2SO_4. After cooling the contents of the tube, absorbance was measured at 490 nm against a reagent blank using Spectronic Genesys 10 UV-Visible spectrophotometer. Glucose was used as standard and the results are expressed as percentage of dry weight of algae.

Low molecular weight carbohydrates (LMWC)

LMWC contents were determined by the same method as total carbohydrates after extraction into 80% ethanol. The extraction was carried out as follows.

To 5 mg of dried algal powder taken in a test tube, 10 ml of 80% ethanol (neutralised with $CaCO_3$) was added and boiled on a steam bath for 1 hr. The solution was decanted into a 10 ml volumetric flask and the solids were comminuted in a high-speed blender with 80% ethanol. The blended material was boiled for 30 minutes, cooled and centrifuged. The centrifugate was transferred to the volumetric flask and diluted to the volume with 80% ethanol. 1 ml of the extract was analysed by phenol – H_2SO_4 method as described for total carbohydrates. The results are given as percentage of dry weight of algae.

Polysaccharides

Polysaccharide contents of algae were determined by taking the difference between total carbohydrate content and LMWC content. The results are expressed as percentage of dry weight of algae.

2.5.4 Lipids

Total lipid contents of algae were determined colourimetrically by sulphophosphovanillin method (Barnes and Blackstock, 1973) after its extraction into 2:1 $CHCl_3$: CH_3OH solvent mixture (Folch *et al.*, 1957).

To 50 mg of dried algal powder taken in a screw capped test tube, 10 ml of 2:1 $CHCl_3$: CH_3OH solvent mixture was added. The tube was loosely capped and heated in a water bath at 60 ^{0}C for 30 min. After cooling the solution, the volume was made up to 10 ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of conc. H_2SO_4 by boiling in a water bath for 10 min. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 min for colour development. The absorbance was then measured at 520 nm against a reagent blank using Spectronic Genesys 10 UV-Visible spectrophotometer. Cholesterol was used as standard and the results are expressed as percentage of dry weight of algae.

2.5.5 Calorific value (Caloric content)

The caloric contents of algae were determined by converting the amounts of major biochemical constituents into calorific values using standard calorific equivalents, *viz*., 5.65 for proteins, 9.45 for lipids and 4.20 for carbohydrates (Dare and Edwards, 1975). The calorific values are expressed as kcal g^{-1} on dry weight basis.

Calorific value (kcal g^{-1}) = (5.65 x P + 9.45 x L + 4.20 x C) / 100

Where P = Total protein content (%)

L = Total lipid content (%)

C = Total carbohydrate content (%)

2.5.6 Fibres

The crude fibre contents of algae were determined by the method described by Larsen (1978). This method is based on the removal of all water-, acid- and alkali-soluble materials present in the algae by extraction at elevated temperatures and the determination of organic part of the remainder as weight loss upon combustion.

1.5 g of dried algal powder was taken in a conical flask equipped with reflux condenser. 150 ml of dil. H_2SO_4 (1.25 g /100 ml) was added and boiled under reflux for 30 min in a water bath. After cooling to room temperature, the mixture was filtered with moderate suction through a coarse sintered glass filter equipped with a piece of linen cloth on the sintered plate. The filter cake was washed with 200 ml of milli-Q water and then transferred to the conical flask with 100 ml of milli-Q water. 100 ml of 2.5% NaOH was added and refluxed for another 30 min. The mixture was then filtered through a Gooch sintered crucible containing a layer of 2-3 mm of acid-washed asbestos on top of the sintered plate. The filter cake was washed with 200 ml of boiling milli-Q water followed by 15 ml of ethanol. The crucible containing the final residue was heated at 105 ^{0}C until constant weight, W_1 was obtained. It was then heated at 450 ^{0}C overnight and the weight, W_2 was recorded. The weight loss, $W_1 - W_2$ represented the fibre content of the sample and it was expressed as the percentage of dry weight of algae.

2.5.7 Ash

The ash content of algae, which represents the total inorganic materials present, was determined by ashing 2 g of the dried algal powder taken in a porcelain crucible at 500 ^{0}C for 6 hrs in a muffle furnace (Larsen, 1978). The weight of the residue, which represented the ash content, was recorded and the results are given as percentage of dry weight of algae.

2.5.8 Chlorine

The chlorine content of algae was estimated argentometrically by Volhard's method after converting chlorine present in the sample into alkali chloride by treating with an alkali salt (AOAC, 1995).

5 g of dried algal powder taken in a silica crucible was moistened with 20 ml of 5% Na_2CO_3 solution. After evaporation to dryness, the sample was charred on a hot plate under hood followed by combustion at 500 ^{0}C for 24 hrs in a muffle furnace. The residue was dissolved in 10 ml of 5N HNO_3 and diluted to 25 ml with milli-Q water. This solution was titrated with standardized 0.1N $AgNO_3$ solution until the precipitation of AgCl stopped and then a slight excess of $AgNO_3$ solution was added. After stirring well, the solution was filtered through Whatman No. 41 filter paper and the residue was washed thoroughly with milli-Q water. To the filtrate, 5 ml of a saturated solution of $FeNH_4(SO_4)_2.12\ H_2O$ was added followed by 3 ml of 12N HNO_3. The excess of $AgNO_3$ present in this solution was then titrated with standardized 0.1N KSCN solution until a pale-rose colouration to the

solution. From the net volume of $AgNO_3$ solution consumed, the chloride content was calculated using the relation,

1 ml of 0.1N $AgNO_3 \equiv 3.506$ mg of chloride

The results are expressed as mg g^{-1} on dry weight basis.

2.5.9 Iodine

Iodine content of algae was determined colourimetrically following the method described by Saenko *et al.* (1978). This method is based on the transformation of all iodine compounds into molecular form, which subsequently interacts with bromide ion to form a complex ion I_2Br^- that yields a salt-like substance with brilliant green. The resultant coloured compound is extracted with toluene and absorbance is measured at 680 nm.

100 mg of dried algal powder was placed in a silica crucible. Iodine present in this sample was first converted to alkali iodide by moistening with 30% K_2CO_3 and ashing in a muffle furnace at 500 ^{0}C for 5 hrs. After transferring the contents of crucible into a 100 ml beaker, 25 ml of 20% NaCl solution was added and iodide ions were salted out for 1 hr. The mixture was then filtered. 5 ml of the filtrate was pipetted into a graduated stoppered test tube and diluted to 10 ml with 20% NaCl solution. Then, 0.25 ml each of freshly prepared 0.5% $NaNO_2$ solution, 20% NaBr solution and 0.5% brilliant green solution were added. Subsequent agitation was followed by the addition of 5 ml of toluene and 1 ml of 5N H_2SO_4. It was shaken for 2 min and allowed to stand for 20 min for the separation of the layers. The blue-green toluene layer was then removed through a separatory funnel and the absorbance was measured at 680 nm against a reagent blank using Spectronic Genesys 10 UV-Visible spectrophotometer. KI was used as standard and the results are expressed as mg g^{-1} on dry weight basis.

2.5.10 Trace Metals

Trace metal contents (Cu, Zn, Mn, Cr, Co, Fe, Ni, Sr, Ag, Cd and Pb) of algae were determined by flame atomic absorption spectrophotometry after digestion of the sample in triple acid mixture (HNO_3 + $HClO_4$ + H_2SO_4) (Rao *et al.*, 1995; Reeta, 1996).

0.5 g of dried algal powder taken in a digestion tube was digested in triple acid mixture (10 ml of HNO_3 + 4 ml of $HClO_4$ + 1 ml of H_2SO_4) until the mixture became colourless. It was then cooled and centrifuged. The centrifugate was transferred to a 50 ml volumetric flask and diluted to the volume with milli-Q water. It was then transferred to a 50 ml plastic bottle and metal concentrations were measured using Perkin Elmer 3110 atomic absorption spectrophotometer. The results are expressed as $\mu g\ g^{-1}$ on dry weight basis.

2.5.11 Amino acids

The total amino acid pool of algae was estimated colourimetrically after liberating amino acids bound in proteins and peptides by acid hydrolysis and separating individual amino acids by two-dimensional paper chromatographic technique (Jayaraman, 1981; Munda and Gubensek, 1986).

100 mg of dried algal powder was hydrolysed with 2 ml of 6N HCl at 105 ^{0}C for 48 hrs in an evacuated and sealed tube. The mixture was then cooled and centrifuged. The centrifugate was transferred to a graduated test tube and volume was made to 3 ml with milli-Q water. 20 μl of this solution was spotted on Whatman No.1 chromatographic paper and developed by two-dimensional paper chromatographic technique. The solvent for first run was butanol : acetic acid : water (4:1:5 v/v) and that for second run was

phenol : water (4:1 v/v). After development, the chromatogram was air-dried and sprayed with ninhydrin reagent for colour development. It was then dried for 1hr at room temperature followed by heating at 100 ^{0}C in an oven for 10 min for complete colour development. Amino acid spots appeared pink in colour except proline and hydroxyproline, which appeared as yellow spots. The spots were identified by comparing with a master chromatogram prepared under identical conditions using known amino acids. The individual spots were cut out and eluted separately in 3 ml of 80% aqueous ethanol. The amino acids were then estimated colourimetrically at 570 nm except proline and hydroxyproline, which were estimated at 440 nm. The results are expressed as mg of amino acid per g dry weight of algae (mg g^{-1}).

2.5.12 Agar

Extraction and Quantitative Determination

3 g of dried algal powder was defatted by sequential extraction with acetone, 80% ethanol, 95% ethanol and diethyl ether. The defatted sample was first dried in air and then in *vacuo* over P_2O_5. The dried defatted sample was soaked overnight in 200 ml milli-Q water. It was then boiled for 6 hrs to extract the agar. The hot solution was filtered through a cloth filter and the residue was washed with hot milli-Q water. The filtrate was poured into a petri-dish and allowed to gel at room temperature. The gel was then cut into 2 cm strips and frozen overnight at – 4 ^{0}C. The frozen gel was allowed to thaw at room temperature and the free liquid was filtered off with suction through Whatman No. 54 filter paper covered with nylon mesh. The residue was washed with milli-Q water followed by 80% ethanol, 95% ethanol and diethyl ether. It was then dried overnight then in *vacuo* over P_2O_5. Dried

agar was weighed and expressed as percentage of agar yield on dry weight basis (Craigie and Leigh, 1978; Valiente *et al.*, 1992).

Qualitative Determination

Melting Temperature

1.5% agar solution was prepared in boiling water (milli-Q). 1 ml of this solution was taken in a test tube and cooled overnight at 4 ^{0}C. A 5 mm glass bead was placed on the top of the agar gel and the tube was equilibrated in a water bath at room temperature for 10 min. The water bath was then heated at approximately 1 ^{0}C /min and the temperature, at which the glass bead sank through the agar was recorded using a thermometer. This temperature represented the gel melting temperature (^{0}C) (Craigie and Leigh, 1978).

Gelling Temperature

1 ml of hot 1.5% agar solution was taken in a test tube and allowed to cool gradually at room temperature. A thermometer was introduced into the agar solution and the temperature was noted when the withdrawal of thermometer left a permanent deformation of the meniscus. This temperature represented the gelling temperature (^{0}C) of agar (Bird and Hinson, 1992).

Gel Strength

Gel strength was determined using 1.5% agar gel allowed to cure overnight at room temperature. A gelometer (Funaki and Kojima, 1951) was used to determine gel strength. The analysis was carried out at Regional

Centre of Central Marine Fisheries Research Institute, Mandapam. The result is expressed in g cm^{-2}.

Chemical Analysis

3,6 – Anhydrogalactose (3,6-AG)

3,6–AG content of agar was determined colourimetrically by modified resorcinol method (Yaphe and Arsenault, 1965) as described by Friedlander *et al.* (1981).

1 mg of agar taken in a test tube was dissolved in 1ml of milli-Q water and 5 ml of resorcinol reagent was added. It was mixed thoroughly and heated in a boiling water bath for exactly 2.5 min. After cooling in an ice bath, absorption was measured at 490 nm. D-fructose was used as standard and the results are expressed as percentage of dry weight of agar.

Sulphate

Sulphate content of agar was measured by a modification of $BaCl_2$ turbidimetric method of Tabatabai (1974) as described by Craigie *et al.* (1984).

20 mg of dry agar was hydrolysed in 0.5 ml of 2N HCl at 100 ^{0}C for 2 hrs in a sealed tube. The contents were then transferred to a 10 ml volumetric flask and diluted to the volume. Humic substances were removed by centrifugation. 2 ml of this solution, 18 ml of milli-Q water and 2 ml of 0.5N HCl were mixed in a 50 ml Erlenmeyer flask. 1 ml of $BaCl_2$-gelatin reagent was added and swirled. After keeping for 30 min, the contents of the flask were again mixed by swirling and the turbidity was measured at 550 nm against a reagent blank. K_2SO_4 was used as standard and the results are expressed as percentage of dry weight of agar.

IR spectrum

Dry agar sample was milled in a mortar with KBr and then pressed into a thin disc. It was analysed by Fourier transform infrared (FTIR) spectroscopic technique using Bruker IFS 66V FTIR spectrometer. The analysis was carried out at RSIC, IIT-Madras, Chennai.

2.5.13 Statistical Analysis

Specieswise variations in organic and inorganic constituents of algae were assessed using one-way ANOVA at 95% probability level. Multi-comparisons of means were made using Tukey's HSD (honestly significant difference) test. Classwise and spatial variations were compared using Student's t-test. The relationships between various constituents of algae were tested using Pearson correlations. All statistical tests were carried out using SPSS (Statsoft).

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Chapter 3

MAJOR BIOCHEMICALS AND CALORIFIC VALUE

3.1 Introduction

A great many kinds of marine macroalgae are edible and have entered as marine vegetables into the diets of human beings. They are consumed as staple food item or as food-supplements in many countries. They are also used as feed for fish and farm animals. Many of the marine macroalgae can act as potential food source for marine invertebrates either directly or indirectly as organic detritus. The marine algae such as *Ulva* are widely used by abalones and other aquaculture species.

The use of marine algae as food for humans, animals and aquaculture purposes is based on their high nutritive value arising from the richness of biochemical constituents such as proteins, carbohydrates, lipids, vitamins and trace minerals. The major biochemical compounds, *viz.*, proteins, carbohydrates and lipids function as the energy-yielding nutrients that provide the energy required by the human and animal body to manufacture the daily requirements of high-energy phosphates (mainly ATP) and reducing equivalents (2H) needed to power all the body functions (Murray *et al.*, 1996). The lipids possess higher energy content per gram compared to proteins and carbohydrates. The caloric equivalents are 9.45

kcal g^{-1} for lipids, 5.65 kcal g^{-1} for proteins and 4.20 kcal g^{-1} for carbohydrates.

Lipids and carbohydrates are the principal dietary sources of energy. Proteins normally provide the body's requirement for amino acid nitrogen and specific amino acids. The energy derived from carbohydrates and lipids affects protein requirements because it spares the use of protein as an energy source. To use expensive dietary protein efficiently and to reduce requirements for it to a minimum, it is necessary to ensure adequate provision of energy from non-protein sources such as carbohydrates and lipids. Dietary lipids, apart from providing significant proportion of the dietary requirement for energy, have two essential functions in nutrition. They act as the vehicle for lipid-soluble vitamins and they supply essential polyunsaturated fatty acids that body is unable to synthesize.

Some carbohydrates present in the diet are indigestible by humans and hence do not provide energy. These indigestible carbohydrates form part of a group of substances known as dietary fibre. It usually consists of cell wall components. The dietary fibre is required for optimal health. Consumption of significant quantities of dietary fibre has been shown to be beneficial to human nutrition helping reduce the risk of certain type of cancer, coronary heart disease, diabetes and constipation (Murray *et al*, 1996). Many of the macroalgae have been found to contain fairly high amounts of fibres (e.g., *Chaetomorpha*).

In the context of use of marine macroalgae as a staple item of diet as well as a potential source of food for animals and in mariculture, several studies have been done on the biochemical aspects of marine algae in order to evaluate their nutritional value. The algae have been found to contain substantial amounts of proteins, carbohydrates, lipids and fibres. They contain various carbohydrates different from those in higher land plants. The algal proteins have many essential amino acids including iodine containing ones (Scott, 1954). Many marine algae have been reported to contain proteins in amounts somewhat higher than that in other food materials such as cereals, eggs and fish (Rao, 1964). Proteins can be extracted from these algae and as such the dry powders of marine algae can be added to various protein deficient foods or taken along with other foodstuffs in small quantities.

Several studies have been undertaken on algal proteins and their quality (Ito and Hori, 1989; Amano and Noda, 1990; Fleurence, 1999). Dam *et al.* (1986) carried out a study on the utilization of algae as a protein source for humans. Fleurence (1999) showed that marine algae contain considerable amounts of proteins (10-47% dry weight) with potential for human and animal nutrition. Fleurence *et al.* (1999) determined the nutritional value of proteins obtained from *Ulva* sp. Wong and Cheung (2001) carried out nutritional evaluation of subtropical marine algae and observed that proteins of algae such as *Ulva lactuca, Hypnea charoides* and *Hypnea japonica* have *in vitro* digestibility over 85% and they contain high amounts of essential amino acids. Digestion of algal proteins by proteolytic enzymes such as pepsin, pancreatin, pronase, trypsin, chymotrypsin etc. has been reported (Fujiwara *et al.*, 1984; Indergaard and Minsaas, 1991; Fleurence *et al.*, 1999).

The biochemical composition of algae has been found to vary with factors such as season of the year, habitat, age, plant-part and growing conditions especially light, nutrients and temperature (Black, 1950; Lobban and Harrison, 1997). Marine algae have been reported to exhibit marked variations in biochemical composition not only among different classes and genera, but also among different species of the same genus (Dhargalkar *et al*, 1980; Dave *et al.*, 1987). Rao and Tipnis (1964) reported the protein content of different species of marine algae from Gujarat coast. Parekh *et al.* (1977) studied the chemical composition of marine algae from Saurashtra coast and found significant variations among different genera of algae. The same species collected from different localities at different periods also showed considerable variations. Murthy and Radia (1978) examined the biochemical contents of seaweeds from Port Okha in relation to ecological factors and reported monthly variations in protein, fat, carbohydrate and crude fibre. Dhargalkar (1979) carried out biochemical studies on the chlorophyte *Ulva reticulata* and reported variations in major metabolites such as proteins, carbohydrates and lipids. Seasonal changes in biochemical composition of marine algae from Goa coast were studied by Solimabi *et al.* (1980) and Sumitra *et al.* (1980). Dave and Chauhan (1985) determined protein contents of algae from Gujarat coast. Parekh and Chauhan (1987) estimated the total lipid content of marine algae from Tamil Nadu and Gujarat coast. Reeta *et al.* (1990) examined the biochemical composition of chlorophyceae and found significant variations between different genera. Saji (1991) studied the biochemical aspects of marine algae from Kerala coast. Kumar (1993) estimated the biochemical constituents of 21 species of marine algae from Tuticorin coast to assess their nutritive value. He observed species specific and seasonal variations in the biochemical content of algae. Ganesan and Kannan (1994) reported seasonal variations in biochemical constituents of economically important seaweeds from Gulf of Mannar. Devi *et al.* (1996) examined organic constituents such as carbohydrates, lipids and proteins of marine algae of Cape Comorin. Dave and Parekh (1997) determined amino acid contents of some marine green algae from Saurashtra coast. Selvi *et* al. (1999) carried out studies on biochemical contents of macroalgae such as *Enteromorpha intestinalis, Ulva lactuca, Chaetomorpha linum* etc. from two estuaries of Tamil Nadu. The chemical constituents of marine algae of Lakshadweep were studied by Koya (2000). Roslin (2001) studied seasonal variations in the lipid contents of some marine algae in relation to environmental parameters. Rajasulochana *et al.* (2002) carried out biochemical analysis of marine algae from Tamil Nadu coast for constituents such as carbohydrates, proteins and lipids.

Many studies have also been carried out on the biochemical composition of marine algae from other parts of the world. Behariya and El-Sayed (1983) studied the biochemical composition of marine algae from Jedda coast, Soudi Arabia. Studies of Rosell and Srivastava (1985) revealed seasonal variation in the biochemical content of marine algae. Qari (1988) studied the seasonal changes in biochemistry of marine algae from Karachi. Hayee-Memon *et al.* (1991) examined the red alga *Gracilaria foliifera* for the presence of carbohydrates. Rodriguez-Montesinos and Hernandez-Carmona (1991) studied seasonal and geographic variations in the chemical composition of *Macrocystis* sp. from the Western coast of Baja, California and showed that lipid levels increase in winter and decrease in summer. Abbas *et al.* (1992) estimated protein content of benthic marine algae from Bahrain coastline. Mishra *et al.* (1993) examined seasonal changes in lipids of algae. Fan-Xiao *et al.* (1993) investigated the nutrient components of edible marine algae from China Sea area. Qari and Qasim (1993) determined the biochemical constituents of seaweeds from Karachi coast. Mercer *et al.* (1993) examined the effects of algal diet on growth and biochemical composition of two species of abalone. During the study, they found evidence for temporal variability in algal lipid composition. Seasonal variation in lipid content of *Laminaria* was studied by Honya *et al.* (1994). Castro-Gonzalez *et al.* (1994) studied the chemical composition of *Macrocystis* collected in summer and winter and its possible use in animal feeding. Ilyas and Sukan (1994) studied seasonal variation in the chemical constituents of *Gracilaria verrucosa* from Aegian coast of Izmir. The chemical composition of common marine algae from Hong Kong was determined by Kaehler and Kennish (1996). Castro-Gonzalez *et al.* (1996) studied the chemical composition of the green alga *Ulva lactuca*. Haroon *et al.* (2000) studied the variations in protein, lipid and carbohydrate contents of *Enteromorpha* spp. from the Gulf of Gdansk coast on the Southern Baltic Sea over a seven-month period. They observed maximum protein contents at the beginning of growing season and maximum carbohydrates in summer. Maximum lipid levels were recorded in spring. Tao and He (2000) analysed nutrient components including proteins and carbohydrates in marine macroalgae from Dalian coastal waters. Nelson *et al.* (2002) studied the seasonal lipid composition in macroalgae of North-eastern Pacific Ocean and observed highest lipid content in winter and spring seasons.

Several studies have been carried out on the fibres of marine algae. Indergaard and Knutsen (1990) studied seasonal variations in fibre content of *Furcellaria lumbricalis*. Fleury and Lahaye (1991) carried out chemical and physicochemical characterization of fibres from *Laminaria digitata*. Qari and Qasim (1993) estimated crude fibre content of marine algae from Karachi coast. Mabeau (1995) carried out nutritional evaluation of dietary fibre ingredients from marine algae and algal products. Michel *et al.* (1996) examined the *in vitro* fermentation by human foetal bacteria of total and purified dietary fibres from marine algae. Bobin-Dubigeon *et al.* (1997) studied the chemical composition, physicochemical properties, enzymatic inhibition and fermentation characteristics of dietary fibres from edible marine algae. Lahaye and Kaeffer (1997) studied the structure, physico-chemical and biological properties of algal dietary fibres. Li *et al.* (2000) carried out an evaluation of physiological functions of dietary fibres from seaweeds. Tao and He (2000) estimated the fibre content of seaweeds collected from the coastline of Dalian. Ruperez and Saura-calixto (2001) studied dietary fibre and physicochemical properties of edible marine algae.

Caloric contents of marine algae were determined by several authors. Paine and Vadas (1969) carried out studied on calorific values of benthic marine algae and their relation to invertebrate food preference. Seasonal changes in calorific values of marine algae from Pacific coast and their significance to some marine invertebrate herbivores were studied by Himmelman and Carefoot (1975). Sumitra *et al* (1975) and Rajagopal *et al* (1976) studied calorific contents of algae from Goa coast. Variation in the caloric content of macroalgae from St. Lawrence estuary was studied by Breton-Provencher and Cardinal (1976). Sumitra *et al* (1980) examined seasonal variations in the caloric content of macroalgae from Goa. Calorific values of marine algae from Maharashtra coast were determined by Dhargalkar *et al.* (1980). McQuaid (1985) studied seasonal variations in the calorific values of intertidal algae from Cape of Good Hope. Saji (1991) determined caloric content of macroalgae from Kerala coast. Qari and Qasim (1993) estimated both ash-free and ash-inclusive calorific values of seaweeds from Karachi coast. Lamare and Wing (2001) measured caloric content of 28 species of marine algae from New Zealand.

Considerable amount of information on the biochemical composition of marine algae from different parts of the world have been accumulated during the last four decades. However, several areas of Indian coast still remain unexplored. Most of the studies on Indian marine algae are confined to a few coastal regions of Tamil Nadu, Maharashtra, Goa and Gujarat. Taking into consideration, the ever-growing demand of non-conventional food for human consumption and other purposes such as aquaculture and animal feed, it seems essential to explore all the available seaweed resources and evaluate their nutritional value. The variations shown by marine algae in their biochemical composition and nutritive value depending on the season of the year and habitat have necessitated an assessment of the seasonal and geographical variations in the biochemical contents of algae. In this context, it has been thought worthwhile to study the biochemical composition and caloric content of some marine algae from selected localities of Kerala coast. The present study provides information on interspecies as well as interclass variability of the major biochemical constituents such as proteins, carbohydrates and lipids on a spatial and temporal scale. It also presents data on the crude fibre content and calorific value of marine algae. This study helps to find out marine algae with more nutritive and calorific value that inhabit the study area. It provides a baseline data necessary to a possible future cultivation of the selected marine algae. The examination of inter-species and intra-annual variability in biochemical composition and nutritive value of marine algae may be useful in mariculture studies.

3.2 Results

The major biochemical constituents such as proteins, lipids and carbohydrates were determined in different species of marine algae collected in different seasons from Ettikkulam and Narakkal. Crude fibre content was also determined. The energy content of marine algae was determined by calculating their calorific value from the data on major biochemical constituents. The methodologies used for the analyses are detailed in Chapter 2. The results are presented in Tables 3.1 – 3.8.

3.2.1 Proteins

Proteins are common to all living cells in one or more forms as vital constituents. The proteins of algae, like those of other plants, consist of many different molecular species and are perhaps predominantly enzymes possessing specific biological roles. Proteins complexed with pigments are involved in algal photosynthesis (e.g., phycobiliproteins). Cell walls of marine algae also contain a protein component.

In the present study, the total protein contents of marine algae varied from 6.11 to 25.77% of dry weight of algae (Table 3.1). The maximum value was exhibited by the rhodophyte *Centroceras clavulatum* collected from Narakkal in the early winter monsoon season and the minimum value by the same species collected from Ettikkulam in the late hot season. Among the chlorophyceae, the highest and lowest protein contents were recorded by *Chaetomorpha antennina* (20.84%) and *Enteromorpha intestinalis* (6.56%) respectively both collected from Ettikkulam in the summer monsoon season.

At Ettikkulam, the amount of protein was in the range 6.11 – 20.84%, whereas at Narakkal it was in the range 6.94 – 25.77%. For chlorophyceae, the ranges of protein content were 6.56 – 20.84% at Ettikkulam and 6.94 – 18.86% at Narakkal. At both the locations, highest values in chlorophyceae were exhibited by *Chaetomorpha antennina* and lowest by *Enteromorpha intestinalis* collected in the same period (summer monsoon season). Rhodophyceae contained proteins in the range 6.11 – 13.61% at Ettikkulam and a wider range of 8.91 – 25.77% at Narakkal. *Centroceras* *clavulatum* collected in the late hot season showed lowest value at Ettikkulam, while the same species collected in the early winter monsoon showed highest protein content at Narakkal. At Ettikkulam, *Grateloupia filicina*, which occurred only in the retreating monsoon season, recorded the highest protein content among the rhodophyceae members. The same species collected in the same period showed lowest protein content at Narakkal.

Table 3.1. Protein contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	20.84	9.64	16.73	13.69	12.71	8.50
Ulva lactuca	10.72	14.40	19.08	14.36	16.18	7.09
Enteromorpha intestinalis	6.56	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	12.46	7.60	10.75	11.43	11.20	8.07
Centroceras clavulatum	*	6.43	*	11.05	*	6.11
Grateloupia filicina	*	13.61	*	*	*	*
Average	**12.64**	**10.34**	**15.52**	**12.63**	**13.37**	**7.44**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	18.86	10.42	*	*	*	*
Enteromorpha intestinalis	6.94	*	*	*	*	13.39
Rhodophyceae						
Centroceras clavulatum	20.62	15.40	25.77	19.74	18.79	13.16
Grateloupia filicina	15.79	8.91	19.92	*	18.69	16.30
Average	**15.55**	**11.58**	**22.85**	**19.74**	**18.74**	**14.28**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

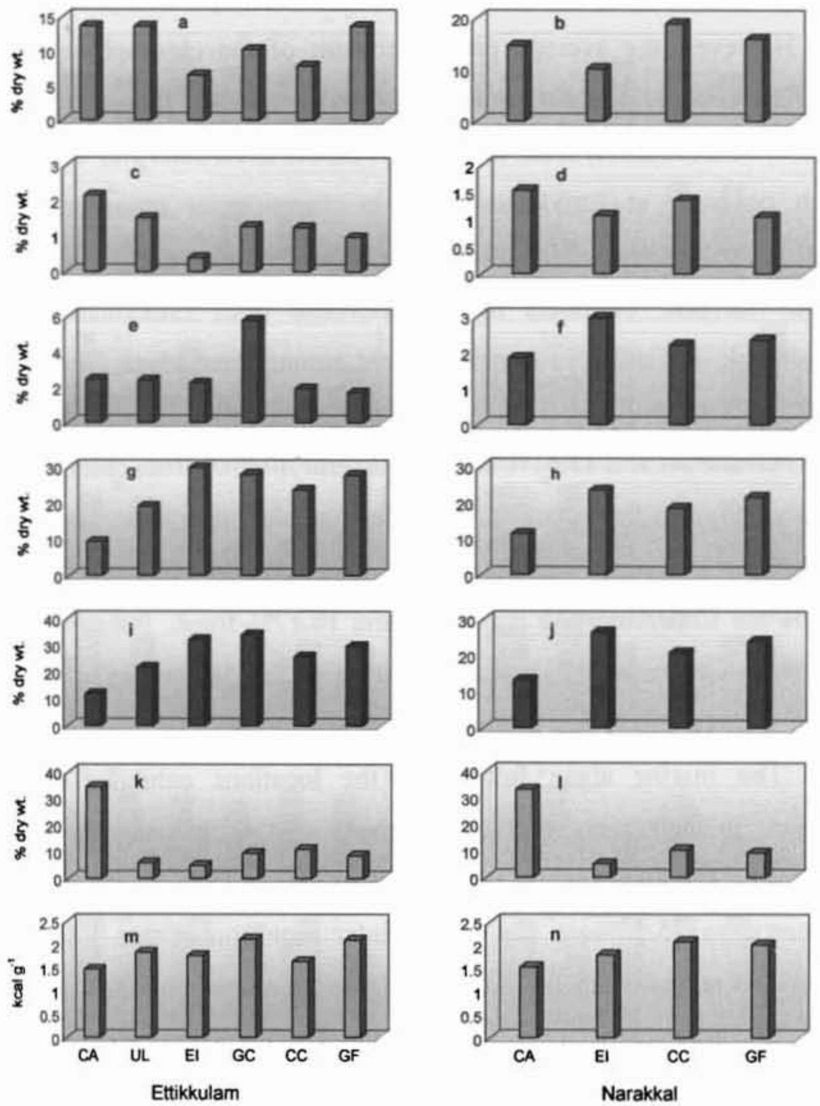

Fig. 3.1. Interspecies variations in the levels of proteins (a, b), lipids (c, d), LMWC (e, f), polysaccharides (g, h), total carbohydrates (i, j), fibres (k, l) and calorific values (m, n) of marine algae (CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

The protein content of algae exhibited interspecies variations (Fig. 3.1). However, the average protein content of the chlorophyte *Chaetomorpha antennina* from Ettikkulam (13.69%) was almost the same as that of *Ulva lactuca* (13.64%) from the same location. *Enteromorpha intestinalis*, which occurred at Ettikkulam only in the summer monsoon season, contained only half the amount of protein (6.56%) shown by *C. antennina* and *U. lactuca*. Members of rhodophyceae from Ettikkulam showed considerable variations in protein content among themselves. The average protein contents were 10.25% for *Gracilaria corticata*, 7.86% for *Centroceras clavulatum* and 13.61% for *Grateloupia filicina*. Both chlorophyceae and rhodophyceae from Narakkal recorded notable interspecies variability in their protein contents. Among chlorophyceae, average protein content was 14.64% for *Chaetomorpha antennina* and 10.17% for *E. intestinalis*. The rhodophyte *Centroceras clavulatum* contained 18.91% proteins, whereas *G. filicina* showed an average protein content of 15.92%.

The marine algae from both the locations exhibited temporal variations in their average protein contents. At Ettikkulam, the average value for all the algal species taken together ranged from 7.44% in the late hot season to 15.52% in the early winter monsoon (Table 3.1) and at Narakkal, it ranged from 14.28% to 22.85% in the late hot season and early winter monsoon respectively. Considering the interclass variations in the protein content with respect to time of collection (Fig. 3.2), it was found that chlorophyceae from Ettikkulam recorded the highest value in the early winter monsoon season (17.90%) and the lowest in the late hot season (7.80%). At Narakkal, chlorophyceae members occurred only during the late hot season, summer monsoon and retreating monsoon seasons. The species

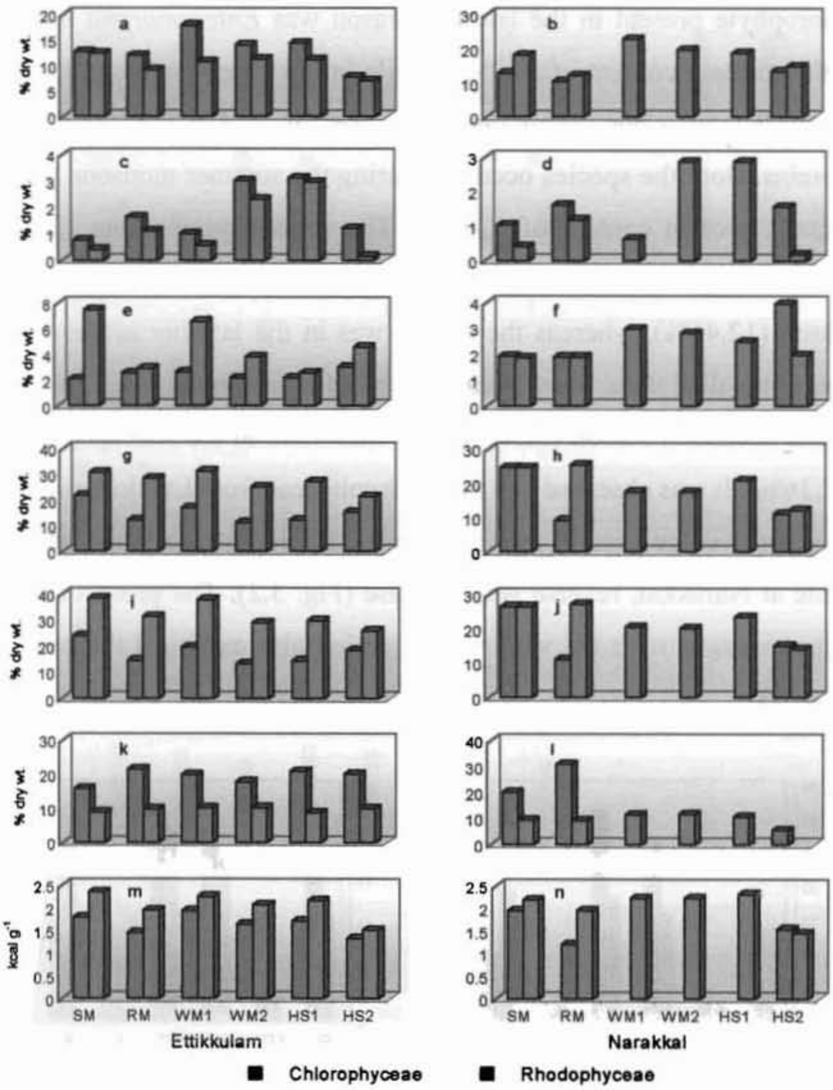

Fig. 3.2. Temporal variations in the levels of proteins (a, b), lipids (c, d), LMWC (e, f), polysaccharides (g, h), total carbohydrates (i, j), fibres (k, l) and calorific values (m, n) of different classes of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

composition was found to be different in different seasons. The only chlorophyte present in the late hot season was *Enteromorpha intestinalis* with a protein content of 13.39%, while in the retreating monsoon season, only *Chaetomorpha antennina* was present which contained 10.42% proteins. Both the species occurred during the summer monsoon giving an average protein content of 12.90%. The rhodophyceae from Ettikkulam exhibited highest value for average protein content in the summer monsoon season (12.46%), whereas the lowest was in the late hot season (7.09%). The same algal class from Narakkal recorded maximum value in the early winter monsoon season (22.85%) and minimum in the retreating monsoon (12.16%). It was observed that the chlorophyceae from Ettikkulam exhibited a higher average protein content than the rhodophyceae in all the seasons while at Narakkal, reverse was the case (Fig. 3.2). The protein content of algae averaged over the whole study period also exhibited the same trend (Fig. 3.3).

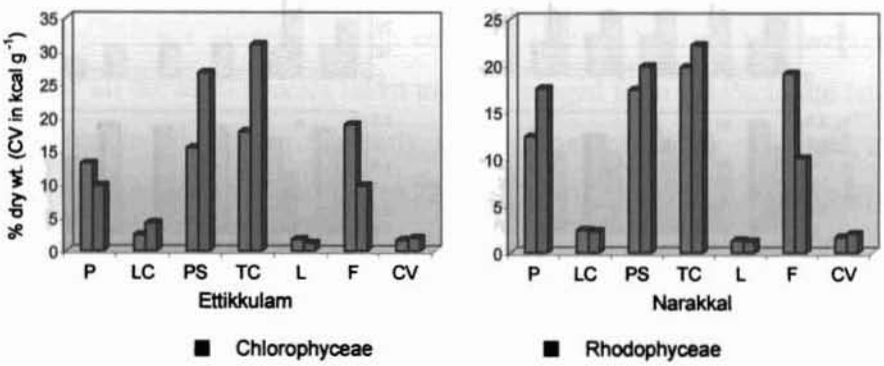

Fig. 3.3. Interclass variations in the major biochemical constituents and calorific value of marine algae (P: Proteins LC: Low-molecular-weight carbohydrates PS: Polysaccharides TC: Total carbohydrates L: Lipids F: Crude fibre CV: Calorific value).

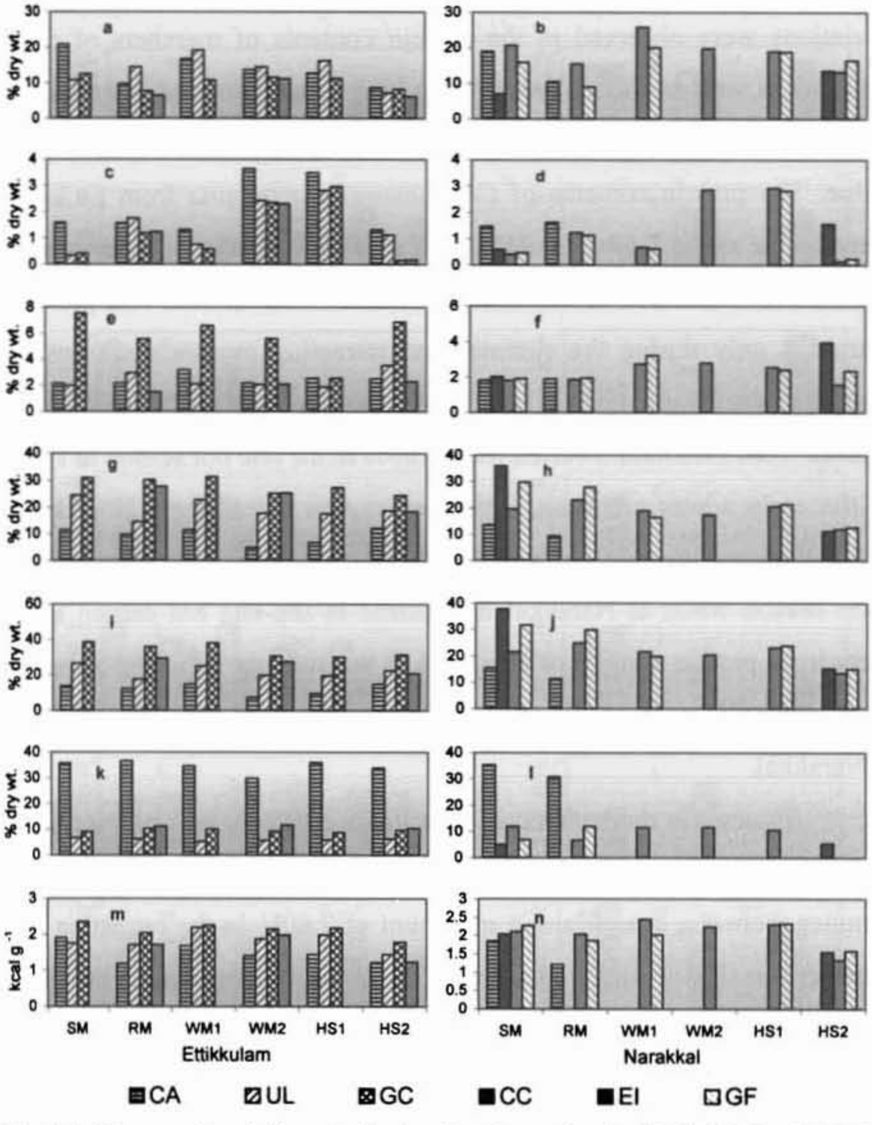

Fig. 3.4. Temporal variations in the levels of proteins (a, b), lipids (c, d), LMWC (e, f), polysaccharides (g, h), total carbohydrates (i, j), fibres (k, l) and calorific values (m, n) of different species of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

Considering the temporal variations in individual species, marked variations were observed in the protein contents of members of chlorophyceae as well as rhodophyceae from both Ettikkulam and Narakkal (Fig. 3.4). In most cases, the maximum value was almost double the minimum value. The protein contents of *Chaetomorpha antennina* from Ettikkulam were in the range 8.50 – 20.84% with maximum in the summer monsoon season and minimum in the late hot season. This species was present at Narakkal only during the summer and retreating monsoon seasons with protein contents of 18.86% and 10.42% respectively. Proteins in *Ulva lactuca* from Ettikkulam varied from 7.09% in the late hot season to 19.08% in the early winter monsoon. This species was absent from Narakkal. At Ettikkulam, *Enteromorpha intestinalis* occurred only in the summer monsoon season while at Narakkal it occurred in the late hot season also. It recorded a protein content of about 7% in the summer monsoon at both the locations and almost two times higher value in the late hot season (13.39%) at Narakkal.

Among the rhodophyceae, *Gracilaria corticata* was present only at Ettikkulam and it recorded a maximum protein content of 12.46% in the summer monsoon season and a minimum of 7.60% in the retreating monsoon season (Fig. 3.4). *Centroceras clavulatum* was present at Ettikkulam only during the late hot season, retreating monsoon and mid winter monsoon season with a maximum protein content in the winter monsoon (11.05%) and minimum in the late hot season (6.11%). The same species collected from Narakkal exhibited a wider variation in protein content with highest value in the early winter monsoon (25.77%) and lowest in the late hot season (13.16%). In *Grateloupia filicina* from Narakkal, the amount of protein varied from 8.91% in the late hot season to 19.92% in the early

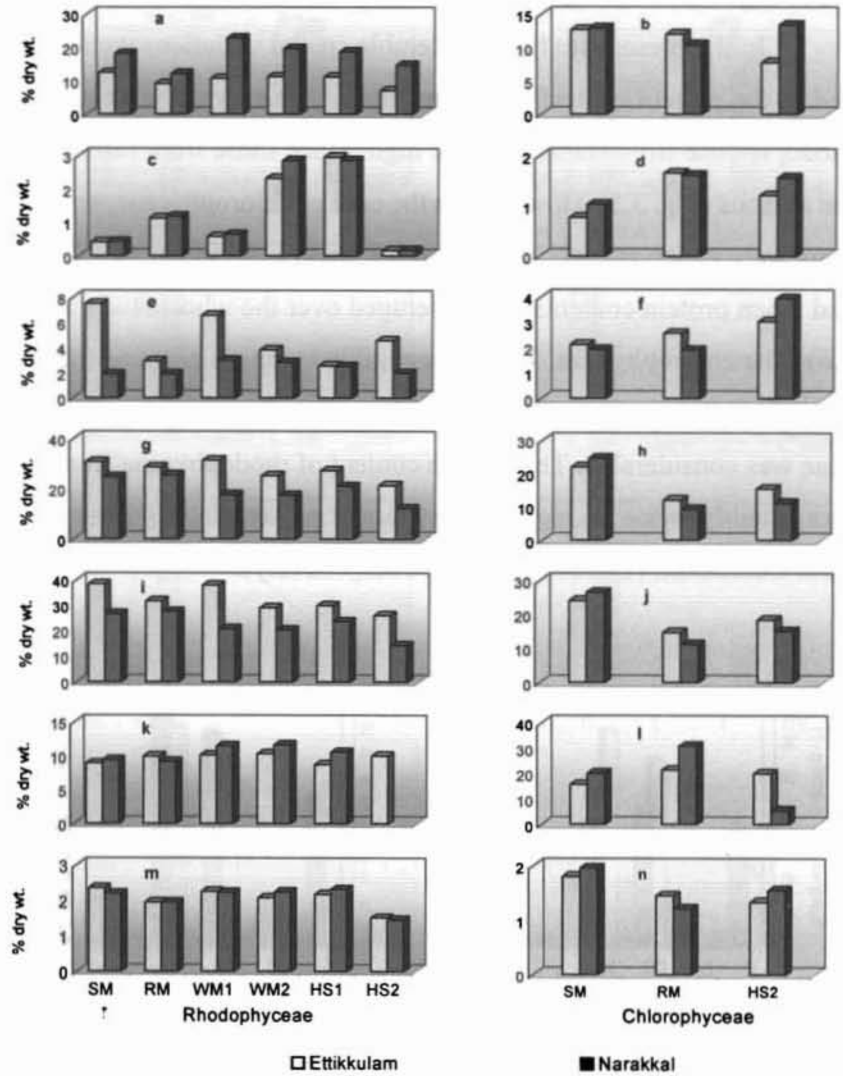

Fig. 3.5. Spatial variations in the levels of proteins (a, b), lipids (c, d), LMWC (e, f), polysaccharides (g, h), total carbohydrates (i, j), fibres (k, l) and calorific values (m, n) of different classes of marine algae in different periods (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

winter monsoon. This species was obtained from Ettikkulam only in the retreating monsoon season with a protein content of 13.61%.

In the present study, considerable spatial variations were also observed in the protein content of marine algae. The average protein contents of rhodophyceae from Narakkal were higher than those from Ettikkulam in all the seasons (Fig. 3.5). However, in the case of chlorophyceae, notable variations were observed only in the late hot season. A similar trend was observed when protein contents were averaged over the whole study period (Fig. 3.6). The chlorophyceae showed negligible variation between the two locations (Ettikkulam: 13.12% and Narakkal: 12.40%), while that for rhodophyceae was considerable. The protein content of rhodophyceae from Narakkal was roughly twice as high as the amount present in the same algal class from Ettikkulam (17.55% and 9.87% respectively).

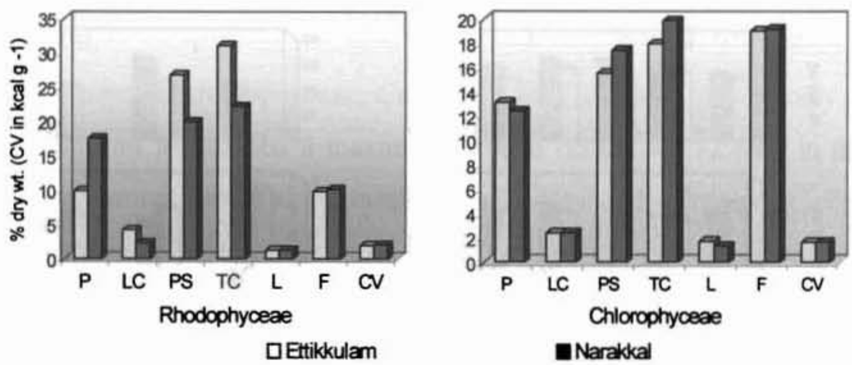

Fig. 3.6. Spatial variations in the mean levels of major biochemical constituents and calorific value of different classes of marine algae (P: Proteins LC: Low-molecular-weight carbohydrates PS: Polysaccharides TC: Total carbohydrates L: Lipids F: Crude fibre CV: Calorific value).

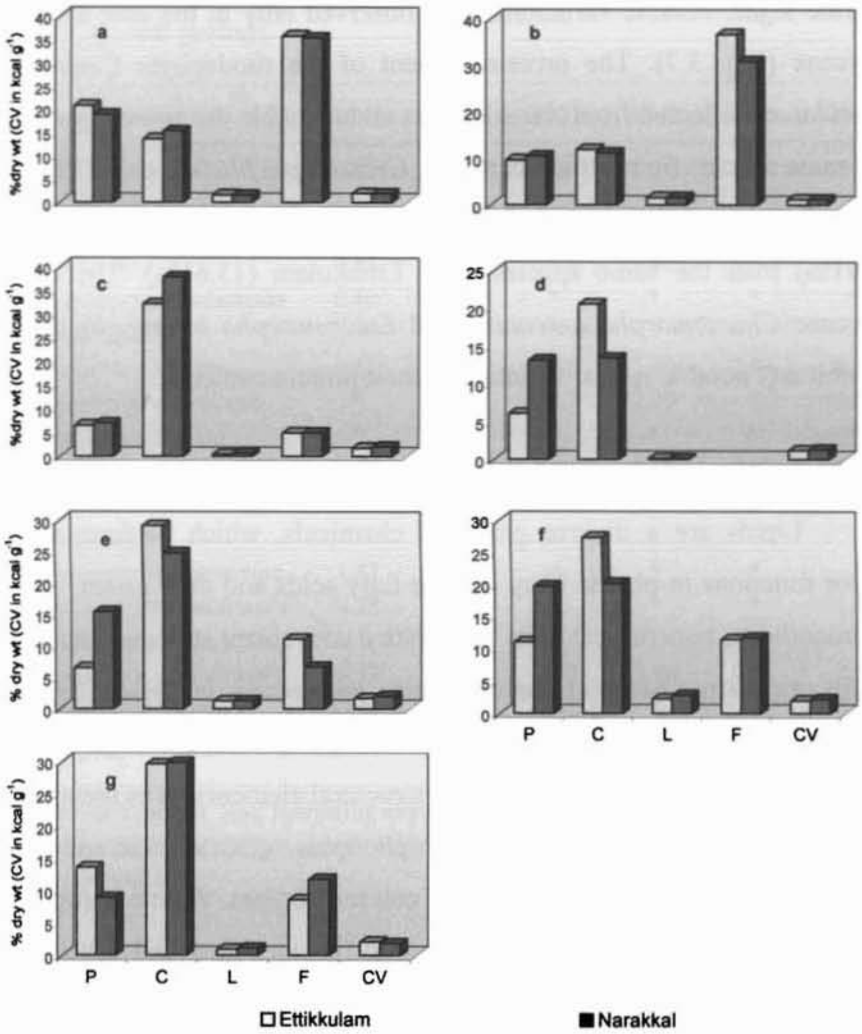

Fig. 3.7. Spatial variations in major biochemical constituents and calorific value of different species of marine algae i) *Chaetomorpha antennina* in summer monsoon season (a), and retreating monsoon season (b) ii) *Enteromorpha intestinalis* in summer monsoon season (c) iii) *Centroceras clavulatum* in late hot season (d), retreating monsoon season (e), and winter monsoon season (f), and iv) *Grateloupia filicina* in retreating monsoon season (g) (P: Proteins C: Total carbohydrates L: Lipids F: Crude fibre CV: Calorific value).

Considering spatial distribution of proteins in individual species of marine algae, notable variations were observed only in the case of rhodophyceae (Fig. 3.7). The protein content of the rhodophyte *Centroceras clavulatum* collected from Narakkal was about double the amount present in the same species from Ettikkulam. But, *Grateloupia filicina*, collected in the retreating monsoon season from Narakkal showed a lower protein content (8.91%) than the same species from Ettikkulam (13.61%). The chlorophyceae *Chaetomorpha antennina* and *Enteromorpha intestinalis* did not exhibit any notable spatial variation in their protein content.

3.2.2 Lipids

Lipids are a diverse group of chemicals, which perform several major functions in plants. They include fatty acids and their esters, sterols, hydrocarbons, terpenes etc. Fats constitute a convenient storage material for living organisms. Most of the cell lipids are present in chloroplast. The lipids of algae, like those of chloroplasts of higher plants, are largely surfactant molecules, which function both as structural element and as metabolites in the photosynthetic organelles. Phospholipids, galactolipids and sterol esters form the hydrophobic barrier of cell membranes. Active transport of carbohydrates through cell membrane may be mediated and specifically controlled by the reversible formation of galactolipids in the membrane. Lipids may also serve a variety of less well-defined functions such as hormones, defence chemical etc.

In the present study, the total lipid contents of marine algae varied from 0.13 to 3.64% of dry weight of algae (Table 3.2). The minimum value was shown by the rhodophyte *Centroceras clavulatum* collected from Narakkal in the late hot season and the maximum value by the chlorophyte

Table 3.2. Lipid contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	1.59	1.57	1.30	3.64	3.47	1.30
Ulva lactuca	0.33	1.74	0.74	2.42	2.80	1.11
Enteromorpha intestinalis	0.40	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	0.41	1.18	0.57	2.35	2.96	0.15
Centroceras clavulatum	*	1.23	*	2.29	*	0.18
Grateloupia filicina	*	0.97	*	*	*	*
Average	**0.68**	**1.34**	**0.87**	**2.68**	**3.08**	**0.69**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	1.47	1.61	*	*	*	*
Enteromorpha intestinalis	0.58	*	*	*	*	1.56
Rhodophyceae						
Centroceras clavulatum	0.39	1.23	0.67	2.86	2.91	0.13
Grateloupia filicina	0.45	1.13	0.62	*	2.82	0.23
Average	**0.72**	**1.32**	**0.64**	**2.86**	**2.86**	**0.64**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

Chaetomorpha antennina from Ettikkulam in the mid winter monsoon season. The minimum lipid content in chlorophyceae (0.33%) was recorded by *Ulva lactuca* collected in the summer monsoon from Ettikkulam. Among the rhodophyceae members, *Gracilaria corticata* collected in the mid hot season from the same location exhibited the maximum lipid content (2.96%).

The marine algae collected from Ettikkulam were found to contain lipids in the range 0.15 – 3.64%, while those from Narakkal exhibited the range 0.13 – 2.91%. The chlorophyceae from Ettikkulam showed a wider range in the lipid content than those from Narakkal. The ranges were 0.33 – 3.64% and 0.58 – 1.61% respectively. At both the locations, highest values were shown by the same algal species (*Chaetomorpha antennina*). At Ettikkulam, lowest value was shown by *Ulva lactuca* and at Narakkal by *Enteromorpha intestinalis* both collected in the summer monsoon season. Rhodophyceae from both the locations exhibited more or less the same range of lipids. The ranges were 0.15 – 2.96% at Ettikkulam and 0.13 – 2.91% at Narakkal. Maximum and minimum values were shown by the same species in different periods. *Gracilaria corticata* at Ettikkulam and *Centroceras clavulatum* at Narakkal recorded maximum lipid content in the mid hot season and minimum in the late hot season.

The total lipid contents of marine algae were observed to show species to species variability (Fig. 3.1). Among the chlorophyceae from Ettikkulam, *Chaetomorpha antennina* recorded an average lipid content of 2.15% and the value for *Ulva lactuca* was 1.52%. During the study period, *Enteromorpha intestinalis* was present at Ettikkulam only during the summer monsoon season and it recorded a lipid content of 0.40%. The average lipid contents of the rhodophyceae members *Gracilaria corticata* and *Centroceras clavulatum* did not vary significantly (1.27% and 1.24% respectively). The rhodophyte *Grateloupia filicina*, which occurred at Ettikkulam only in the retreating monsoon season recorded 0.97% lipid content. At Narakkal, both rhodophyceae and chlorophyceae exhibited interspecies variations in the average lipid contents. The values shown by the chlorophyceae members *Chaetomorpha antennina* and *Enteromorpha intestinalis* were 1.54% and 1.07% respectively. The average amount of lipids in the rhodophyte *Centroceras clavulatum* was 1.36% and that in *Grateloupia filicina* was 1.05%.

Considerable temporal variations were observed in the average lipid content of marine algae from both the locations. The average value for the whole algae collected from Ettikkulam varied from 0.68% (in the summer monsoon season) to 3.08% (in the mid hot season) (Table 3.2). At Narakkal, the range was 0.64 – 2.86% with maximum recorded in both the mid winter monsoon and the mid hot season and minimum in both early winter monsoon and late hot season. Both chlorophyceae and rhodophyceae from Ettikkulam showed highest average lipid content in the mid hot season (3.14% and 2.96% respectively) (Fig. 3.2). Rhodophyceae from Narakkal recorded high values in both the mid winter monsoon and the mid hot season (2.86%) whereas chlorophyceae, which was absent from Narakkal in winter monsoon and most of the hot season, showed maximum value in the retreating monsoon season (1.61%). At both the locations, rhodophyceae recorded minimum values in the late hot season (Ettikkulam: 0.17% and Narakkal: 0.18%) and chlorophyceae in the summer monsoon season (Ettikkulam: 0.77% and Narakkal: 1.03%). A classwise comparison showed that at both the locations, chlorophyceae exhibited a higher average lipid content than rhodophyceae in all the seasons (Fig. 3.2). However, when the lipid contents were averaged over the whole study period, the variations were not much pronounced (Fig. 3.3).

Considering temporal variations in the lipid contents of individual species, members of both chlorophyceae and rhodophyceae were observed to exhibit marked variations (Fig. 3.4). All the algal species contained lipids over 2% in the mid winter monsoon and the mid hot season. Lipid contents less than 1% were recorded by all but one species in the summer monsoon and the early winter monsoon seasons. The exception was the chlorophyte *Chaetomorpha antennina*, which recorded lipid contents of about 1.5% in the summer monsoon and 1.3% in the early winter monsoon. In the retreating monsoon season, the lipid contents of algae were in between 1 and 2% of dry weight. In the late hot season, members of chlorophyceae showed lipid contents over 1%, whereas rhodophyceae contained amounts below 0.25%. Among the chlorophyceae from Ettikkulam, *Chaetomorpha antennina* showed low lipid contents in both the late hot season and the early winter monsoon (1.30%) and high values in both the mid winter monsoon (3.64%) and the mid hot season (3.47%). The amount of total lipids in *Ulva lactuca* varied from 0.33% in the summer monsoon to 2.80% in the mid hot season. *Enteromorpha intestinalis*, which was present only in the summer monsoon season, recorded a lipid content of 0.40%. The rhodophyceae members *Gracilaria corticata* and *Centroceras clavulatum* exhibited more or less the same range of lipids. In the former, the range was 0.15 – 2.96% and in the latter 0.18 – 2.29%. Both the species recorded the lowest value in the late hot season. *G. corticata* recorded the maximum value in the mid hot season, whereas C. *clavulatum* which was absent in the mid hot season showed the maximum in the mid winter monsoon. *Grateloupia filicina*, which occurred only in the retreating monsoon season, contained 0.97% total lipids. At Narakkal, the chlorophyte *Chaetomorpha antennina* was obtained only in the summer and the retreating monsoon seasons with lipid contents of 1.47% and 1.61% respectively. *Enteromorpha intestinalis*, which occurred only during the late hot season and the summer monsoon, recorded the lipid content of 1.56% and 0.58% respectively. The rhodophyceae members *Centroceras clavulatum* and *Grateloupia filicina* exhibited comparable ranges of lipids (0.13 – 2.91% and 0.23 – 2.82%

respectively). Both the species recorded minimum value in the late hot season and maximum value in the mid hot season. The rhodophyte *Gracilaria corticata* and the chlorophyte *Ulva lactuca* were absent from Narakkal.

Spatial variations in the lipid contents were negligible in the marine algae selected for the present study (Fig. 3.5). Classwise as well as specieswise comparisons showed that marine algae of Ettikkulam contained almost the same amount of total lipids as those from Narakkal in all the seasons.

3.2.3 Carbohydrates

Plants are composed predominantly of carbohydrate materials. The major functions of carbohydrates in plant tissues are the provision of source of energy, immediate or delayed, and the provision of structural strength. The carbohydrates present in algae may be classified into low-molecular-weight carbohydrates (LMWC) and polysaccharides. The polysaccharides may be further divided into storage and structural polysaccharides. The marine algae are found to contain characteristic LMW carbohydrates. The principal LMWC in chlorophyceae is sucrose. In rhodophyceae, the main LMWC is floridoside except in Ceramiales, which contains digeneaside as the principal LMWC. Floridoside consists of glycerol plus galactose, whereas digeneaside consists of glyceric acid plus mannose. The marine algae are also noted for the diversity of their storage and structural polysaccharides. Several characteristic storage polysaccharides are found in marine algae most of which are branched or unbranched chains of glucose units (i.e. glucans). Most chlorophyceae, like higher plants, store starch, a mixture of amylose (unbranched molecular chain of 1,4 linked α-D-glucose units) and amylopectin (branched molecule containing 1,6 linked α-D-glucose units also). Rhodophyceae store primarily floridean starch, a branched glucan similar to amylopectin except for having a few 1,3 linked glucose units. Phaeophyceae store laminaran, which, like starch, comprises branched and unbranched molecules. The structural polysaccharides include cellulose, mannan and xylan, which constitute the fibrillar layer of cell walls. Various mucilaginous polysaccharides are also found in the wall-matrix. These include the sulphated polysaccharides such as agars and carrageenans of rhodophyceae and alginates of phaeophyceae. The chlorophyceae also contains sulphated polysaccharides such as sulphated xylo-arabinogalactans (e.g., *Chaetomorpha*), sulphated glucuronoxylorhamnans (e.g., *Ulva* and *Enteromorpha*) and sulphated glucuronoxylo rhamnogalactans (e.g., *Acetabularia*).

The total carbohydrate content of marine algae determined in the present study includes all the carbohydrates except the microfibrillar polysaccharides that constitute the algal cell wall and are insoluble in most solvents. These are determined separately as the crude fibre content of the algae. Thus, the polysaccharide contents determined by subtracting LMWC content from total carbohydrate content also exclude the fibrillar polysaccharides.

Total Carbohydrates

The total carbohydrate content of marine algae showed variations in the range 7.01 – 38.33% of dry weight of algae (Table 3.3). The chlorophyte *Chaetomorpha antennina* collected in the mid winter monsoon season from Ettikkulam contained the lowest amount of carbohydrates, whereas the rhodophyte *Gracilaria corticata* collected in the summer monsoon season from the same location showed the maximum value. Among the chlorophyceae, the highest carbohydrate content was found in *Enteromorpha intestinalis* (37.81%) in the summer monsoon season at Narakkal. In the case of rhodophyceae, the lowest amount of carbohydrates was found in *Centroceras clavulatum* (13.40%) in the late hot season at Narakkal.

Table 3.3. Total carbohydrate contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	13.62	11.90	14.66	7.01	9.32	14.62
Ulva lactuca	26.39	17.46	24.78	19.67	19.42	22.18
Enteromorpha intestinalis	32.17	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	38.33	35.71	37.89	30.62	29.85	31.21
Centroceras clavulatum	*	29.20	*	27.34	*	20.58
Grateloupia filicina	*	29.61	*	*	*	*
Average	**27.63**	**24.78**	**25.78**	**21.16**	**19.53**	**22.15**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	15.25	11.15	*	*	*	*
Enteromorpha intestinalis	37.81	*	*	*	*	15.06
Rhodophyceae						
Centroceras clavulatum	21.32	24.79	21.58	20.19	23.05	13.40
Grateloupia filicina	31.72	29.81	19.60	*	23.74	14.85
Average	**26.53**	**21.92**	**20.59**	**20.19**	**23.40**	**14.44**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

At Ettikkulam, chlorophyceae recorded a wider range of total carbohydrate content (7.01 – 32.17%) compared to rhodophyceae (20.58 – 38.33%). Both the algal classes recorded the maximum value in the summer monsoon season (*E. intestinalis* among chlorophyceae and *G. corticata* among rhodophyceae). The minimum amount of carbohydrate in chlorophyceae was shown by *Chaetomorpha antennina* in the mid winter monsoon. *Centroceras clavulatum* collected in the late hot season exhibited the lowest carbohydrate content among the rhodophyceae and the amount was about three times the minimum value shown by the chlorophyceae. The range of total carbohydrates in marine algae from Narakkal was 11.15 – 37.81% of dry weight of algae with minimum and maximum values shown by the members of chlorophyceae in different periods (*Chaetomorpha antennina* in the retreating monsoon season and *Enteromorpha intestinalis* in the summer monsoon respectively). Among the rhodophyceae, *Centroceras clavulatum* collected in the late hot season recorded the lowest value (13.40%) and the highest value (31.72%) by *Grateloupia filicina* in the summer monsoon. Thus, chlorophyceae and rhodophyceae from both the locations recorded the highest amount of total carbohydrates in the summer monsoon season.

Marked interspecies variations were observed in the total carbohydrate contents of marine algae from both Ettikkulam and Narakkal (Fig. 3.1). The average amount of total carbohydrates in the chlorophyte *Ulva lactuca* from Ettikkulam was about two times that in *Chaetomorpha antennina* (21.65% and 11.86% respectively). *Enteromorpha intestinalis*, which occurred only during the summer monsoon season at Ettikkulam, recorded an average carbohydrate content of 32.17%, which was about three times the average amount in *C. antennina*. At Narakkal, *E. intestinalis* recorded about two times the average carbohydrate content of *C. antennina* (26.44% and 13.20% respectively). The variations in the average carbohydrate contents of rhodophyceae were not as much pronounced as in chlorophyceae. At Ettikkulam, *Gracilaria corticata* recorded an average value of 33.93% and *Centroceras clavulatum*, 25.70%. *Grateloupia filicina*, which was present only in the retreating monsoon season recorded total carbohydrate content of 29.61%. At Narakkal, *C. clavulatum* recorded an average value of 20.72% and *G. filicina*, 23.95%.

The marine algae from both Ettikkulam and Narakkal exhibited notable temporal variations in the average carbohydrate content. At Ettikkulam, the average value for the whole marine algae selected for the present study varied from 19.53% in the mid hot season to 27.63% in the summer monsoon season (Table 3.3). At Narakkal, the variations were in the range 14.44 – 26.53% with minimum in the late hot season and maximum in the summer monsoon. The chlorophyceae and rhodophyceae from Ettikkulam exhibited widely different ranges of average carbohydrate content (13.34 – 24.06% and 25.89 – 38.33% respectively) with lowest values in the mid winter monsoon and the late hot season respectively (Fig. 3.2). Both the classes showed highest values in the summer monsoon. At Narakkal, the ranges were comparable, 11.15 – 26.53% for chlorophyceae and 14.12 – 27.30% for rhodophyceae. Chlorophyceae showed maximum value in the summer monsoon and minimum value in the retreating monsoon season while rhodophyceae recorded maximum in the retreating monsoon and minimum in the late hot season. The value for rhodophyceae in the summer monsoon was also high (26.52%).

A classwise comparison showed that rhodophyceae from Ettikkulam contained higher amount of total carbohydrates than chlorophyceae in all the seasons and the variation was more than double in some seasons (Fig. 3.2). However, such a trend was observed in the marine algae of Narakkal only in the retreating monsoon season and the average carbohydrate contents in chlorophyceae and rhodophyceae were comparable in the late hot season and the summer monsoon. The carbohydrate contents averaged over the whole study period for chlorophyceae and rhodophyceae were 17.94% and 31.03% respectively at Ettikkulam and 19.82% and 22.19% respectively at Narakkal (Fig. 3.3).

Considering the individual species, notable temporal variations were found in the total carbohydrate contents of marine algal species belonging to both chlorophyceae and rhodophyceae (Fig. 3.4). The chlorophyte *Chaetomorpha antennina* contained low amounts of carbohydrates (< 15%) in all the seasons. At Ettikkulam, the lowest value was recorded in the mid winter monsoon (7.01%) and the highest in the early winter monsoon (14.66%) as well as in the late hot season (14.62%). At Narakkal, this species recorded total carbohydrate contents of 15.25% in the summer monsoon season and 11.15% in the retreating monsoon. The carbohydrate content of *Ulva lactuca* from Ettikkulam varied from 17.46% in the retreating monsoon to 26.39% in the summer monsoon. *Enteromorpha intestinalis* recorded high amounts of carbohydrates in the summer monsoon season at both Ettikkulam and Narakkal (32.17% and 37.81% respectively). This species showed a low value of 15.06% in the late hot season at Narakkal.

The rhodophyceae *Gracilaria corticata* recorded the highest amounts of total carbohydrates in different seasons. The values varied from 29.85% in the mid hot season to 38.33% in the summer monsoon (Fig. 3.4). For *Centroceras clavulatum*, the carbohydrate contents varied from 20.58% in the late hot season to 29.20% in the retreating monsoon at Ettikkulam where this species was absent during the early hot period as well as the summer and winter monsoons. At Narakkal, total carbohydrates of this species varied from 13.40% in the late hot season to 24.79% in the retreating monsoon. Carbohydrates of *Grateloupia filicina* ranged from 14.85% in the late hot season to 31.72% in the summer monsoon at Narakkal and at Ettikkulam, this species occurred only in the retreating monsoon season with a carbohydrate content of 29.61%.

In the present study, considerable spatial variations were observed in the total carbohydrate content of rhodophyceae only (Fig. 3.5). The rhodophyceae from Ettikkulam exhibited higher values for the average amounts of carbohydrates than those from Narakkal in all the seasons. Former contained 4 – 17% more carbohydrates than the latter depending on the collection period. The total carbohydrates averaged over the whole study period for rhodophyceae were 31.03% and 22.19% at Ettikkulam and Narakkal respectively (Fig. 3.6). The same for chlorophyceae were 17.94% and 19.82% respectively.

Considering the individual species of algae, rhodophyte *Centroceras clavulatum* showed notable spatial variations with higher values at Ettikkulam, whereas *Grateloupia filicina* collected in the retreating monsoon season exhibited almost the same amount of total carbohydrates at both the locations (Fig. 3.7). The chlorophyte *Enteromorpha intestinalis* collected in the summer monsoon from Narakkal contained about 5% more carbohydrates than the same species from Ettikkulam. *Chaetomorpha antennina* recorded comparable amounts of carbohydrates at the two locations.

Table 3.4. Low-molecular-weight carbohydrate contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	2.11	2.16	3.18	2.18	2.53	2.51
Ulva lactuca	1.99	2.96	2.10	2.08	1.86	3.55
Enteromorpha intestinalis	2.25	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	7.52	5.55	6.58	5.59	2.55	6.89
Centroceras clavulatum	*	1.49	*	2.06	*	2.27
Grateloupia filicina	*	1.71	*	*	*	*
Average	**3.47**	**2.77**	**3.95**	**2.98**	**2.31**	**3.81**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	1.83	1.89	*	*	*	*
Enteromorpha intestinalis	2.01	*	*	*	*	3.94
Rhodophyceae						
Centroceras clavulatum	1.81	1.86	2.74	2.80	2.56	1.57
Grateloupia filicina	1.92	1.92	3.23	*	2.41	2.34
Average	**1.89**	**1.89**	**2.98**	**2.80**	**2.48**	**2.62**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

Low-Molecular-Weight Carbohydrates (LMWC)

In the present study, the total amounts of LMWC varied from 1.49% to 7.52% of dry weight of algae (Table 3.4). The minimum and maximum values were shown by the members of rhodophyceae collected from the same location in different periods, *viz.*, *Centroceras clavulatum* in the retreating monsoon season and *Gracilaria corticata* in the summer monsoon respectively at Ettikkulam. Chlorophyceae exhibited a narrower range of LMWC. The range was 1.83 – 3.94% with minimum value shown by

Chaetomorpha antennina in the summer monsoon season and maximum by *Enteromorpha intestinalis* in the late hot season both collected from Narakkal. The rhodophyceae from Narakkal also exhibited a narrow range comparable to that shown by chlorophyceae. The values varied from 1.57% in *Centroceras clavulatum* in the late hot season to 3.23% in *Grateloupia filicina* in the early winter monsoon season. *Ulva lactuca* collected in the late hot season recorded the highest amount of LMWC (3.55%) and that in the mid hot season recorded the lowest amount (1.86%) among the chlorophyceae of Ettikkulam.

Interspecies variations in the LMWC contents were notable only in the case of rhodophyceae from Ettikkulam (Fig. 3.1). Here, *Gracilaria corticata* contained more than two times the amount of LMWC present in other species of rhodophyceae. *G. corticata* recorded an average value of 5.78%, whereas *Centroceras clavulatum* and *Grateloupia filicina* showed average values of 1.94% and 1.71% respectively.

The average amount of LMWC in marine algae showed temporal variations. The variation was well pronounced in rhodophyceae of Ettikkulam, while only slight variations were observed in others (Fig. 3.2). In rhodophyceae of Ettikkulam, the average value varied from 2.55% in the mid hot season to 7.52% in the summer monsoon season. The variation observed in chlorophyceae was less than 1% of LMWC (2.12% in both the summer monsoon and the mid winter monsoon season to 3.03% in the late hot season). Rhodophyceae from Narakkal also exhibited a variation of only about 1% LMWC (1.86% in the summer monsoon to 2.98% in the early winter monsoon season) while chlorophyceae showed a variation of about 2% LMWC (1.89% in the retreating monsoon and 3.94% in the late hot season).

A classwise comparison of the average amount of LMWC in marine algae showed that rhodophyceae of Ettikkulam contained more LMWC than chlorophyceae in all the seasons (Fig. 3.2). This difference was high in the summer monsoon and the early winter monsoon seasons compared to other seasons. At Narakkal, chlorophyceae recorded a higher amount than rhodophyceae in the late hot season and the two algal classes showed comparable amounts in the summer monsoon and the retreating monsoon seasons. Considering the individual species, marked temporal variations in LMWC contents were observed only in the rhodophyte *Gracilaria corticata* at Ettikkulam (Fig. 3.4). In this species, the amount varied from 2.55% in the mid hot season to 7.52% in the summer monsoon season. All other species showed a temporal variation of about 1 – 2% LMWC only.

Notable spatial variations were shown by rhodophyceae in their LMWC content. The average amount of LMWC was higher in rhodophyceae of Ettikkulam than those from Narakkal in all the seasons (Fig. 3.5). The highest difference was observed in the summer monsoon season (Ettikkulam: 7.52% and Narakkal: 1.86%) and lowest in the mid hot season (Ettikkulam: 2.55% and Narakkal: 2.48%). The chlorophyceae from Ettikkulam showed a higher value for the average amount of LMWC than those from Narakkal in both the summer monsoon and the retreating monsoon seasons, while reverse was the case in the late hot season. A comparison of the amounts of LMWC in algae averaged over the whole study period (Fig. 3.6) showed that chlorophyceae from both Ettikkulam and Narakkal contained the same average amount (2.42%) while rhodophyceae from Ettikkulam contained more amount than those from Narakkal (4.22% and 2.29% respectively).

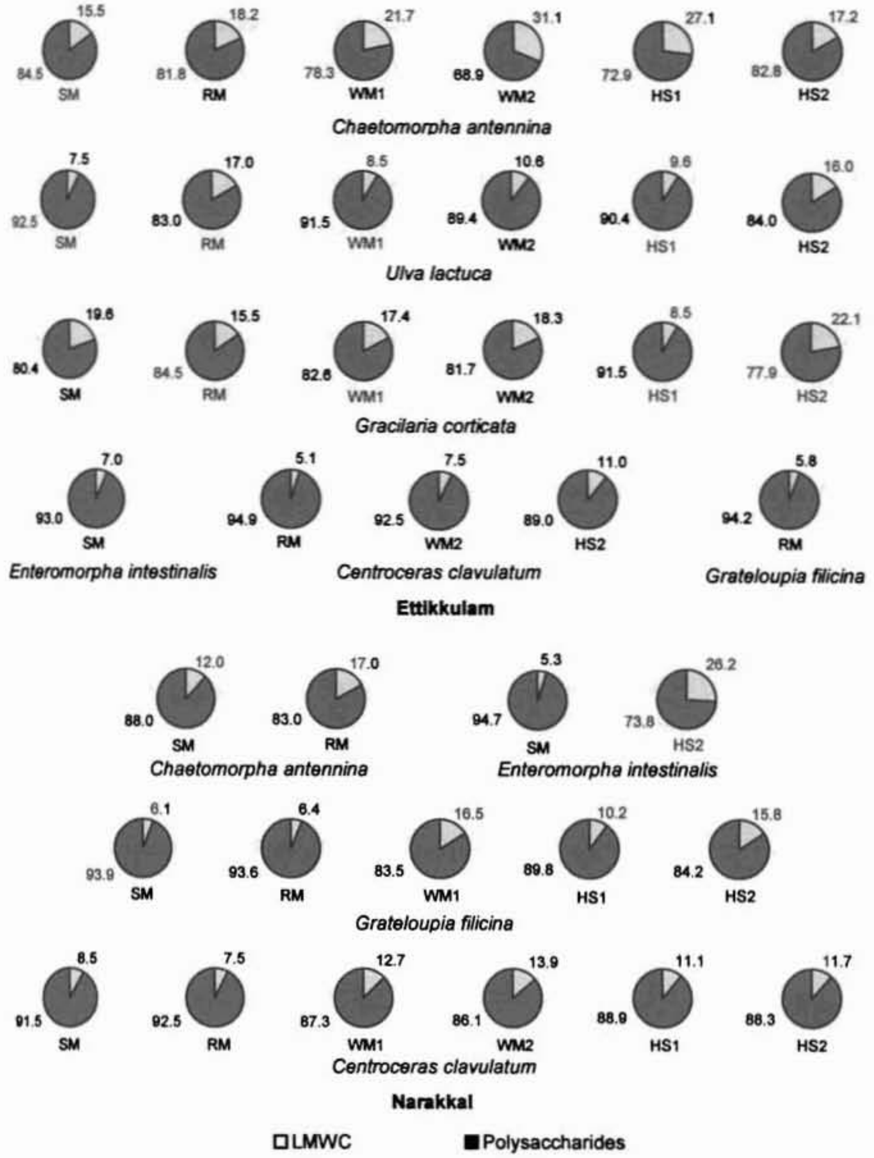

Fig. 3.8. Low-molecular-weight carbohydrate (LMWC) and polysaccharide contents of marine algae expressed as percentage of total carbohydrates (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

Expressing LMWC content of marine algae as percentage of their total carbohydrates, it was found that the former constituted only a small proportion of the latter (Fig. 3.8). The values were found to vary from 5.1 to 31.1% of the total carbohydrate content of algae, with *Centroceras clavulatum* collected in the retreating monsoon season and *Chaetomorpha antennina* collected in the mid winter monsoon season, both from Ettikkulam, showing the minimum and maximum values respectively. Only 13% of the samples (5 out of 38) were found to contain LMWC in amounts greater than 20% of the total carbohydrates. 34% of the samples showed LMWC levels below 10% of total carbohydrates and majority of the samples contained LMWC in amounts in between 10 – 20% of the total carbohydrate contents.

Polysaccharides

The polysaccharide contents of marine algae were found to vary between 4.83% and 35.81% of dry weight of algae (Table 3.5). The highest and lowest amounts were recorded by the members of chlorophyceae, viz., *Enteromorpha intestinalis* from Narakkal in the summer monsoon season and *Chaetomorpha antennina* from Ettikkulam in the mid winter monsoon season respectively. *Centroceras clavulatum* collected in the late hot season from Narakkal showed the lowest value (11.83%) among the rhodophyceae, whereas *Gracilaria corticata* of Ettikkulam collected in the early winter monsoon season showed the highest value (31.30%).

At Ettikkulam, chlorophyceae recorded widely varying amounts of polysaccharides in the range 4.83 – 29.92% (Table 3.5) with an average of 15.52%. *Chaetomorpha antennina* recorded the lowest value (in the mid winter monsoon season) and *Enteromorpha intestinalis*, which was present

Table 3.5. Polysaccharide contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	11.51	9.74	11.48	4.83	6.79	12.11
Ulva lactuca	24.41	14.51	22.68	17.59	17.56	18.64
Enteromorpha intestinalis	29.92	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	30.81	30.16	31.30	25.03	27.30	24.32
Centroceras clavulatum	*	27.71	*	25.28	*	18.30
Grateloupia filicina	*	27.90	*	*	*	*
Average	**24.16**	**22.00**	**21.82**	**18.18**	**17.22**	**18.34**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	13.42	9.25	*	*	*	*
Enteromorpha intestinalis	35.81	*	*	*	*	11.12
Rhodophyceae						
Centroceras clavulatum	19.51	22.94	18.84	17.39	20.49	11.83
Grateloupia filicina	29.80	27.89	16.38	*	21.33	12.51
Average	**24.64**	**20.03**	**17.61**	**17.39**	**20.91**	**11.82**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

only in the summer monsoon season, recorded the highest value. Rhodophyceae contained a greater amount of polysaccharides (average: 26.81%) than chlorophyceae and it varied in a narrower range of 18.30 – 31.30% with minimum in *Centroceras clavulatum* (late hot season) and maximum in *Gracilaria corticata* (early winter monsoon season). The chlorophyceae of Narakkal contained polysaccharides in the range 9.25% - 35.81%. At this location also, *Chaetomorpha antennina* recorded the minimum value (in the retreating monsoon season) and *Enteromorpha intestinalis*, the maximum value (in the summer monsoon season). In the case of rhodophyceae, polysaccharide content varied from 11.83% in *Centroceras clavulatum* (late hot season) to 29.80% in *Grateloupia filicina* (summer monsoon season).

Interspecies variations in the polysaccharide contents of marine algae were similar to the variations in the total carbohydrate content (Fig. 3.1). At Ettikkulam, the chlorophyte *Ulva lactuca* recorded two times higher and *Enteromorpha intestinalis* recorded three times higher values than the average polysaccharide content of *Chaetomorpha antennina*. The values were 19.23%, 29.92% and 9.41% respectively. At Narakkal, the average polysaccharide content of *E. intestinalis* was more than double the amount recorded by *C. antennina*. Among the rhodophyceae of Ettikkulam, the average amounts of polysaccharides were 28.15% in *Gracilaria corticata*, 23.76% in *Centroceras clavulatum* and 27.90% in *Grateloupia filicina*. Rhodophyceae of Narakkal recorded lower values (18.50% by *C. clavulatum* and 21.58% by *G. filicina*). Temporal (Fig. 3.2) and spatial (Fig. 3.5) variations in polysaccharide contents also followed more or less the same trend as the total carbohydrates.

Expressing polysaccharide content as percentage of total carbohydrates, it was found that most of the carbohydrates of marine algae were constituted by polysaccharides (Fig. 3.8). The values varied from 68.9 to 94.9% of the total carbohydrate content of algae. *Chaetomorpha antennina* collected from Ettikkulam in the mid winter monsoon season recorded the lowest value and *Centroceras clavulatum* collected in the retreating monsoon season from the same location showed the highest value. 34% of the samples (13 out of 38) showed polysaccharide contents greater than 90% of the total carbohydrate contents and 53% recorded values in between 80 – 90% of the total carbohydrates. Only 13% of the samples showed polysaccharide levels lower than 80% of the total carbohydrates.

3.2.4 Fibre

The crude fibre is considered to be the material remaining after the extraction of algae with hot dilute solutions of acid and alkali (Larson, 1978). This generally contains the fibrous materials constituting the cell walls of algae. These include polysaccharides such as cellulose, xylan and mannan. They provide structural strength to the algal cell walls.

The marine algae selected for the present study recorded widely different amounts of crude fibre varying in the range 4.91 – 36.61% (Table 3.6). The minimum and maximum values were shown by the members of chlorophyceae from Ettikkulam, viz., *Enteromorpha intestinalis* in the summer monsoon season and *Chaetomorpha antennina* in the retreating monsoon season respectively. *E. intestinalis* collected in the summer monsoon from Narakkal also recorded fibre content close to the lowest value (4.94%). The highest fibre content at Narakkal was 35.32% recorded by *C. antennina* itself in the summer monsoon season. Among rhodophyceae, the fibre contents varied in a narrow range of 6.57 – 11.87%. The lowest and highest values were recorded by the same species collected from Narakkal in different periods. *Centroceras clavulatum* recorded the lowest value in the retreating monsoon season and the highest in the summer monsoon. The crude fibre contents of *Grateloupia filicina* from Narakkal were close to the lowest value in the summer monsoon season (6.85%) and to the highest value in the retreating monsoon season (11.80%). However, this species collected in the retreating monsoon season recorded the lowest value (8.60%) among the rhodophyceae of Ettikkulam.

Table 3.6. Fibre contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	35.73	36.61	34.54	29.86	35.93	33.83
Ulva lactuca	6.61	6.16	5.25	5.61	5.83	6.25
Enteromorpha intestinalis	4.91	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	8.92	10.17	10.08	9.11	8.71	9.59
Centroceras clavulatum	*	11.04	*	11.47	*	10.41
Grateloupia filicina	*	8.60	*	*	*	*
Average	**14.04**	**14.52**	**16.62**	**14.01**	**16.82**	**15.02**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	35.32	30.83	*	*	*	*
Enteromorpha intestinalis	4.94	*	*	*	*	5.45
Rhodophyceae						
Centroceras clavulatum	11.87	6.57	11.46	11.64	10.54	*
Grateloupia filicina	6.85	11.80	*	*	*	*
Average	**14.75**	**16.40**	**11.46**	**11.64**	**10.54**	**5.45**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent.

Gracilaria corticata collected in the mid hot season also showed a low fibre content (8.71%). The maximum value for the rhodophyceae of Ettikkulam was shown by *Centroceras clavulatum* in the mid winter monsoon season (11.47%).

The crude fibre contents of marine algae showed marked variations between some genera of chlorophyceae. *Chaetomorpha antennina* contained a very high amount of fibre (above 30%), whereas other chlorophyceae members like *Enteromorpha intestinalis* and *Ulva lactuca* recorded fibre contents below 7% (Fig. 3.1). At Ettikkulam, *C. antennina* recorded an average fibre content of 34.42%, whereas *E. intestinalis* and *U. lactuca* recorded very low fibre contents of 4.91% and 5.95% respectively. At Narakkal, *C. antennina* showed an average fibre content of 33.08%, which is as high as the amount shown by the same species from Ettikkulam. Here, *U. lactuca* was absent and *E. intestinalis* recorded an average value of 5.20%. The members of rhodophyceae from both Ettikkulam and Narakkal recorded very low interspecies variability in their fibre content. At Ettikkulam, average fibre contents were 8.60% for *Grateloupia filicina*, 9.43% for *Gracilaria corticata* and 10.97% for *Centroceras clavulatum*. At Narakkal, *C. clavulatum* recorded a value of 10.42% while *G. filicina* showed average fibre content of 9.33%.

In the present study, no significant variations were found in the crude fibre content of marine algae with respect to the space or time of their occurrence.

3.2.5 Calorific Value

The energy content of a food is usually expressed as its calorific value. In the energy flow studies, caloric content of an organism is an important parameter, which is used for converting the biomass values to energy units. The calorific values of marine algae are important for the determination of feeding habits of certain invertebrate animals. It is also important from the point of view of using marine macroalgae as food for humans and animals.

The calorific values of marine algae selected for the present study varied from 1.19 to 2.43 kcal g^{-1} dry weight of algae (Table 3.7). The

Table. 3.7. Calorific values (kcal g^{-1} dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	1.90	1.19	1.68	1.41	1.44	1.22
Ulva lactuca	1.75	1.71	2.19	1.87	1.99	1.44
Enteromorpha intestinalis	1.76	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	2.35	2.04	2.25	2.15	2.17	1.78
Centroceras clavulatum	*	1.71	*	1.99	*	1.23
Grateloupia filicina	*	2.10	*	*	*	*
Average	**1.94**	**1.75**	**2.04**	**1.86**	**1.87**	**1.42**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	1.85	1.21	*	*	*	*
Enteromorpha intestinalis	2.04	*	*	*	*	1.54
Rhodophyceae						
Centroceras clavulatum	2.10	2.03	2.43	2.23	2.30	1.32
Grateloupia filicina	2.27	1.86	2.01	*	2.32	1.57
Average	**2.07**	**1.70**	**2.22**	**2.23**	**2.31**	**1.48**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent

caloric content was lowest in the chlorophyte *Chaetomorpha antennina* from Ettikkulam in the retreating monsoon season and highest in the rhodophyte *Centroceras clavulatum* from Narakkal in the early winter monsoon season. The highest calorific value in chlorophyceae was shown by *Ulva lactuca* (2.19 kcal g^{-1}) in the early winter monsoon and the lowest value in rhodophyceae by *C. clavulatum* in late hot season (1.23 kcal g^{-1}), both collected from Ettikkulam. Thus, in the case of chlorophyceae, maximum and minimum calorific values were shown by different species from the same location while in the case of rhodophyceae, by the same species from different locations.

At Ettikkulam, the calorific values of marine algae varied in the range 1.19 – 2.35 kcal g^{-1} (Table 3.7). Among the chlorophyceae, *Chaetomorpha antennina* collected in the retreating monsoon season showed the minimum caloric content (1.19 kcal g^{-1}) and *Ulva lactuca* in the early winter monsoon recorded the maximum (2.19 kcal g^{-1}). In the case of rhodophyceae, *Centroceras clavulatum* collected in the late hot season recorded the minimum calorific value (1.23 kcal g^{-1}) and *Gracilaria corticata* in the summer monsoon season exhibited the maximum value (2.35 kcal g^{-1}). The caloric content of marine algae from Narakkal exhibited a range similar to that from Ettikkulam (1.21 – 2.43 kcal g^{-1}). In the case of chlorophyceae, the calorific value varied from 1.21 kcal g^{-1} (*Chaetomorpha antennina* in the retreating monsoon season) to 2.04 kcal g^{-1} (*Enteromorpha intestinalis* in the summer monsoon season). Among the rhodophyceae, *Centroceras clavulatum* collected in the late hot season showed the lowest value (1.32 kcal g^{-1}), whereas the same species collected in the early winter monsoon season recorded the highest value (2.43 kcal g^{-1}).

Specieswise comparison of caloric contents of algae showed notable variations in some cases. The rhodophyceae members *Gracilaria corticata* and *Grateloupia filicina* from Ettikkulam showed average calorific values above 2 kcal g^{-1} (2.12 and 2.10 kcal g^{-1} respectively), whereas *Centroceras clavulatum* recorded the value 1.64 kcal g^{-1} (Fig. 3.1). All the members of chlorophyceae recorded average caloric contents below 2 kcal g^{-1}. *Chaetomorpha antennina* showed the lowest average value (1.47 kcal g^{-1}). *Ulva lactuca* exhibited average calorific value of 1.83 kcal g^{-1}.

Enteromorpha intestinalis, which occurred only in the summer monsoon season recorded 1.76 kcal g^{-1}. At Narakkal also, *C. antennina* recorded the lowest value (1.53 kcal g^{-1}). *E. intestinalis* contained an average energy content of 1.79 kcal g^{-1}. The rhodophyceae members *Centroceras clavulatum* and *Grateloupia filicina* showed comparable average calorific values (2.07 and 2.01 kcal g^{-1} respectively).

Notable temporal variations were observed in the calorific values of marine algae selected for the present study. Considering all the algal species together, the average caloric content of marine algae from Ettikkulam varied from 1.42 kcal g^{-1} in the late hot season to 2.04 kcal g^{-1} in the early winter monsoon season (Table 3.7). The marine algae from Narakkal showed a similar range of variation (1.48 kcal g^{-1} in the late hot season to 2.23 kcal g^{-1} in the winter monsoon season). Chlorophyceae from Ettikkulam exhibited more or less the same range of caloric content as that from Narakkal. The ranges were 1.33 (late hot season) – 1.94 kcal g^{-1} (early winter monsoon) and 1.21 (retreating monsoon) – 1.95 kcal g^{-1}(summer monsoon) respectively (Fig. 3.2). Similarly, rhodophyceae from the two locations recorded variations in almost the same range. At Ettikkulam, the calorific value varied from 1.51 kcal g^{-1} in the late hot season to 2.35 kcal g^{-1} in the summer monsoon season and at Narakkal, from 1.45 kcal g^{-1} in the late hot season to 2.31 kcal g^{-1} in the mid hot season. From a classwise comparison it was found that at both the locations, the average value of caloric content was more for rhodophyceae than for chlorophyceae in almost all the seasons (Fig. 3.2). The caloric content averaged over the whole study period also exhibited the same trend (Fig. 3.3).

Considering the temporal variations of calorific value in individual species, marked variations were observed in some cases. Among the chlorophyceae from Ettikkulam, *Chaetomorpha antennina* recorded low calorific values (<2 kcal g^{-1}) compared to other algae. It recorded a maximum value of 1.90 kcal g^{-1} in the summer monsoon season and minimum of 1.19 kcal g^{-1} in the retreating monsoon (Fig. 3.4). *Ulva lactuca* showed calorific values varying in the range 1.44 – 2.19 kcal g^{-1} with minimum in the late hot season and maximum in the early winter monsoon season. *Enteromorpha intestinalis* recorded an energy content of 1.76 kcal g^{-1} in the summer monsoon season. The rhodophyceae *Gracilaria corticata* showed calorific values above 2 kcal g^{-1} in all the seasons except in the late hot season when it recorded a value of 1.78 kcal g^{-1}. The highest value was recorded in the summer monsoon season (2.35 kcal g^{-1}). C*entroceras clavulatum* showed calorific values below 2 kcal g^{-1}, with a minimum of 1.23 kcal g^{-1} in the late hot season and a maximum of 1.99 kcal g^{-1} in the mid winter monsoon. *Grateloupia filicina,* which was obtained only in the retreating monsoon season, showed a calorific value of 2.10 kcal g^{-1}. At Narakkal, *Chaetomorpha antennina* recorded calorific value of 1.85 kcal g^{-1} in the summer monsoon season and 1.21 kcal g^{-1} in the retreating monsoon. *Enteromorpha intestinalis* showed calorific values of 2.04 kcal g^{-1} in the summer monsoon season and 1.54 kcal g^{-1} in the late hot season. Among the rhodophyceae, C*entroceras clavulatum* showed calorific values above 2 kcal g^{-1} in all the seasons except in the late hot season when it recorded a value of 1.32 kcal g^{-1}. Highest caloric content was found in the early winter monsoon season (2.43 kcal g^{-1}) and lowest in the late hot season (1.32 kcal g^{-1}). In *Grateloupia filicina*, the caloric content varied from 1.57 kcal g^{-1} in the late hot season to 2.32 kcal g^{-1} in the mid hot season. A high value of 2.27 kcal g^{-1} was observed in the summer monsoon season also. Thus, in general, almost all the algal species selected for the present study showed high calorific values in the summer or winter monsoon seasons and low values in the late hot season or the retreating monsoon season.

Considerable spatial variations were not observed in the calorific values of marine algae selected for the present study (Fig. 3.5). The caloric content of chlorophyceae and rhodophyceae from Ettikkulam averaged over the whole study period was found to be almost the same as that from Narakkal (Fig. 3.6). For chlorophyceae, the average value was 1.66 kcal g^{-1} at both the locations and for rhodophyceae, it was 1.98 kcal g^{-1} at Ettikkulam and 2.04 kcal g^{-1} at Narakkal. A comparison for different seasons also showed that both chlorophyceae and rhodophyceae exhibited little spatial variations in their energy content (Fig. 3.5). Considering individual species, *Centroceras clavulatum* from Narakkal exhibited a slightly higher average caloric content than that from Ettikkulam (Fig. 3.7). The former contained about 430 calories more energy per gram dry weight than the latter. Average caloric contents of all the other algal species were comparable between the two locations. *Grateloupia filicina* from Ettikkulam recorded about 240 calories more energy per gram than the same species from Narakkal. *Chaetomorpha antennina* showed almost the same values at both the locations. *Enteromorpha intestinalis* collected in the summer monsoon season from Narakkal contained 280 calories more energy per gram compared to that from Ettikkulam.

The mean values of major biochemical constituents and caloric content in different species of marine algae from Kerala coast are given in Table 3.8.

Table 3.8. Mean values of major biochemical constituents and caloric content of marine algae from Kerala coast.

	Proteins (% dry wt)	Carbohydrates (% dry wt)			Lipids (% dry wt)	Crude Fibre (% dry wt)	Calorific value (kcal g^{-1})
		LMWC	PS	TC			
C. antennina	13.92	2.30	9.89	12.19	1.99	34.08	1.49
U. lactuca	13.64	2.42	19.23	21.65	1.52	5.95	1.83
E. intestinalis	8.96	2.73	25.62	28.35	0.85	5.10	1.78
G. corticata	10.25	5.78	28.15	33.93	1.27	9.43	2.12
C. clavulatum	15.22	2.13	20.25	22.38	1.32	10.62	1.93
G. filicina	15.54	2.25	22.64	24.89	1.03	9.08	2.03

LMWC: Low-molecular-weight carbohydrates PS: Polysaccharides TC: Total carbohydrates

3.2.6 Statistical Analysis

The significance of the observed variations in the biochemical constituents and calorific values of marine algae were tested statistically. Interspecies variations were tested by one-way ANOVA, while interclass and spatial comparisons were made by Student's t-test. The analysis showed that interspecies variations in the total carbohydrate contents of marine algae from Ettikkulam were highly significant ($P<0.001$, Table 3.9). Both LMWC and polysaccharides showed highly significant variations. Protein as well as lipid contents of different species of algae were not statistically different ($P>0.05$). However, calorific values showed significant specieswise variations ($P<0.05$), probably due to the highly significant variations in the total carbohydrate contents. Fibre contents also exhibited significant variations ($P<0.001$). At Narakkal, statistically significant interspecies variations were observed only for fibre contents ($P<0.001$, Table 3.10). Comparison of different species of algae on the basis of ANOVA and

Table 3.9. One-way ANOVA results for interspecies variations in major biochemical constituents and calorific values of marine algae from Ettikkulam.

		Sum of Squares	df	Mean Square	F	P
Proteins	Between Groups	133.058	5	26.612	1.988	0.132
	Within Groups	227.522	17	13.384		
	Total	360.580	22			
Lipids	Between Groups	4.516	5	0.903	0.787	0.573
	Within Groups	19.507	17	1.147		
	Total	24.023	22			
TCH	Between Groups	1613.975	5	322.795	24.735	0.000
	Within Groups	221.850	17	13.050		
	Total	1835.825	22			
LMWC	Between Groups	54.926	5	10.985	9.912	0.000
	Within Groups	18.841	17	1.108		
	Total	73.767	22			
PS	Between Groups	1274.352	5	254.870	21.170	0.000
	Within Groups	204.663	17	12.039		
	Total	1479.015	22			
Fibres	Between Groups	3130.833	5	626.167	316.970	0.000
	Within Groups	33.583	17	1.975		
	Total	3164.416	22			
Calorific value	Between Groups	1.436	5	0.287	4.088	0.013
	Within Groups	1.194	17	0.070		
	Total	2.631	22			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

Table 3.10. One-way ANOVA results for interspecies variations in major biochemical constituents and calorific values of marine algae from Narakkal.

		Sum of Squares	df	Mean Square	F	P
Proteins	Between Groups	122.235	3	40.745	1.986	0.175
	Within Groups	225.629	11	20.512		
	Total	347.865	14			
Lipids	Between Groups	0.515	3	0.172	0.152	0.926
	Within Groups	12.463	11	1.133		
	Total	12.978	14			
TCH	Between Groups	220.119	3	73.373	1.493	0.271
	Within Groups	540.756	11	49.160		
	Total	760.875	14			
LMWC	Between Groups	1.340	3	0.447	1.103	0.389
	Within Groups	4.455	11	0.405		
	Total	5.795	14			
PS	Between Groups	191.389	3	63.796	1.168	0.366
	Within Groups	600.775	11	54.616		
	Total	792.164	14			
Fibres	Between Groups	986.586	3	328.862	54.849	0.000
	Within Groups	41.971	7	5.996		
	Total	1028.557	10			
Calorific value	Between Groups	0.502	3	0.167	1.241	0.342
	Within Groups	1.483	11	0.135		
	Total	1.984	14			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

Table 3.11. Results of post hoc test (Tukey's HSD) for parameters (major biochemical constituents and calorific value) showing significant inter-species variations in one-way ANOVA.

Ettikkulam					Narakkal				
Parameter	Species	CA	UL	GC	Parameter	Species	CA	EI	CC
TCH	UL	SIG			Fibres	EI	SIG		
	GC	SIG	SIG			CC	SIG	NS	
	CC	SIG	NS	SIG		GF	SIG	NS	NS
LMWC	UL	NS							
	GC	SIG	SIG						
	CC	NS	NS	SIG					
PS	UL	SIG							
	GC	SIG	SIG						
	CC	SIG	NS	NS					
Fibres	UL	SIG							
	GC	SIG	SIG						
	CC	SIG	SIG	NS					
Calorific value	UL	NS							
	GC	SIG	NS						
	CC	NS	NS	NS					

CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina* TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates PS: Polysaccharides SIG: Significant at 0.05 level NS: Not significant

Tukey's post hoc test is given in Table 3.11 (multiple comparison tables are given as appendices).

As with the interspecies variations, marine algae from Ettikkulam showed significant interclass variations for carbohydrates (LMWC, $P<0.05$; polysaccharides, $P<0.001$; total carbohydrates, $P<0.001$), fibres ($P<0.001$) and calorific values ($P<0.05$) (Table 3.12). Proteins and lipids did not show

Table 3.12. Results of t-test for classwise comparisons of major biochemical constituents and calorific values of marine algae from Ettikkulam.

Parameter	Class	N	Mean	Standard Deviation	t	df	P
Proteins	Chlorophyceae	6	13.1517	3.3202	1.809	10	0.101
	Rhodophyceae	6	10.3267	1.8988			
Lipids	Chlorophyceae	6	1.8050	1.0340	0.869	10	0.405
	Rhodophyceae	6	1.2610	1.1320			
TCH	Chlorophyceae	6	17.4283	4.0938	-5.538	10	0.000
	Rhodophyceae	6	32.0746	5.0202			
LMWC	Chlorophyceae	6	2.4467	0.3629	-2.665	10	0.024
	Rhodophyceae	6	4.6625	2.0038			
PS	Chlorophyceae	6	14.9883	4.0823	-5.489	10	0.000
	Rhodophyceae	6	27.4117	3.7515			
Fibres	Chlorophyceae	6	19.2833	2.1357	10.540	10	0.000
	Rhodophyceae	6	9.6567	0.6659			
Calorific value	Chlorophyceae	6	1.6467	0.2254	-2.640	10	0.025
	Rhodophyceae	6	2.0500	0.2988			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

any significant variations between different classes of algae. At Narakkal, biochemical composition and calorific values of rhodophyceae were statistically indistinguishable from those of chlorophyceae (Table 3.13). Considering the spatial variations for different classes of algae (Tables 3.14 and 3.15), significant variations were observed only for rhodophyceae. They showed significant spatial variations in the levels of proteins ($P<0.01$) and

Table 3.13. Results of t-test for classwise comparisons of major biochemical constituents and calorific values of marine algae from Narakkal.

Parameter	Class	N	Mean	Standard Deviation	t	df	P
Proteins	Chlorophyceae	3	12.2354	1.5944	-2.352	7	0.051
	Rhodophyceae	6	17.7383	3.7823			
Lipids	Chlorophyceae	3	1.4002	0.3217	0.059	7	0.954
	Rhodophyceae	6	1.3567	1.2105			
TCH	Chlorophyceae	3	17.5788	7.9953	-1.060	7	0.324
	Rhodophyceae	6	22.0200	4.8546			
LMWC	Chlorophyceae	3	2.5827	1.1730	0.478	7	0.647
	Rhodophyceae	6	2.3283	0.4934			
PS	Chlorophyceae	3	14.9967	8.3863	-1.078	7	0.317
	Rhodophyceae	6	19.6933	5.0038			
Fibres	Chlorophyceae	3	18.8033	12.7419	1.545	6	0.173
	Rhodophyceae	5	10.4380	1.1423			
Calorific value	Chlorophyceae	3	1.5667	0.3707	-2.066	7	0.078
	Rhodophyceae	6	2.0583	0.3219			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

carbohydrates (LMWC, $P<0.05$; polysaccharides, $P<0.05$; total carbohydrates, $P<0.01$).

Parameter Relationships

The correlation matrix and Pearson correlation coefficients for major biochemical constituents and calorific values are given in Tables 3.16 and 3.17. Total carbohydrate contents showed positive correlations with LMWC

Table 3.14. Results of t-test for spatial comparisons of major biochemical constituents and calorific values of chlorophyceae from Kerala coast.

Parameter	Location	N	Mean	Standard Deviation	t	df	P
Proteins	Ettikkulam	6	13.1517	3.3202	0.442	7	0.672
	Narakkal	3	12.2354	1.5944			
Lipids	Ettikkulam	6	1.8050	1.0340	0.643	7	0.541
	Narakkal	3	1.4002	0.3217			
TCH	Ettikkulam	6	17.4283	4.0938	-0.039	7	0.970
	Narakkal	3	17.5788	7.9953			
LMWC	Ettikkulam	6	2.4467	0.3629	-0.276	7	0.791
	Narakkal	3	2.5827	1.1730			
PS	Ettikkulam	6	14.9883	4.0823	-0.002	7	0.998
	Narakkal	3	14.9967	8.3863			
Fibres	Ettikkulam	6	19.2833	2.1357	0.096	7	0.926
	Narakkal	3	18.8033	12.7419			
Calorific value	Ettikkulam	6	1.6467	0.2254	0.412	7	0.693
	Narakkal	3	1.5667	0.3707			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

and polysaccharides ($P<0.01$). This was expected, as the latter two parameters constitute the former. A negative correlation was observed between fibre contents and carbohydrates, especially polysaccharides ($P<0.01$). This is due to the fact that the inclusion of more photosynthetic carbon into the microfibrillar polysaccharides constituting the cell walls decreases the amount of other polysaccharides. LMWC contents of algae

Table 3.15. Results of t-test for spatial comparisons of major biochemical constituents and calorific values of rhodophyceae from Kerala coast.

Parameter	Location	N	Mean	Standard Deviation	t	df	P
Proteins	Ettikkulam	6	10.3267	1.8988	-4.290	10	0.002
	Narakkal	6	17.7383	3.7823			
Lipids	Ettikkulam	6	1.2610	1.1320	-0.141	10	0.890
	Narakkal	6	1.3567	1.2105			
TCH	Ettikkulam	6	32.0746	5.0202	3.527	10	0.005
	Narakkal	6	22.0200	4.8546			
LMWC	Ettikkulam	6	4.6625	2.0038	2.771	10	0.020
	Narakkal	6	2.3283	0.4934			
PS	Ettikkulam	6	27.4117	3.7515	3.023	10	0.013
	Narakkal	6	19.6933	5.0038			
Fibres	Ettikkulam	6	9.6567	0.6659	-1.419	9	0.189
	Narakkal	5	10.4380	1.1423			
Calorific value	Ettikkulam	6	2.0500	0.2988	-0.046	10	0.964
	Narakkal	6	2.0583	0.3219			

TCH: Total carbohydrates LMWC: Low-molecular-weight carbohydrates
PS: Polysaccharides

were found to be positively correlated with polysaccharides ($P<0.05$). This is because of the fact that during the periods of growth, algae synthesize both LMWC and polysaccharides, and the faster synthesis of LMWC than its conversion to polysaccharides results in the accumulation of both the substances. The lipid contents of algae showed negative correlation with total carbohydrates ($P<0.01$), especially polysaccharides. This may be due

Table 3.16. Correlation matrix and Pearson correlation coefficients for major biochemical constituents and calorific values of marine algae from Ettikkulam.

Variables	Lipids	TC	LMWC	PS	Fibres	CV
Proteins	0.334	-0.364	-0.202	-0.361	0.237	0.350
Lipids		-0.531[a]	-0.336	-0.516[b]	0.332	-0.083
TC			0.585[a]	0.983[a]	-0.717[a]	0.716[a]
LMWC				0.429[b]	-0.196	0.417[b]
PS					-0.755[a]	0.705[a]
Fibres						-0.545[a]

TC: Total carbohydrates LMWC: Low-molecular-weight carbohydrates PS: Polysaccharides
[a] Significant at 0.01 level ($P< 0.01$)
[b] Significant at 0.05 level ($P< 0.05$)

Table 3.17. Correlation matrix and Pearson correlation coefficients for major biochemical constituents and calorific values of marine algae from Narakkal.

Variables	Lipids	TC	LMWC	PS	Fibres	CV
Proteins	0.157	-0.263	0.288	-0.282	0.068	0.570[b]
Lipids		-0.061	0.298	-0.085	0.167	0.303
TC			-0.191	0.996[a]	-0.637[b]	0.611[b]
LMWC				-0.273	-0.350	0.130
PS					-0.589	0.587[b]
Fibres						-0.487

TC: Total carbohydrates LMWC: Low-molecular-weight carbohydrates PS: Polysaccharides
[a] Significant at 0.01 level ($P< 0.01$)
[b] Significant at 0.05 level ($P< 0.05$)

to the fact that during the periods of slow growth, more photosynthetic carbons are incorporated into the substances like lipids thereby increasing their amount and decreasing the level of carbohydrates including polysaccharides. Proteins and carbohydrates (LMWC, polysaccharides and total carbohydrates) showed positive correlations with the caloric content of algae ($P<0.05$). This was expected because both proteins and carbohydrates are energy-yielding constituents. Fibre content showed a negative correlation with calorific value ($P<0.01$), probably due to its negative correlation with the energy-yielding carbohydrates.

3.3 Discussion

In the present study, marine algae from Kerala coast were observed to exhibit interspecies and interclass variations in their biochemical compositions and nutritive values. They also showed considerable temporal variations. Spatial variations were observed in some cases. In most cases, carbohydrates were found to make up the largest proportions compared to proteins and lipids. There were a few exceptions with proteins showing the highest proportion. In the case of the chlorophyte *Chaetomorpha antennina*, fibres represented the largest component. In all the marine algae studied, lipids exhibited relatively smallest proportion.

During the present investigation, total protein contents of marine algae varied from 6.11 to 25.77% on dry weight basis. Total carbohydrates varied in the range 7.01 – 38.33% and total lipids in the range 0.13 – 3.64% dry weight of algae. An earlier study on the biochemical composition of marine algae from other parts of Kerala coast (Saji, 1991) had recorded values in the ranges 3.0 – 18.6% for proteins, 5.9 – 55% for carbohydrates and 0.5 – 7.35% for lipids in the algal species same as those selected for the present study. The protein content of chlorophyceae members varied from 6.56% to 20.84% during the present study. A more or less similar range of protein content (7.22 – 25.48%) was reported by Rao and Tipnis (1964) for the chlorophyceae of Gujarat coast. Dave and Chauhan (1985) recorded a range of 4.98 – 29.38% for the protein content of marine algae from Gujarat coast. Lewis and Gonzalves (1960) observed protein levels greater than 28% in algae from Bombay (Mumbai) coast while in the present study protein contents were less than 26%. Dhargalkar *et al.* (1980) reported proteins in the range 10 – 33% and carbohydrates in the range 28 – 75% in marine algae from Maharashtra coast. The values recorded by them were found to be considerably higher than the protein and carbohydrate contents observed in the same algal species from Kerala coast during the present study.

The protein contents of marine algae from Kerala coast were found to be higher than that reported by Qari and Qasim (1993) for marine algae from Karachi coast. They observed protein contents in the range 4.33 – 15% on dry weight basis. However, carbohydrate and lipids levels were observed to be considerably higher in the algae from Karachi. The ranges of values were 14.00 – 62.64% for carbohydrates and 2.26 – 11.91% for lipids. Kumar (1993) estimated biochemical constituents of marine algae from Tuticorin coast of Tamil Nadu and reported values on fresh weight basis. He recorded protein contents in the range 1.23– 8.62% and carbohydrates in the range 10.5 – 30% for green and red algae. Roslin (2001) recorded lipid contents in the range 0 – 6.5% in different species of marine algae from Tamil Nadu coast. The lipid contents reported by Selvi *et al.* (1999) for the chlorophyceae members *Ulva lactuca, Enteromorpha intestinalis* and *Chaetomorpha* sp. from two estuaries in Tamil Nadu were higher than the lipid levels obtained for the same algae in the present study. Protein contents of marine algae from Bahrain coast (Abbas *et al.*, 1992) were found to be lower than that from Kerala coast. Koya (2000) studying the chemical constituents of marine macroalgae from Lakshadweep coast reported protein levels in the range 4.5 – 15.9% in rhodophyceae members. Carbohydrates varied in the range 14.63 – 21.80%. He observed that the carbohydrate contents were more than protein and lipid contents in all the rhodophyceae members studied. Reeta *et al.* (1990) also obtained the same result for 27 species of chlorophyceae. Chennubhotla (1992) reported lipid levels varying in the range 0.25 – 1.65% in rhodophyceae from Lakshadweep. The average protein content varied from 4.49% to 6.30%. Devi *et al.* (1996) studied the biochemical composition of marine algae from Cape Comorin and reported values in the ranges 1.38 – 12.75% for proteins, 0.91 – 9.66% for carbohydrates and 0.36 – 1.78% for lipids. Protein values obtained in the present study were found to be comparable with that reported by Sumitra *et al.* (1980) for the marine algae from Goa coast. They obtained a range of 8.62 - 13.12% for the average protein content, while in the present study the range was 8.96 – 15.54%. The carbohydrate and lipid values reported by them were much higher than the present values. They obtained average carbohydrate contents above 50% and observed that the carbohydrate levels were almost similar in all the algae studied.

Haroon *et al.* (2000) studying the biochemical composition of *Enteromorpha* spp. from Gulf of Gdansk coast on the Southern Baltic Sea recorded values in the ranges 9.42- 20.60% for proteins, 29.09 – 39.81% for carbohydrates and 1.88 – 7.77% for lipids. In the present study, *E. intestinalis* recorded protein values varying from 6.56% to 13.39% (average = 8.96%), carbohydrates from 15.06% to 37.81% (average = 28.35%) and lipids from 0.40% to 1.56% (average = 0.85%). A similar range of protein content (5.8– 13.0%, average = 9.37%) was reported by Saji (1991) for *E. compressa* from Saudi on the central Kerala coast. However, carbohydrates recorded lower values in the range 6.75 – 27.75% (average = 17.52%) and lipids showed higher values varying from 1.2 – 3.3% (average = 1.99%) compared to those in the present study. *E. intestinalis* from Maharashtra exhibited much higher values of protein (23.62%) and carbohydrate (53.10%) contents (Dhargalkar *et al.*, 1980). *E. tuberosa* from Tuticorin showed protein values in the range 6.26 – 8.62% on fresh weight basis (Kumar, 1993). At Cape Comorin, *E. linza* recorded 8.56% proteins, 1.82% carbohydrates and 0.52% lipids (Devi *et al.*, 1996)

In the present study, *Chaetomorpha antennina* recorded protein contents in the range 8.50 – 20.84% (average = 13.92%). Carbohydrates ranged from 7.01% to 15.25% (average = 12.19%) and lipids from 1.30% to 3.64% (average = 1.99%). Earlier report from Kerala on the same species (Saji, 1991) showed lower protein contents ranging from 3% to 14.5%. Lipid levels varied in the range 0.9 – 7.35% with mean value higher than that in the present study. Although carbohydrate contents ranged from 5.9% to 22.5%, mean value was almost similar to that observed in the present study. Devi *et al.* (1996) recorded very low values of biochemical constituents in *C. antennina* from Cape Comorin. They obtained 6.56% proteins, 2.5% carbohydrates and 0.57% lipids. The same species exhibited a protein content of 10.13% at Mandapam coast (Reeta *et al.*, 1990). An average protein content of 22.6% was shown by *C. antennina* from Lakshadweep (Koya, 2000) where the carbohydrate content was 20.4%. *C. media* from Goa coast recorded average protein content of 13% (Sumitra *et al.*, 1980), while that from Maharashtra showed a protein level of 20.31% (Dhargalkar *et al.*, 1980). At both the coastal regions, the average carbohydrate values were above 50%. *C. antennina* from Karachi was found to contain lower levels of proteins (Qari and Qasim, 1993) and higher levels of carbohydrates and lipids compared to that obtained in the present study.

The chlorophyte *Ulva lactuca* also showed variations in the biochemical composition in comparison with earlier reports. The ranges of values obtained in the present study were 7.09 – 19.08% for proteins (average = 13.64%), 17.46 – 26.39% for carbohydrates (average = 21.65%) and 0.33 - 2.80% for lipids (average = 1.52%). Saji (1991) reported lower values of proteins ranging from 1.4% to 16.0% and higher values of carbohydrates and lipids varying in the ranges 16 – 38% and 1.0 – 5.2% respectively. Roslin (2001) recorded lipid contents varying in the ranges 0 – 5.4% in *U. lactuca* and 0 – 1.4% in *U. fasciata*, both collected from Arockiapuram coast of Tamil Nadu. Dhargalkar *et al.* (1980) observed protein contents of 10.81% in *U. lactuca*, 12.88% in *U. fasciata* and 17.63% in *U. reticulata* from Maharashtra coast. All these species recorded very high values (above 47%) of carbohydrates. Dhargalkar (1986) reported protein contents in the range 10.9 – 19.8% in *U. reticulata*. Kumar (1993) obtained protein contents ranging from 4.36% to 5.62% on fresh weight basis in *U. lactuca* and from 5.16% to 6.75% in *U. reticulata* from Tuticorin. Carbohydrates were in the ranges 14.00– 28.50% and 22.80 – 30.00% respectively. *U. fasciata* from Karachi recorded lower protein contents (Qari and Qasim, 1993), while that from Goa exhibited a protein content (Sumitra *et al.*, 1980) comparable to the mean protein content of *U. lactuca* in the present study. The carbohydrate and lipid contents were higher in the algae from Karachi as well as Goa coast. Abbas *et al.* (1992) and Selvi *et al.* (1999) recorded low values of proteins in *U. lactuca*.

During the present study, the rhodophyte *Gracilaria corticata* recorded protein contents ranging from 7.60% to 12.46% (average = 10.25%), carbohydrates from 29.85% to 38.33% (average = 33.93%) and lipids from 0.15% to 2.96% (average = 1.27%). Earlier study on the same species from Kerala (Saji, 1991) recorded protein, carbohydrate and lipid contents in the ranges 4.8 – 13.8%, 9.75 – 37.5% and 0.85 – 5% respectively. The mean levels of proteins and carbohydrates were found to be lower and that of lipids higher than the mean levels recorded in the present study. *G. corticata* from Goa exhibited values in the ranges 3 – 20% for proteins, 40 – 60% for carbohydrates and 7-15% for lipids (Sumitra *et al.*, 1980). Dhargalkar *et al.* (1980) reported high values of protein (24.37%) and carbohydrate (71.03%) contents in *G. corticata* from Maharashtra. The same species from Tuticorin showed protein contents varying from 3.43 to 5.81% on fresh weight basis while carbohydrate levels varied from 16.20 – 30.00% (Kumar, 1993). *G. corticata* from Cape Comorin showed very low values of biochemical contents (Proteins: 3.31%, carbohydrates: 9.66% and lipids: 0.45%; Devi *et al.*, 1996).

The ranges of biochemical values recorded by *Grateloupia filicina* in the present study were 8.91 – 19.92% for proteins (average = 15.54%), 14.85 – 31.72% for carbohydrates (average = 24.89%) and 0.23 – 2.82% for lipids (average = 1.03%) while the respective ranges in *Centroceras clavulatum* were 6.11 – 25.77% (average = 15.22%), 13.40 – 29.20% (average = 22.38%) and 0.13 – 2.91% (average = 1.32%). Saji (1991) reported protein, carbohydrate and lipid levels in the ranges 6.90 – 18.60%, 17.25 – 55.00% and 0.85 – 5.20% respectively for *G. filicina* and 7.20 – 15.40%, 10.25 – 20.25% and 0.50 – 1.50% respectively for C. *clavulatum*. Higher values of protein and carbohydrate contents were recorded by both the species from Maharashtra coast (Dhargalkar *et al.*, 1980). C. *clavulatum* from Gujarat coast recorded a protein content of 20.12% (Rao and Tipnis, 1964). Rajasulochana *et al.* (2002) reported values varying in the range 12.73 – 16.83% for proteins, 58.5 – 66.6% for carbohydrates and 0 – 0.44% for lipids in *Grateloupia lithophila* from Tamil Nadu coast.

From the ongoing discussion, it can be seen that biochemical composition of marine algae from Kerala coast exhibit considerable variations from that of marine algae from other coastal regions. However, it was found that variations were not much pronounced between the locations selected for the present study. This may be due to the fact that the various biochemical constituents of marine algae are influenced primarily by the environmental factors such as temperature, light available for photosynthesis, nutrient concentration in the ambient seawater etc. (Percival and McDowell, 1967) and these factors exhibit more or less similar conditions at different locations of the study area (Rao *et al.*, 1973; Subramanyan, 1973). During the present study, it was found that chlorophyceae members did not show any notable spatial variation in the major biochemical constituents such as proteins, carbohydrates and lipids ($P>0.05$). No spatial variation was observed in the lipid levels of rhodophyceae also. However, protein and carbohydrate levels showed some spatial variations when all the members of rhodophyceae were taken together ($P<0.01$). Rhodophyceae from Narakkal showed higher value for average protein content than that from Ettikkulam, while a reverse trend was observed in the average carbohydrate content. The observed spatial variations may be due to the difference in the dominant species of rhodophyceae at the two locations. At Ettikkulam, the dominant species was *Gracilaria corticata* that contained high levels of carbohydrates and low levels of proteins while at Narakkal, dominant species were

Centroceras clavulatum and *Grateloupia filicina* with high levels of proteins. A specieswise comparison showed that C. *clavulatum* from Ettikkulam contained lower levels of proteins compared to that from Narakkal, while reverse was the case in *G. filicina.* This may be due to the difference in the uptake rate of nitrogen nutrients which is affected by various environmental factors as well as biological factors such as the type of tissue, the age of the plant part, its nutrient past history, interplant variability etc. (Lobban and Harrison, 1997).

The foregoing discussion reveals some interspecies variations in the biochemical composition of marine algae. The algae were also found to exhibit interclass variations in some of the major biochemical constituents. In the present study, it was observed that chlorophyceae members from northern Kerala (Ettikkulam) contained more amounts of protein than rhodophyceae members, while at the location on the central Kerala coast (Narakkal), reverse was the case. However, these variations were not statistically significant at 95% level ($P>0.05$). Similar pattern of variations was observed earlier by Saji (1991) for algae from Kerala coast. She observed that chlorophyceae members from Elathur and Thikkotti near Kozhikkode on the northern Kerala coast contained more proteins than rhodophyceae while at Saudi on the central Kerala coast, rhodophyceae recorded more proteins than chlorophyceae. Dhargalkar *et al.* (1980) reported higher protein contents in rhodophyceae members than in chlorophyceae from Maharashtra coast, whereas Sumitra *et al.* (1980) observed higher values of protein in chlorophyceae members compared to rhodophyceae members from Goa coast. Chlorophyceae from many coastal regions including Tuticorin, Mandapam, Lakshadweep and Karachi also exhibited higher levels of protein than rhodophyceae (Chennubhotla *et al.*, 1987; Kaliaperumal *et al.*, 1987; Kumar, 1993; Qari and Qasim, 1993; Koya, 2000). Gutirrez *et al.* (1990) observed that rhodophyceae members contained considerably higher protein contents than chlorophyceae. In the present study, carbohydrate contents of marine algae from Ettikkulam showed a trend reverse to that of proteins. Here, rhodophyceae exhibited higher levels of total carbohydrates than chlorophyceae and the differences were highly significant ($P<0.001$). This may be due to the high phycocolloid content of rhodophyceae (Chapman and Chapman, 1980). Such a trend was observed in the marine algae of Narakkal in the retreating monsoon season only and here, both the algal classes showed comparable values in the late hot season and the summer monsoon season (chlorophyceae were absent from Narakkal during the winter monsoon and early hot season). This discrepancy may be due to the changes in the species composition of chlorophyceae of Narakkal in different seasons. In the late hot season, only *Enteromorpha intestinalis* was present, while in the retreating monsoon season, only *Chaetomorpha antennina* with low carbohydrate content was present. Both these species were present in the summer monsoon season. Saji (1991) observed that rhodophyceae from Elathur and Saudi contained more carbohydrates than chlorophyceae. Similar results were reported from other coastal regions also (Dhargalkar *et al.*, 1980; Chennubhotla *et al.*, 1987; Kaliaperumal *et al.*, 1987; Qari and Qasim, 1993). Sumitra *et al.* (1980) and Kumar (1993) observed that chlorophyceae and rhodophyceae members contained comparable amounts of carbohydrates. It was observed during the present study that the average lipid content of chlorophyceae was higher than that of rhodophyceae at both the locations, but the variations were statistically insignificant ($P>0.05$). Studying the lipid content of algae from Kerala coast Saji (1991) also observed higher values for chlorophyceae than for rhodophyceae. Marine algae from Lakshadweep (Kaliaperumal *et al.*, 1987; Koya, 2000) and Karachi coasts (Qari and Qasim, 1993) also showed the same trend.

Several authors have also observed that the biochemical composition of marine macroalgae exhibit considerable temporal variations (Murthy and Radia, 1978; Solimabi *et al.*, 1980; Rosell and Srivastava, 1985; Qari, 1988; Saji, 1991; Kumar, 1993; Mercer *et al.*, 1993, Ganesan and Kannan, 1994; Ilyas and Sukan, 1994; Haroon *et al.*, 2000; Nelson *et al.*, 2002). Ganesan and Kannan (1994) observed that biochemical contents of marine algae from Gulf of Mannar were generally more during the monsoon and post monsoon seasons. Qari (1988) found that protein and lipid contents of marine algae from Karachi coast were high in winter months while carbohydrate contents were high in summer months. Kumar (1993) observed that many of the algae from Tuticorin coast contained maximum protein in November and maximum carbohydrate in September. Solimabi *et al.* (1980) recorded maximum carbohydrate in October, November and May and minimum in January and February in macroalgae from Goa. Proteins showed a gradual increase from October to May. Haroon *et al.* (2000) observed that *Enteromorpha* spp. from Gulf of Gdansk contained maximum protein and minimum carbohydrate in spring and autumn. Minimum protein and maximum carbohydrate were observed in summer. Maximum lipid content was noted in spring. Nelson *et al.* (2002) recorded highest levels of lipids in winter and spring seasons in macroalgae of north-eastern Pacific Ocean. Marine algae from Kerala coast were also observed to show significant seasonal variations in biochemical constituents such as proteins, carbohydrates and lipids (Saji, 1991). During the present study, all the algal species collected from Kerala coast exhibited considerable temporal variations in their biochemical composition. In general, protein and carbohydrate contents were high in the summer monsoon and the early winter monsoon seasons. Their levels remained fairly high during the period from the mid winter monsoon to mid hot season. Lipid levels showed higher values in this period compared to other seasons. Retreating monsoon season also recorded fairly high lipid levels.

The accumulation of different biochemical constituents in marine algae can be regarded as the result of a number of enzymic reactions and diffusion processes. Various factors, which affect the rates of these processes, are found to be responsible for the variation in the biochemical composition of marine algae (Percival and McDowell, 1967). These factors include temperature, light, nutrients, water motion, time of emersion (exposure to atmosphere), level of desiccation (dehydration) etc. Most of these factors seem to exert significant influence on the biochemical composition of marine algae from Kerala coast during the hot season particularly in the late hot season, which is the hottest period in Kerala (Menon and Rajan, 1989). The high temperature increases the respiration rate and affects the metabolism of algae (Chapman, 1976). Since the hot season represents the transition period between the winter monsoon (north-east monsoon) and summer monsoon (south-west monsoon), more or less stagnant conditions exist along the west coast of India during this period (Rao *et al.*, 1973). Such a stagnant condition is likely to increase the emersion time of marine algae because it is the splash from breaking waves that keeps many of the intertidal algae moist and cool during the period of low tides (Dring, 1982). Also, a decrease in the turbulence and water movements caused by the stagnant conditions may affect the nutrient uptake rate of marine algae (DeBoer, 1981).

The emersion has been found to be a stress to marine algae, to which different species are more or less tolerant (Lobban and Harrison, 1997). The most obvious result of emersion will be desiccation, but there will also be a rather abrupt change in the temperature experienced by the algae and this may exert a more immediate influence. The dehydration rates are affected by temperature and there tends to be more water loss during the hot weather period than the rest of the year. It has been found that on a hot sunny day, even in cool temperate climates, exposed rock surfaces quickly reach a temperature that is about 10 – 15 ^{0}C higher than the water temperature (Dring, 1982). Thus, the temperature changes associated with emersion and submersion during the hot weather period may cause severe stress to the marine algae by affecting their metabolic rates as well as the rates of drying and the degree of desiccation.

The emersion also deprives seaweeds of their source of nutrients such as nitrogen, phosphorus and inorganic carbon. Since marine algae have no roots, they take up mineral salts only when they are covered by water. Thus, prolonged emersion can affect the growth rate of algae when they become unable to obtain sufficient nutrients during short periods of their submersion (Dring, 1982). However, if the relative humidity is high enough to prevent the desiccation during emersion, photosynthesis may continue at normal rate (Lobban and Harrison, 1997). This may result in a decline in protein levels and an accumulation of carbohydrates. It has been found that even if desiccation occurs, there will be an initial increase in photosynthesis. This may be because of an initial increase in the rate of diffusion of CO_2 from air into the algal cells as the surface water dries, but it is soon followed by a steady decline in photosynthetic rate if the water loss continues (Dring, 1982; Lobban and Harrison, 1997).

In the present study, all the algal species from Ettikkulam showed low protein contents in the late hot season. They also recorded fairly high values of carbohydrates. This suggests that in all these species, photosynthesis takes place at a fairly high rate during the period of emersion in the hot season, but the growth is limited by the restricted availability of essential nutrients especially nitrogen. The marine algae from Narakkal exhibited a reverse trend in the contents of proteins and carbohydrates during the late hot season. This indicates that the marine algae from Narakkal are subjected to desiccation stress during emersion with the resultant reduction in the photosynthetic rate. The high temperature condition of the season also increases the respiration rate and the resulting carbon losses may not be sufficiently compensated by the photosynthetic carbon assimilation due to its reduced rate. Thus reserve carbohydrates may be used as carbon source to support metabolic activity. The use of storage carbohydrates in response to high respiration activity has been reported (Gomez *et al.*, 1998). The decrease in the carbohydrate level is accompanied by a decrease in the dry solid content of the algae. Although the protein level also declines, its change is very small compared to carbohydrates and hence, the proportion of proteins calculated on the dry weight basis remains fairly high.

All the marine algae except *Enteromorpha intestinalis* and *Grateloupia filicina* recorded higher protein contents in the summer monsoon season compared to that in the late hot season. Protein levels in *G. filicina* were more or less the same in these two seasons, while that in *E. intestinalis* was two times higher in the late hot season. Carbohydrate contents of marine algae also showed higher values in the summer monsoon season except in the case of *Chaetomorpha antennina*, which showed a slightly lower value. Most of the algal species recorded their highest carbohydrate levels in this season. Lipid levels also showed greater values except in the case of *Ulva lactuca* and *E. intestinalis*. The observed increase in the biochemical constituents may be due to an increase in the growth rate of algae resulting from the favourable environmental conditions of the summer monsoon season. The high temperatures observed in the hot season are brought down by the advent of southwest monsoon which gives rise to extensive clouding and rainfall (Menon, 1979). However, enough light is available for a good rate of photosynthesis even when the sky is overcast (Shah, 1973). High levels of nutrients are present in the ambient seawater, which is brought about by an increased land drainage and river run-off during the monsoon rains as well as by the coastal upwelling (Bhattathiri, 1992; Nair *et al*, 1992). Several authors have reported the occurrence of strong upwelling during the whole of the southwest monsoon season all along the west coast of India from Cape Comorin to Karwar (Ramasastry, 1959; Rao, 1973; Rao *et al*., 1973; Subramanyan, 1973; Johannessen, *et al*, 1987) which makes the coastal water rich in nutrients giving high primary production. The phytoplankton production on the west coast of India was found to be maximum during the summer monsoon season (Jones and Banerji, 1973; Rao, 1973; Rao *et al*., 1973; Subramanyan, 1973). It was also found that many species of marine algae occur only in the wet summer monsoon season and several perennial algae resume their growth and form new branches during this season (Luning, 1990). Murthy *et al*. (1978) observed that the northwest coast of India was repopulated with marine algae during the summer monsoon. The growth of marine algae along the west coast of India during the wet summer monsoon season is usually considered reminiscent of the new growth that occurs at temperate latitudes in winter and early spring (Luning, 1990).

The low level of proteins exhibited by *Enteromorpha intestinalis* in the summer monsoon season may be related to the accumulation of large amount of carbohydrates (over 30%). During the growth of algae the synthesis of carbohydrate may be continued at a high rate after the other constituents such as proteins have built up to a constant level, thus increasing the dry solid content. This results in a decrease in the proportion of proteins on dry weight basis. In the present study, *E. intestinalis* showed more than two-fold increase in the carbohydrate content supporting the above argument. The same argument may also be applied to *Grateloupia filicina* from Narakkal, which recorded lower protein content in the summer monsoon compared to that in the late hot season. This species also recorded high levels of carbohydrates (over 30%) in the summer monsoon season. However, in this case the protein synthesis may be taking place at a rate that keeps the proportion of protein on dry weight basis more or less the same as that in the late hot season.

The chlorophyte *Chaetomorpha antennina* contained most of its carbohydrates in the form of fibrillar components of cell walls as suggested by the high level of crude fibre content (over 30%) observed in the present study. The mean level of non-cell wall carbohydrates was only about 12% of the dry weight. The fibrillar components did not show any seasonal variation, while the non-cell wall carbohydrates exhibited notable variations. A slightly lower level of non-cell wall carbohydrates observed in the summer monsoon season compared to that in the hot season may be related to the high protein contents recorded in this season. A high level of protein suggests that a major part of the assimilated carbon were utilized for the synthesis of carbon skeleton of proteins. Proteins were synthesized at a higher rate compared to carbohydrates with the result that the proportion of carbohydrates on dry weight basis showed a decrease, while that of proteins showed an increase.

The low level of lipids shown by *Enteromorpha intestinalis* in the summer monsoon season may be due to the same reason as in the case of its protein level. However, this may not be applicable to *Ulva lactuca* because the increase in the carbohydrate level of this species was very low compared to that in *E. intestinalis*. A three-fold decrease in the lipid levels shown by this species may be due to a probable utilization of available lipids in osmo-regulation (Lobban and Harrison, 1997) during low salinity conditions of summer monsoon season. Another probability is that fat synthesis in *Ulva* may be limited by some enzyme activity as in the case of *Chlorella* spp. (Fogg, 1964).

The early retreating monsoon season is characterized by increasing temperature and light conditions due to the cessation of summer monsoon rains. The nutrient levels become comparatively low due to the weakening of coastal upwelling and decreased land drainage. Since nitrates are highly water soluble, most of it may be leached away from the soil during the initial run-off period (early summer monsoon) and this may result in a decrease in the nitrate concentration during the late summer monsoon even if the river discharge is high (Xavier *et al*, 1999). Also, the high rate of primary production during the summer monsoon may cause depletion in the nutrient levels (Balachandran, 2001). This may be the reason for the lower protein levels shown by most of the algal species in the retreating monsoon season compared to that in the summer monsoon season during the present study. Most of the algal species also showed lower carbohydrate contents, even though light and temperature conditions permitted a good rate of photosynthesis. This suggests a switch from vegetative growth to reproductive development under the prevailing environmental conditions. In the reproductive stage most of the marine algae involve very little growth (Lobban and Harrison, 1997) and during the spore formation stage in reproduction, carbohydrates are used up (Percival and McDowell, 1967). The initiation of reproduction usually takes place in response to some environmental cue. Nitrogen availability has been shown to influence reproduction in some algae. High nitrogen levels favour vegetative growth and low nitrogen levels stimulate sexual reproduction (DeBoer, 1981). Thus, a fall in the nitrogen nutrients at the end of summer monsoon season may be initiating reproduction in algae. The increasing temperature, salinity and light conditions may also have some influence. The decreased turbulence due to the cessation of monsoon rains and the stable conditions of the transition period between the southwest and northeast monsoons may be favouring the settlement of spores or other reproductive structures.

In the present study, most of the algal species recorded an increase in the lipid content on dry weight basis during the retreating monsoon season compared to that in the summer monsoon season. This may be because of the reduction in the levels of proteins and carbohydrates. Under this condition, more photosynthetic intermediates can be utilized in the synthesis of lipid molecules. The proportion of fats was found to increase with decreasing cell nitrogen content (Fogg, 1956). Also, the reproductive stages of algae were found to contain high levels of fats (Miller, 1962). The increasing salinity and light conditions may also favour the production of fats (Fogg, 1956; Ganesan *et al.*, 1999).

In the winter monsoon season, the light, temperature and nutrient conditions are favourable for a second peak in the algal growth. Nutrients are brought to the coastal waters by the increased land drainage and river run-off during the late retreating monsoon season, which is the secondary rainy season of Kerala called "Thulavarsha" (Menon, 1979). Nutrients are also regenerated by the decomposition of dead matter. These conditions favour the active growth of algae and a peak in growth have been observed for many macroalgae in this season (Rao, 1972 & 1975). Phytoplankton also exhibits a peak in the primary production during this season (Rao, 1973). In the present study, all the algal species recorded higher protein contents during the early winter monsoon compared to that in the retreating monsoon season. Most of them recorded increase in the carbohydrate level as well. This may be attributed to an expected increase in the growth rate of algae. A slight decrease in the carbohydrate content on the dry weight basis shown by the rhodophyceae of Narakkal may be due to the large increase in their protein contents. Lipid levels on dry weight basis recorded a decrease in the early winter monsoon season. This may be due to an increase in the dry solid content accompanying the increase in protein and carbohydrate levels.

The period from late winter monsoon to mid hot season records progressively increasing temperature and light conditions due to the near clear sky and meagre rainfall (Rao *et al.*, 1973; Menon, 1979). The increase in temperature and the minimum input of freshwater from land increases the salinity of coastal waters (Rao *et al.*, 1973). The high temperature increases all the bacterial processes thereby increasing the active regeneration of nutrients (Nair *et al*, 1973). As a result the marine algae can continue their growth. However, the increase in temperature results in an increased rate of respiration with a consequent reduction in the net photosynthesis. This may be the reason for the slightly lower carbohydrate and protein levels observed during this period compared to those in the early winter monsoon season. All the algal species recorded comparatively high levels of lipids during this period. This may be attributed to various environmental factors. The comparatively high salinity of the season may have an influence on the fat synthesis. Ganesan *et al.* (1999) has observed an increased fat synthesis under the high salinity conditions. Fat production is also stimulated by light (Fogg, 1956). High fat contents may also be resulted from water deficiency induced in the living cells during the exposure to atmosphere in the dry season (Collyer and Fogg, 1955).

Seasonal variations in the polysaccharide fraction of the total carbohydrates of marine algae were found to follow more or less the same trend as the total carbohydrates. This may be due to the fact that polysaccharides, as in the case of higher plants, constitute most of the carbohydrates in marine algae. In the present study, polysaccharides of most of the algae constituted more than 80% of the total carbohydrates. As with the total carbohydrates, spatial variations in the polysaccharide contents were notable only in rhodophyceae. The average amounts of polysaccharides in the rhodophyceae from Ettikkulam were higher than those from Narakkal in all the seasons. This may be due to the occurrence at Ettikkulam of the species *Gracilaria corticata* that contained high levels of polysaccharides. Chlorophyceae from both Ettikkulam and Narakkal exhibited comparable values.

The low-molecular-weight carbohydrate (LMWC) fraction of the total carbohydrates was found to constitute only a small proportion of the dry weight of algae. These compounds are the principal photosynthetic products in algae. They are used for the synthesis of the functional and structural components of the cells while some go into storage compounds.

They are also used in respiration. The rapid utilization of LMWC results in their low proportion in algae. Comparatively high levels of LMWC observed in *G. corticata* may be due to a faster rate of its synthesis compared to the rate of its utilization. This species also contained high levels of polysaccharides suggesting a high rate of photosynthesis. This polysaccharide fraction was mainly constituted by the phycocolloid, agar. The lowest LMWC content in *G. corticata* was observed in the hot season (April). This may be due to the utilization of more low-molecular-weight photosynthetic products in the synthesis of lipids and proteins. The highest level of lipids in this species was recorded during this period and protein level was also fairly high. Due to the comparatively high temperatures of the season, respiration was also high.

Most of the algal species recorded relatively high levels of LMWC in the late hot season. This may be due to the observed reduction in the synthesis of proteins and lipids under the prevailing environmental conditions of the season, resulting in the accumulation of more LMWC. Also, a reduction in the dry solid content increases the proportion of LMWC on the dry weight basis. Majority of the algal species recorded relatively low levels of LMWC in the summer monsoon season. This may be due to the rapid utilization of these compounds in the synthesis of macromolecules during the active growth of algae. However, during the winter growth, most of the algae recorded relatively high levels of LMWC. Due to the more favourable light conditions in the winter monsoon season than in the summer monsoon (when the irradiance is comparatively low due to cloud cover) photosynthetic rate may be higher in the winter monsoon. As a result, the algae may be synthesising LMWC at a faster rate than the rate of its utilization. This may be the reason for the observed high levels of LMWC in winter monsoon.

The crude fibre content of marine algae did not exhibit any notable seasonal variation in the present study. It also showed little spatial variations. However, marked variations were observed between some algal species. The crude fibre contents of different species of marine algae were found to vary in the range 4.91 – 36.61%. The chlorophyte *Chaetomorpha antennina* showed fibre content above 30% while all the other algal species recorded fibre contents below 12%. The chlorophyceae members *Enteromorpha intestinalis* and *Ulva lactuca* contained fibres below 7%. Qari and Qasim (1993) also obtained the highest fibre content in *C. antennina* among different algal species from Karachi coast. They recorded fibre contents in the range 2.20 – 18.85% in different species of algae. They observed an average fibre content of 4.45% in *U. fasciata*, while in the present study *U. lactuca* showed an average fibre content of 5.95%. As in the case of present study, the marine algae from Karachi coast were also found to show comparable values of crude fibre between different locations. Indergaar and Knutsen (1990) studying the seasonal difference in crude fibre content of the rhodophyte *Furcellaria lumbricalis* obtained values in a narrow range of 3.4 – 4.9% of dry weight suggesting little seasonal variations.

The crude fibre of marine algae is generally considered to consist of the materials that constitute the basic structure of algal cell walls (Larsen, 1978). In some algae, this material consists almost entirely of cellulose (cellulosic group of algae), while some others contain a xylan or a mannan also (Percival and McDowell, 1967). Cellulose is generally present in the cell walls as aggregates of microfibrils embedded in an encrusting material. Xylans also form microfibrils, while mannans form short rods. In higher plants, the encrusting materials are classified as hemicellulose, pectic substances, gums and mucilages, while in algae they are generally grouped together as mucilages and are usually constituted by sulphated polysaccharides (Percival and McDowell, 1967). The cellulose of algal cell walls can exist in different physical forms. Highly crystalline cellulose that gives a characteristic well-defined X-ray diffraction pattern is known as cellulose-I. It yields only glucose on hydrolysis. The treatment of cellulose with alkali alters the X-ray diffraction pattern and gives cellulose-II (mercerised cellulose), which has a lower crystallinity than cellulose-I.

The chlorophyte *Chaetomorpha antennina* belongs to the cellulosic group of algae and the hydrolysis of its microfibrils yields only glucose as is to be expected if only cellulose is present (Lobban and Harrison, 1997). The high levels of crude fibre content observed in this species during the present study may be due to the abundance of highly crystalline cellulose-I in the form of thick microfibrils in the cell walls (McCandless, 1981; Percival and McDowell, 1967). The cell walls of *Enteromorpha intestinalis* and *Ulva lactuca* contain cellulose in type-II form that is closely associated with an equal amount of xylan (Cronshaw *et al.*, 1958). Here, cellulose is supposed to form the central core of microfibrils (Dennis and Preston, 1961). The xylan – glucan microfibrils are found to be thinner than the cellulosic microfibrils (Kreger, 1962). The rhodophyceae members also contain microfibrils composed of cellulose with low crystallinity and the content was reported to be very low (McCandless, 1981). The low levels of fibre content exhibited by many algal species especially rhodophyceae members may be due to the abundance of matrix or mucilaginous materials relative to the fibrillar components (Lobban and Harrison, 1997). It has been found that, in rhodophyceae, a larger part of weight of the plant is made up of mucilaginous polysaccharides (Percival and McDowell, 1967).

The energy contents of marine algae expressed as their calorific values were observed to show considerable seasonal variations during the present study. Interspecies and interclass variations were notable for algae from Ettikkulam. However, spatial variations were not much pronounced. The calorific values of different species of marine algae varied in the range 1.19 – 2.43 kcal g^{-1} dry weight. The observed values were comparable with those reported by Saji (1991) for the marine algae from other regions of Kerala coast. However, the values were lower than the caloric contents of marine algae from other coastal regions like Goa, Maharashtra and Karachi (Sumitra *et al.*, 1975 & 1980; Rajagopal *et al.*, 1976; Dhargalkar *et al.*, 1980; Qari and Qasim, 1993). In the present study, highest energy content (2.43 kcal g^{-1}) was exhibited by *Centroceras clavulatum* collected from Narakkal in the early winter monsoon. This was due to the presence of high levels of proteins which was the highest protein content recorded during the present study. The lowest calorific value (1.19 kcal g^{-1}) shown by *Chaetomorpha antennina* collected from Ettikkulam in the retreating monsoon was due to the low levels of proteins and carbohydrates. *Centroceras clavulatum* collected from Ettikkulam in the late hot season also showed low energy content (1.23 kcal g^{-1}) and this was due to the low levels of proteins and lipids.

During the present study, the average energy content of different algal species varied in the range 1.47 – 2.12 kcal g^{-1} with *Chaetomorpha antennina* showing the lowest and *Gracilaria corticata* showing the highest values. Saji (1991) reported caloric contents in the range1.05 – 2.24 kcal g^{-1} for the algal species same as those selected for the present study. She also reported the lowest caloric content in *C. antennina.* Although this species recorded highest lipid levels during the present study, its energy content was found to be the lowest. This may be due to its low levels of carbohydrates (mean = 12.19%). All other species recorded mean values above 20% for carbohydrates. Protein levels of *C. antennina* were also moderate (mean = 13.69%). The high energy content in *G. corticata* may be due to its high level of carbohydrates (34%). Sumitra *et al.* (1975) also obtained highest caloric content in *G. corticata* among different algal species from Goa coast. They reported caloric contents of marine algae varying in the range 1.90 – 4.05 kcal g^{-1} dry weight, while Rajagopal *et al.* (1976) recorded a range of 2.86 – 7.83 kcal g^{-1} ash free dry weight. Qari and Qasim (1993) recorded calorific values above 2 kcal g^{-1} for different species of marine algae from Karachi coast and the values varied in the range 2.0 – 3.6 kcal g^{-1}. Lamare and Wing (2001) studied caloric content of 28 species of New Zealand marine algae and reported values in the range 1.04 – 3.34 kcal g^{-1} dry weight. Himmelman and Carefoot (1975) reported energy contents varying from 2.6 to 3.6 kcal g^{-1} in marine algae from Pacific coast, whereas Paine and Vadas (1969) observed calorific values ranging from 0.65 to 4.56 kcal g^{-1} dry weight. Carefoot (1973) recorded caloric contents in the range 0.77 – 4.61 kcal g^{-1} in marine algae from northeast Pacific coast.

The average energy contents recorded by the chlorophyceae members *Chaetomorpha antennina* and *Ulva lactuca* and the rhodophyte *Grateloupia filicina* (1.49 kcal g^{-1}, 1.83 kcal g^{-1} and 2.03 kcal g^{-1} respectively) from Kerala coast during the present study were comparable to the values reported by Saji (1991) for the same algal species (1.34 kcal g^{-1}, 1.94 kcal g^{-1} and 2.16 kcal g^{-1} respectively). The rhodophyceae members *Gracilaria corticata* and *Centroceras clavulatum* recorded higher values during the present study (2.12 kcal g^{-1} and 1.93 kcal g^{-1} respectively) than those observed by Saji (1991) (1.50 kcal g^{-1} and 1.32 kcal g^{-1} respectively). In the present study, *Enteromorpha intestinalis* showed average caloric content of 1.78 kcal g^{-1} whereas Saji (1991) recorded average caloric content of 1.49 kcal g^{-1} for *E. compressa*. Qari and Qasim (1993) obtained average calorific values of 2.56 kcal g^{-1} for *Chaetomorpha antennina* and 3.17 kcal g^{-1} for *Ulva fasciata* from Karachi coast. Sumitra *et al.* (1975) obtained an average caloric content of 1.90 kcal g^{-1} for *U. fasciata* from Goa coast, which was comparable to the average value shown by *U. lactuca* (1.83 kcal g^{-1}) in the present study. However, Sumitra *et al.* (1980) recorded a minimum caloric content of 2.4 kcal g^{-1} in *U. fasciata* from Goa coast, whereas *U. lactuca* showed a maximum value of 2.19 kcal g^{-1} during the present study. They recorded calorific values above 3 kcal g^{-1} in *Gracilaria corticata,* while the same species showed a maximum of 2.35 kcal g^{-1} in the present study. Paine and Vadas (1969) reported caloric contents above 3 kcal g^{-1} for *U. lactuca, Enteromorpha* sp. and *Grateloupia* sp. from Pacific coast. Carefoot (1973) also obtained a similar result.

Considering the interclass variations, it was found that the average energy content was more in rhodophyceae than in chlorophyceae. The former showed an average calorific value above 2 kcal g^{-1}, while the latter showed average caloric content less than 2 kcal g^{-1}. However, the variations were statistically significant at Ettikkulam only ($P<0.05$). The higher energy content of rhodophyceae from this location may be due to the high level of carbohydrates observed in them. Saji (1991) observed higher caloric content in rhodophyceae than in chlorophyceae from central Kerala, whereas a reverse case was observed at northern stations. Dhargalkar *et al.* (1980) showed that rhodophyceae from Maharashtra coast contained more energy than chlorophyceae. Sumitra *et al.* (1975) also obtained a similar result for the marine algae from Goa coast. They recorded mean caloric contents of 1.90 kcal g^{-1} in chlorophyceae and 3.36 kcal g^{-1} in rhodophyceae.

Notable spatial variations were not observed in the calorific values of marine algae during the present study. In the case of chlorophyceae members, the comparable values of caloric contents observed at Ettikkulam and Narakkal were due to the comparable biochemical composition of the algae from the two locations. The chlorophyceae recorded comparable values of proteins, carbohydrates and lipids between the two locations. Although rhodophyceae members did not show any spatial variation in the lipid levels, considerable variations were observed in protein and carbohydrate levels. However, energy content remained almost the same at both the locations. This may be due to the compensating effects of relative changes in carbohydrates and proteins (Paine and Vadas, 1969). Protein levels were higher in rhodophyceae from Narakkal, while carbohydrate levels were higher at Ettikkulam resulting in comparable values of caloric contents at the two locations. Data collected by Saji (1991) also showed that caloric contents of marine algae from Kerala did not show any notable spatial variation. A similar observation was made by Qari and Qasim (1993) in the marine algae from Karachi coast.

A consideration of temporal variations showed that in general, the energy contents of marine algae from Kerala were high in the summer and winter monsoon seasons and low in the late hot season or retreating monsoon season. Fairly high values were observed in the early hot season. This may be explained by considering the levels of the major biochemical constituents such as proteins, carbohydrates and lipids in the marine algae during different seasons. The high caloric contents during the summer and winter monsoon seasons were due to high levels of protein and carbohydrates accumulated during the active growth of algae. Fairly high calorific values of algae during the early hot season were resulted from fairly high levels of proteins, carbohydrates and lipids. Low energy contents observed in the marine algae from Ettikkulam during the late hot season were due to low levels of proteins and lipids while at Narakkal, low calorific values were resulted from low levels of carbohydrate and lipids. Low caloric contents exhibited by some algal species during the retreating monsoon season were mainly due to the low levels of proteins.

In conclusion, marine algae from Kerala coast were observed to show variations in their biochemical compositions and energy contents. They showed interspecies and interclass variations in the levels of major biochemical constituents such as proteins, carbohydrates, lipids and fibres as well as in their caloric contents. Spatial variations were notable only for the protein and carbohydrate (LMWC, polysaccharides and total carbohydrates) contents of rhodophyceae members. All the algal species studied were found to exhibit considerable temporal variations in their biochemical compositions. Protein and carbohydrate levels were high in the summer monsoon and early winter monsoon seasons. Lipid levels were higher in the period from mid winter monsoon to mid hot season compared to other seasons. Biochemical constituents recorded comparatively low levels in the late hot season. Crude fibre contents of algae did not show any notable seasonal variations. Calorific values were high in the summer monsoon and early winter monsoon seasons and low in late hot seasons. Thus, with respect to the biochemical composition and calorific value, the best seasons for harvest of algae for food purposes appears to be summer monsoon and early winter monsoon seasons while the least suitable period is the late hot season. Among the different species of marine algae studied, C*entroceras clavula-*

tum, Grateloupia filicina, Gracilaria corticata and *Ulva lactuca* were found to have potential as food sources. *C. clavulatum* and *G. filicina* from Narakkal and *G. corticata* and *G. filicina* from Ettikkulam recorded calorific values greater than 2 kcal g^{-1}. The species from Narakkal showed high levels of proteins (over 15%) and carbohydrates (over 20%) in most seasons. They also contained comparatively good levels of lipids and crude fibre. Thus, they can serve as sources of major biochemical constituents and hence can be considered for use as food item. *G. filicina* from Ettikkulam, which was obtained only in the retreating monsoon season, also contained fairly good levels of major biochemical constituents and hence can serve as a food source. However, *C. clavulatum* from this location exhibited comparatively low values for mean caloric content (1.64 kcal g^{-1}) and protein levels. *G. corticata* from Ettikkulam showed mean calorific value of 2.12 kcal g^{-1} which was mainly due to its high carbohydrate content (over 30%). However, a greater proportion of this carbohydrate is possibly constituted by mucilaginous polysaccharides with low nutritive value. This species also contained low levels of proteins (≈ 10%). Thus, *G. corticata* appears to be less nutritive than *C. clavulatum* and *G. filicina.* The chlorophyte *U. lactuca* collected from Ettikkulam recorded mean calorific value of 1.83 kcal g^{-1}. It contained fairly good levels of proteins (mean = 14%), carbohydrates (22%) and lipids (1.5%). This species may also be used as a food source. Although *Enteromorpha intestinalis* showed calorific values comparable to *U. lactuca,* its protein, lipid and crude fibre levels were comparatively low. Thus, this species appears to be inferior to *U. lactuca* as a food source. *Chaetomorpha antennina* with very high levels of crude fibre showed the lowest calorific value (1.5 kcal g^{-1}) and hence least suitable for food purposes.

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Chapter 4

TRACE MINERALS

4.1 Introduction

The use of marine macroalgae as livestock feed and fertilizer is a common practice in many parts of the world. The principal value of algae in such uses lies in their richness of minerals especially trace elements. Marine algae have been found to contain almost all the essential elements required for the development of land plants as well as higher animals. These include C, H, O, N, P, S, Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Co, V, Mo, Si, Se, Cl, I etc. The edible macroalgae are also found to be useful dietary sources of many of the elements required by humans including Na, K, Ca, Mg and P which are required in large quantities (macro minerals); Fe, Mn, I and Cl needed in smaller amounts; and Cu, Zn, Mo, Co, Ni etc. needed only in trace amounts.

Marine algae have been reported to contain more than sixty elements (Silas *et al.*, 1987). Of these, about 21 elements are essential for the metabolic processes in algae, whereas others do not find any important metabolic roles. There is good evidence that C, H, O, N, P, S, K, Ca, Mg, Fe, Cu, Zn, Mn and Mo are required by all algae, although S, K and Ca can be replaced to some extent by other elements. In addition, Na, Co, V, Si, B, Cl and I are probably required by one or more algae (O'Kelley, 1974; DeBoer, 1981). Many of these essential elements are taken up by the marine algae from the surrounding seawater in excess of their requirements and several other elements are taken up but not utilized. Generally, essential as well as non-essential elements are accumulated in the tissues of algae to concentrations above their levels in seawater, giving rise to concentration factors of several orders of magnitude. The elements such as C, H, O, N, P, S, Na, K, Ca and Mg are generally present in marine algae in high amounts. They are known as macro minerals, whereas the other elements, which are present in relatively low levels, are called micro minerals or trace elements.

Since the marine algae grow in a medium containing most of the known chemical elements, they probably contain at least minute traces of all the elements present in their environment. Thus, in addition to the essential elements, marine algae may contain some toxic heavy metals also. These metals may enter the higher trophic levels through direct consumption of marine algae or stepwise transmission through the food web via secondary and tertiary producers. Thus, in the context of use of marine macroalgae as human food material, livestock feed and fish feed, the investigations on the heavy metal contents of marine algae are of utmost importance. The analysis of heavy metal contents in marine algae also finds use as an alternative for metal pollution monitoring in coastal environments (Lobban and Harrison, 1997). Because of their ability to concentrate metals from dilute aqueous solutions, marine algae have great potential as biological monitors. The variations in the concentrations of metals in algae often are taken to reflect the metal concentration in the surrounding water. The rationale for using marine macroalgae as indicators of metal pollution has three main bases (Luoma *et al.*, 1982). The first is that metal concentrations in solution often are near the limits of analytical detection and may be variable with time. Marine algae concentrate metals from solution and integrate short-term temporal fluctuations in concentrations. Second, empirical methods for distinguishing the biologically available fractions of the total concentration of a dissolved metal have not been developed for natural systems. By definition, marine algae will accumulate only those metals that are biologically available. Finally, because algae do not ingest particulate-bound metals, they will accumulate metals only from solution. One drawback with the use of macroalgae as bioindicators is that any factor that tends to alter their growth rate, such as light intensity, temperature etc., can alter their effectiveness as bioindicators (Phillips, 1990). Another problem is that of contamination by fine particles adhering to the surface of the algae. However, despite these problems, the use of macroalgae as bioindicators is one of the best options available when information about the bioavailability of metals is required. The accumulation of metals by algae could also be applied to biological control of metals in natural waters or in industrially polluted waters (Greene and Bedell, 1990). Algae can also be used for the recovery of heavy metals from industrial effluents or process waters. However, algae used for these purposes are usually freshwater forms rather than marine algae.

The accumulation of metals by marine macroalgae occurring in different parts of the world has been discussed by various authors. One of the early qualitative spectroscopic analysis of metals in marine algae was carried out by Cornec (1919) who found traces of metals such as Ag, As, Co, Cu, Mn, Ni, Zn, Bi, Sn, Ga, Mo, Au, Sb, Ge, Ti, W and V. Webb (1937) provided values for Na, K, Ca, Mg, Sr, Ba, B, Al, Mn, Fe, Cu and Pb in marine algae. Rare elements such as Ra, Pr, Nd and Sm have also been observed in marine algae (Weisner, 1938; Servigne and Tchakirian, 1939). Oy (1940) studied the physiological importance and the contents of Fe, Cu, Mn and B in several species of marine algae. Wilson and Fieldes (1941) determined the amount of eighteen trace elements in marine algae. Trace elements in the common Scottish marine algae were analyzed by Black and Mitchell (1952). Wort (1955) examined the elemental composition of marine algae of British Columbia coastal waters. Young and Langille (1958) carried out a survey of the inorganic elements in the marine algae of the Atlantic coast of Canada. The Sr content of marine algae was determined by Yamamota *et al.* (1969). Yoshimura and Oishi (1973 a) studied the distribution of metallic ions in marine algae from Japan. Fuge and James (1973) reported the trace element concentration of marine algae from Cardigan Bay, Wales. Bryan and Hummerstone (1973) studied the use of marine algae as indicator of heavy metals in estuaries in southwest England. The trace metal concentration in marine algae from Bristol Channel was determined by Fuge and James (1974). Haug *et al.* (1974) estimated the heavy metal pollution in Norwegian fjord areas by the analysis of algae. The uptake of Zn and Sr by algae was studied by Skipnes *et al.* (1975). Morris and Bale (1975) reported the accumulation of Cd, Cu, Mn and Zn by macroalgae in Bristol Channel. Foster (1976) determined the levels of polyvalent metals in sixteen species of marine algae from Vostok Bay, Sea of Japan. Melhuus *et al.* (1978) carried out a study on the use of benthic algae as biological indicators of heavy metal pollution in Norway. Sivalingam (1978) estimated the trace metal content of some tropical marine algae. Khristoforova and Bogdanova (1980) studied the mineral composition of marine algae from coral islands of the Pacific Ocean as a function of environmental conditions. Markham *et al.* (1980) determined the effects of Cd on growth and physiology of the marine alga *Ulva lactuca*. The seasonal variation in the levels of major and minor elements in the marine algae from Canada was reported by Whyte and Englar (1980 a). Mercury contamination in tropical algal species of Malaysia was studied by Sivalingam (1980). Krupina (1981) carried out studies on the use of marine algae for monitoring marine pollution by heavy metals. Rice and Lapointe (1981) studied the trace metal chemistry of the chlorophyte *Ulva fasciata*. Burdon-Jones *et al.* (1982) determined the regional and seasonal variations of trace metals in tropical marine algae from North Queensland. Cullinane and Whelan (1982) reported Cu, Cd and Zn contents of marine algae from the south coast of Ireland. Wong *et al.* (1982) determined heavy metals in the chlorophyte *Ulva lactuca* from Tolo Harbour and studied its use as a biomonitor in Hong Kong waters. The accumulation of Zn, Mn and Co in marine algae from the Adriatic Sea was studied by Munda (1984). Wahbeh *et al.* (1985) estimated the concentrations of Zn, Mn, Cu, Cd, Mg and Fe in marine algae from Jordan. Barnett and Ashcroft (1985) reported the heavy metal content of marine algae from Humber estuary. Lacerda *et al.* (1985) studied the variations in the concentration of heavy metals such as Cu, Cr, Cd, Co, Zn and Pb in benthic algae from Brazil. The seasonal variation in the accumulation of Fe, Co and Cu in the marine algae from Greece was determined by Sawidis and Voulgaropoulos (1986). Ho (1987) estimated the metal content of intertidal macroalgae from Hong Kong. The metal content of marine algae from Stockholm was reported by Forsberg *et al.* (1988). Turkan *et al.* (1989) examined the heavy metal accumulation by the macroalgae in the Bay of Izmir, Turkey. Ho (1990) studied the use of *Ulva lactuca* as an indicator of contamination by metals such as Mn, Fe, Cu, Zn and Pb in intertidal waters in Hong Kong. The heavy metal content of macroalgae from Cuba was determined by Ramirez *et al.* (1990). Say *et al.* (1990) studied the use of *Enteromorpha* as a monitor of heavy metals. Constantini *et al.* (1991) evaluated Hg, Cd and Pb levels in marine algae. Munda and Hudnik (1991) determined the trace metal content in marine algae from the northern Adriatic. The uptake of metals by Black Sea algae including *Chaetomorpha*, *Ulva* and *Gracilaria* was studied by Guven *et al.* (1992). Catsiki and Papathanassiou (1993) studied the use of *Ulva lactuca* as indicator of metal pollution. Barreiro *et al* (1993) carried out studies on the heavy metal accumulation in marine algae of northwest Spain. Kucuksezgin and Balci (1994) determined heavy metal contamination in marine algae such as *Ulva*, *Gracilaria* etc. collected from Izmir Bay in Turkey. Karez *et al* (1994) examined the accumulation of trace metals by algae from Brazil. Malea *et al* (1995) estimated the metal content of marine macroalgae from Greece. The heavy metal accumulation pattern in selected algal species of Malaysia was studied by Murugadas *et al.* (1995). Seferlis and Haritonidis (1995) examined the accumulation of Zn, Pb, Cd, Cu and Fe under different salinities and determined the heavy metal burden in the chlorophyte *Ulva rigida*. Gnassia-Barelli *et al.* (1995) studied the heavy metal distribution in marine algae from the northwestern Mediterranean. The seasonal and local variations of Cr, Ni and Co concentrations in the marine algae *Ulva rigida* and *Enteromorpha linza* from Greece were determined by Haritonidis and Malea (1995). Riget *et al.* (1995) reported the seasonal variation of Cd, Cu, Pb and Zn in marine algae. Vasquez and Guerra (1996) carried out studies on the use of marine algae as bioindicators of natural and anthropogenic contaminants in northern Chile. Riget *et al.* (1997) determined the baseline levels and natural variability of elements in marine algae from west Greenland. The suitability of marine algae such as *Enteromorpha* and *Porphyra* for biomonitoring of Cu, Cd, Pb and Hg in the Oporto coastal waters was evaluated by Leal *et al.* (1997). Hardisson *et al.* (1998) estimated the level of Hg in algae of Canary Islands. Haritonidis and Malea (1999) studied the bioaccumulation of metals by the chlorophyte *Ulva rigida* and Fityanos *et al.* (1999) studied the use of *Ulva* and *Enteromorpha* as biological indicators of heavy metal pollution in Thermaikos Gulf in Greece. Wang and Dei (1999) reported the metal accumulation in marine macroalgae. Muse *et al.* (1999) studied the use of marine algae in the assessment of heavy metal pollution in Argentina. Stengel and Dring (2000) determined Cu and Fe concentrations in marine algae from sites in Ireland. Klimmek *et al* (2001) evaluated the capacity of algae to accumulate heavy metals such as Cd, Ni, Pb and Zn. The effects of nutrients on the metal accumulation in the chlorophyte *Ulva fasciata* from Hong Kong was studied by Lee and Wang (2001). Sanchez-Rodriguez *et al.* (2001) determined the concentration levels of trace metals in different species of macroalgae from Mexico. Vasconcelos and Leal (2001) reported the seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae such as *Enteromorpha.* Villares *et al.* (2001) studied the use of *Ulva* and *Enteromorpha* as indicators of pollution caused by metals such as Al, Cu, Fe, Mn, Ni and Zn in coastal waters of northwest Spain. Barreiro *et al.* (2002) determined the levels of Cu, Cr, Mn, Zn, Fe and Al in marine algae from northwest Spain. Seasonal variation and background levels of these metals were studied by Villares *et al.* (2002). Ruperez (2002) estimated the mineral content of edible macroalgae of Spain. The levels of essential and potentially toxic trace metals in marine algae from Antarctica were determined by Farias *et al.* (2002). Lozano *et al.* (2003) estimated the levels of Pb and Cd in marine algae from Canary Islands.

Many studies have been made on the mineral distribution of Indian marine algae also. However, most of these studies are confined to a few coastal regions like Gujarat, Maharashtra, Goa, Mandapam etc. Information on the elemental composition of marine algae of southwest coast of India is rather meager. One of the early studies on the mineral composition of Indian marine algae was carried out by Pillai (1956) on the algae growing in the vicinity of Mandapam. Rao and Tipnis (1967) studied the heavy metal content of marine algae from Gujarat coast. Information regarding the naturally occurring radioactive substances in marine algae was given by Unni (1967). Langalia *et al.* (1967) determined the alkali metal contents of marine algae. The elemental composition of marine algae growing on Visakhapatnam coast was analyzed by Tewari *et al.* (1968). Dhandhukia and Seshadri (1969) estimated arsenic content of marine algae. Joshi and Gowda (1975) reported seasonal variation in metals such as Na, K, Mg and Ca in marine algae from Ratnagiri coast. Zingde *et al.* (1976) studied the distribution of trace elements such as As, Cu, Zn and Mn in marine algae of Goa. Mehta and Baxi (1976) determined the mineral constituents of marine algae from Okha coast. Parekh *et al.* (1977) reported the variation in the mineral content of marine algae from Saurashtra coast. Agadi *et al.* (1978) analyzed seventeen species of marine algae from Goa for the levels of metals such as Co, Cu, Fe, Mn, Ni, Pb and Zn. All these metals showed considerable variations in their concentration. The distribution of metals such as Na, K, Mg, Ca, Mn, Zn, Cu, Ni, Co, Cr, Pb and Cd in marine algae from Saurashtra coast was studied by Rao and Indusekhar (1986, 1987 & 1989). Ganesan *et al.* (1991) studied the trace metal distribution in marine algae from Gulf of Mannar. Rajendran *et al.* (1993) determined the levels of trace metals such as Cu, Fe, Mn and Zn in some Indian marine algae. Rao *et al.* (1995) studied the distribution of trace metals in marine algae from Visakhapatnam coast. Ganesan and Kannan (1995) estimated the Fe and Mn concentrations in marine algae from Tuticorin coast. The studies on the elemental composition of Indian marine algae have been reviewed by Rao (1992).

As with the metal content of marine algae, several studies have been made on their halogen content especially iodine which is considered to be an essential mineral for many organisms. The deficiency of iodine in human and animal organisms has been found to retard the synthesis of thyroxin, which leads to the generation of an endemic goiter. As marine algae have the ability to accumulate iodine from seawater, many of them have become natural sources of iodine and they can meet the dietary requirement of iodine. Thus, diseases like goiter are less prevalent in countries where marine algae form part of the diet. Iodine occurring in the marine algae is in the readily available form and has been found to be superior to iodine obtained from mineral rocks (Thivy, 1960). Iodine extracted from marine algae also finds use in the preparation of iodine chemicals used in pharmaceuticals, chemical industry and photographic purposes.

Iodine, as an element was first isolated from marine algae by Courtois in 1812. Then, several countries started producing iodine from marine algae. Although world's present requirement of iodine is largely met from the mother liquor of Chile saltpetre, iodine is still produced in small quantities from marine algae in many countries. In India, where more than 250 tons of iodine is imported annually, there is growing interest to search for iodine in marine algae. In addition to iodine, marine algae contain other halogens such as chlorine, bromine and fluorine. Among the different halogens, chlorine has been shown to be the major halogen in most, if not all algae. The concentration levels of halogens and their variations in different species of marine algae have been reported from different parts of the world. Kongisser (1931) and Trofimov (1938) carried out studies on the iodine content of marine algae. Seasonal variation in the halogen content of marine algae from Scotland was studied by Black (1949). Black (1950) estimated iodine content of the common British marine algae. Vinogradov (1953) reported halogen contents of marine algae. Scott (1954) made observations on the iodine containing amino acids present in marine algae. Bromine compounds of algae were studied by Saito and Ando (1955). Young and Langille (1958) examined the occurrence of halogens in marine algae from Atlantic coast of Canada. Yoshimura and Oishi (1973 b) reported the iodine content of algae from Japan. Fries (1975) studied the requirement of bromine in marine algae. Whyte and Englar (1976) determined halogen content in marine algae. The concentrations of iodine and bromine in marine algae from the Seas of Japan and Okhotsk were examined by Saenko *et al.* (1978). Whyte and Englar (1980 b) studied the seasonal variation in the halogen content of marine algae from Canada.

Many studies have been carried out on the halogen contents of Indian marine algae also. Joseph *et al.* (1948) studied the iodine content of some of the Indian algae. Pillai (1956) determined the seasonal variations in the halogen contents of eleven species of marine algae growing around Mandapam. Kappanna and Rao (1962) estimated the iodine content of marine algae from Gujarat coast. Kappanna and Rao (1963) and Rao and Tipnis (1967) reported chloride contents of marine macroalgae. Dave *et al.* (1967) considered Indian marine algae as a source of iodine. Tewari *et al.* (1968) estimated the chloride contents of marine algae from Visakhapatnam. The iodine content of algae from Saurashtra coast was determined by Dave *et al.* (1969). Dave *et al.* (1973) and Dave and Sharma (1974) studied the iodine content of marine algae from Indian coast. Joshi and Gowda (1975) determined the seasonal variation in the chloride contents of marine algae from Ratnagiri coast. Mehta and Baxi (1976) reported chloride contents of macroalgae from Okha coast. Solimabi and Das (1977) studied the distribution of iodine in marine algae from Coa region. Dhandhukia *et al.* (1977) carried out study on the recovery of iodine from Indian marine algae. The bromine content of algae from Goa coast was determined by Naqvi *et al.* (1979). Solimabi *et al.* (1981) estimated the bromine and iodine contents in marine algae from Andaman Sea. Mairh and Dave (1982) studied seasonal variations in iodine contents of marine algae in relation to their growth and developmental phase. Oza *et al.* (1983) reported chloride content of algae from Okha coast. Rao (1987) estimated the halogen content of marine algae from Saurashtra coast. Thomas *et al.* (1987) and Thomas and Subbaramaiah (1991) determined the seasonal variations in iodine contents of marine algae from Mandapam region. Rao and Indusekhar (1989) studied the distribution of iodine, chlorine and bromine in marine algae from Saurashtra coast. Seasonal variation in halide contents of algae from Porbandar and Okha coast was reported by Rao and Singbal (1995). Devi *et al.* (1996) determined the abundance of iodine in marine algae of Cape Comorin.

As in the case of trace metal composition, studies on the halogen content of marine algae of southwest coast of India especially Kerala are rather scanty. No detailed studies have been carried out so far on the halogen and trace metal contents of marine algae from central and northern coasts of Kerala. The present study provides information on the levels of halogens, trace metals and the total mineral content (ash) of different species of marine macroalgae from two locations on the central and northern coasts of Kerala. The elements quantified include the halogens - iodine and chlorine, and the trace metals - Fe, Zn, Mn, Cu, Co, Cr, Ni, Sr, Ag, Cd and Pb. In addition to the specieswise and classwise variations in the trace mineral content, temporal variations were also studied since such variations can have positive or negative implications on the nutritional value of marine algae. A consideration of seasonal effects is necessary in the selection of harvesting times of algae. The application of marine algae as bioindicators of mineralization and anthropogenic impact of heavy metals will also be benefited from the elucidation of seasonal variability of trace metal composition. The study also incorporates the spatial variation in the elemental composition.

4.2 Results

Mineral constituents such as trace metals and the halogens (iodine and chlorine) were determined in different species of marine algae collected in different seasons from Ettikkulam and Narakkal. Total mineral content (ash) was also determined. The methodologies used for the analyses are detailed in chapter 2. The results are presented in Tables 4.1 – 4.7.

4.2.1 Ash

The ash content represents the total inorganic materials present in algae. It involves inorganic salts, presumably in solution in the cell sap, as well as the metal ions combined with organic constituents. In the present study, ash content of marine algae exhibited a range of 6.36 – 28.13% of dry weight (Table 4.1). The minimum and maximum values were recorded by the members of rhodophyceae collected from Ettikkulam in different periods, *viz.*, *Gracilaria corticata* in summer monsoon season and *Centroceras clavulatum* in the retreating monsoon season respectively. At Narakkal also, minimum and maximum values were shown by the members

Table 4.1. Ash contents (% dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	8.99	11.00	11.53	17.29	17.28	14.58
Ulva lactuca	14.16	12.54	16.49	17.22	14.40	15.37
Enteromorpha intestinalis	17.51	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	6.36	7.08	7.95	9.30	9.22	6.76
Centroceras clavulatum	*	28.13	*	12.65	*	23.69
Grateloupia filicina	*	12.02	*	*	*	*
Average	**11.76**	**14.15**	**11.99**	**14.12**	**13.63**	**15.10**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	12.04	16.68	*	*	*	*
Enteromorpha intestinalis	19.07	*	*	*	*	23.16
Rhodophyceae						
Centroceras clavulatum	12.57	12.74	20.30	21.90	23.53	*
Grateloupia filicina	12.49	10.75	*	*	16.13	*
Average	**14.04**	**13.39**	**20.30**	**21.90**	**19.83**	**23.16**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Species were absent / ash content was not determined.

of rhodophyceae. Here, *Grateloupia filicina* recorded the minimum value (10.75%) in the retreating monsoon season and *Centroceras clavulatum* showed the maximum value (23.53%) in mid hot season. Thus, at both the locations, the rhodophyte *Centroceras clavulatum* recorded the highest ash content. The chlorophyceae members exhibited ash contents in the range 8.99 - 23.16%. The minimum value was shown by *Chaetomorpha antennina* collected from Ettikkulam in summer monsoon season and maximum value by *Enteromorpha intestinalis* collected from Narakkal in the late hot

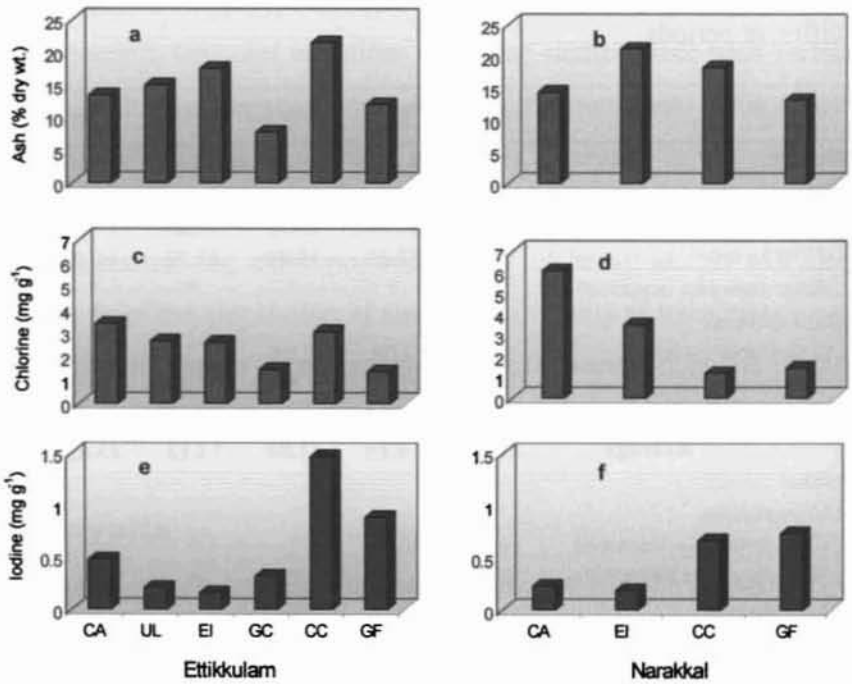

Fig. 4.1. Interspecies variations in ash (a, b), chlorine (c, d) and iodine (e, f) contents of marine algae (CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

season. Thus, it can be seen that both the classes of algae recorded the lowest ash content in the summer monsoon season. At Ettikkulam also, the maximum ash content among the chlorophyceae was shown by *Enteromorpha intestinalis* (17.51% in the summer monsoon season). Similarly, *Chaetomorpha antennina* collected in the summer monsoon season recorded the lowest ash content (12.04%) among the chlorophyceae from Narakkal. Thus, among the chlorophyceae, *Chaetomorpha antennina* recorded the lowest and *Enteromorpha intestinalis* showed the highest ash contents at both the locations.

The ash contents of marine algae showed interspecies variations (Fig. 4.1). The rhodophyte *Gracilaria corticata* recorded ash contents below 10% while all the other species recorded average ash contents above 12%. Among the rhodophyceae from Ettikkulam, *Centroceras clavulatum* showed an average ash content of 21.49%, which was about three times as high as the average ash content of *Gracilaria corticata* (7.78%). *Grateloupia filicina*, which occurred only in the retreating monsoon season recorded ash contents of 12.02%. The chlorophyceae members *Chaetomorpha antennina* and *Ulva lactuca* exhibited average ash contents of 13.45% and 15.03% respectively. *Enteromorpha intestinalis*, which was present only in the summer monsoon season showed an ash content of 17.51%. At Narakkal, *Centroceras clavulatum* recorded average ash content of 18.21%, while *Grateloupia filicina* showed average value of 13.12%. The chlorophyceae members *Chaetomorpha antennina* and *Enteromorpha intestinalis* recorded average ash contents of 14.36% and 21.12% respectively.

The marine algae from both the locations exhibited temporal variations in their ash contents. At Ettikkulam, the average value for all the algal species taken together varied from 11.76% in summer monsoon season to 15.10% in the late hot season (Table 4.1). At Narakkal, the range was 13.39 – 23.16% with minimum recorded in the retreating monsoon season and maximum in the late hot season. The ranges of average ash contents in chlorophyceae were 11.77% (retreating monsoon season) – 17.26% (mid winter monsoon season) at Ettikkulam and 15.56% (summer monsoon season) – 23.16% (late hot season) at Narakkal (Fig. 4.2). Rhodophyceae recorded average ash contents in the range 6.36% (summer monsoon season) – 15.74% (retreating monsoon season) at Ettikkulam and 11.75% (retreating monsoon season) – 21.90% (mid winter monsoon season) at

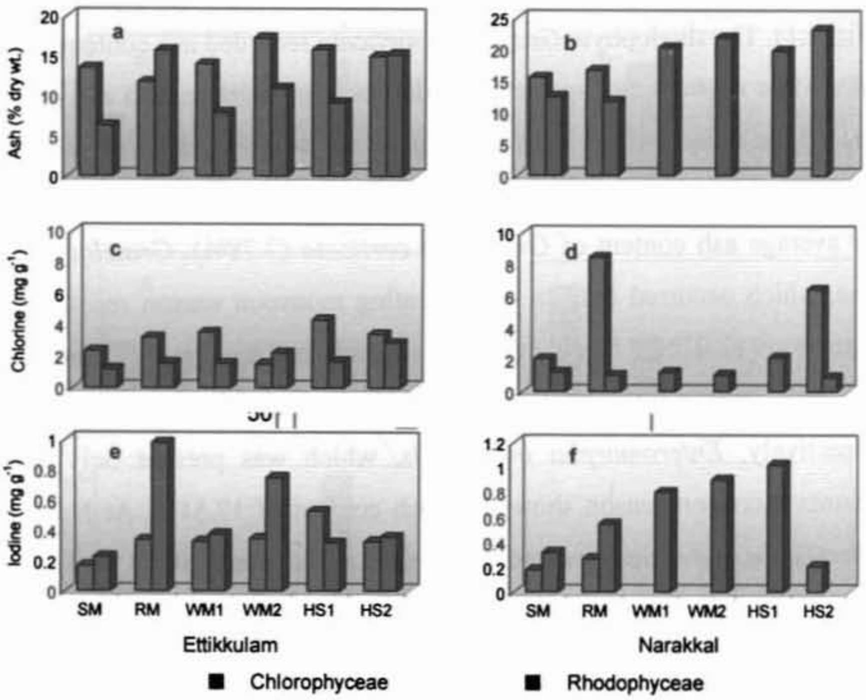

Fig. 4.2. Temporal variations in ash (a, b), chlorine (c, d) and iodine (e, f) contents of different classes of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

Narakkal. A classwise comparison of the average ash content showed that in most seasons, chlorophyceae contained more ash than rhodophyceae (Fig. 4.2). Reverse trend was observed at Ettikkulam in the retreating monsoon and late hot seasons. When ash contents were averaged over the whole study period, chlorophyceae showed higher ash contents than rhodophyceae at both the locations (Fig. 4.3). However, the variation was very small. The values for chlorophyceae and rhodophyceae were 14.49% and 12.32% respectively at Ettikkulam and 17.74% and 16.30% respectively at Narakkal.

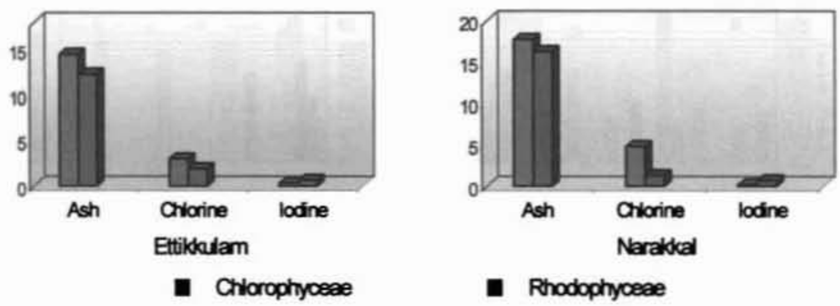

Fig. 4.3. Interclass variations in the mean values of ash (% dry weight), chlorine (mg g^{-1}) and iodine (mg g^{-1}) of marine algae from Kerala coast.

Considering the individual species, most of the algae selected for the present study were observed to show temporal variations in their ash contents (Fig. 4.4). In some cases, the maximum values were roughly twice as high as the minimum values. Among chlorophyceae, *Chaetomorpha antennina* from Ettikkulam recorded ash contents in the range 8.99 – 17.29% with minimum in summer monsoon season and maximum in mid winter monsoon season as well as mid hot season. This species occurred at Narakkal only in the summer monsoon and retreating monsoon seasons and recorded ash contents of 12.04% and 16.68% respectively. *Ulva lactuca* which was obtained only from Ettikkulam, exhibited ash contents varying from 12.54% in the retreating monsoon season to 17.22% in the mid winter monsoon season. *Enteromorpha intestinalis* occurred at Ettikkulam only in the summer monsoon season and it recorded an ash content of 17.51%. This species was present at Narakkal in the summer monsoon and late hot seasons with ash contents of 19.07% and 23.16% respectively. Among rhodophyceae, *Gracilaria corticata* from Ettikkulam exhibited only a slight temporal variation in their ash content. The values varied in a narrow range

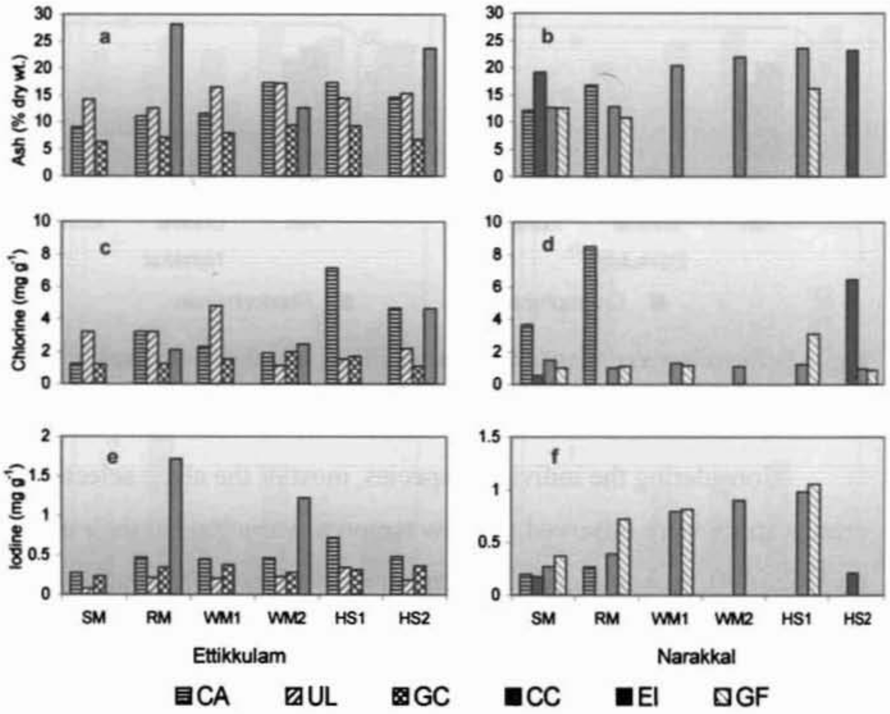

Fig. 4.4. Temporal variations in ash (a, b), chlorine (c, d) and iodine (e, f) contents of different species of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

of 6.36 – 9.30% with minimum in the summer monsoon season and maximum in mid winter monsoon season. The ash content in the mid hot season (9.22%) was close to the highest value. This species was absent from Narakkal. The rhodophyte *Centroceras clavulatum* exhibited considerable temporal variations. At both the locations, maximum value was almost twice as high as the minimum value. At Ettikkulam, minimum ash content recorded was 12.65% in mid winter monsoon season and maximum value

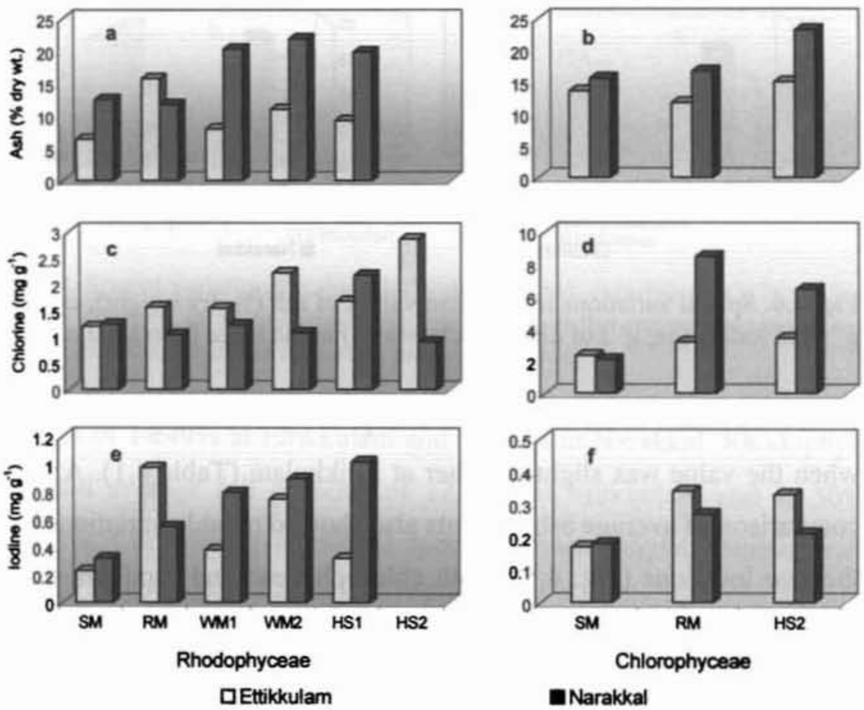

Fig. 4.5. Spatial variations in ash (a, b), chlorine (c, d) and iodine (e, f) contents of different classes of marine algae in different periods (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

was 28.13% recorded in the retreating monsoon season. At Narakkal, values were in the range 12.57% (summer monsoon season) – 23.53% (mid hot season). *Grateloupia filicina* exhibited ash contents varying from 10.75% in the retreating monsoon season to 16.13% in the mid hot season at Narakkal. This species was present at Ettikkulam only in the retreating monsoon season and showed an ash content of 12.02%.

The ash content of marine algae showed considerable spatial variations. The average ash content of algae from Narakkal was higher than that

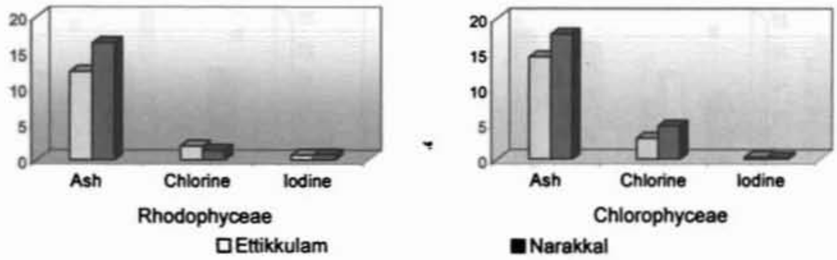

Fig. 4.6. Spatial variations in the mean values of ash (% dry weight), chlorine (mg g^{-1}) and iodine (mg g^{-1}) of different classes of marine algae from Kerala coast.

from Ettikkulam in all the seasons except in the retreating monsoon season when the value was slightly higher at Ettikkulam (Table 4.1). A classwise comparison of average ash contents also showed notable variations between the two locations (Fig. 4.5). Both chlorophyceae and rhodophyceae from Narakkal showed higher ash contents than those from Ettikkulam. The only exception was observed in the retreating monsoon season when rhodophyceae from Ettikkulam showed higher ash contents than those from Narakkal. The ash contents averaged over the whole study period also showed a higher value at Narakkal (Fig. 4.6). Chlorophyceae recorded average ash

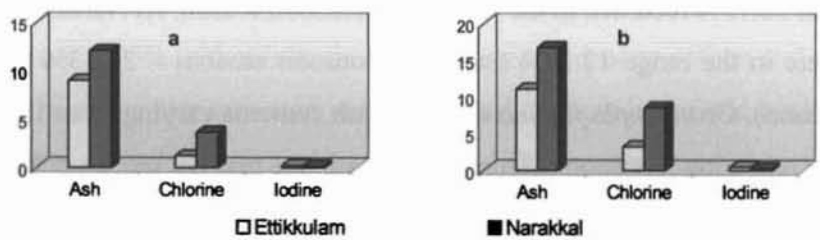

Fig. 4.7. Spatial variations in the ash (% dry weight), chlorine (mg g^{-1}) and iodine (mg g^{-1}) contents of *Chaetomorpha antennina* in a) summer monsoon and b) retreating monsoon seasons.

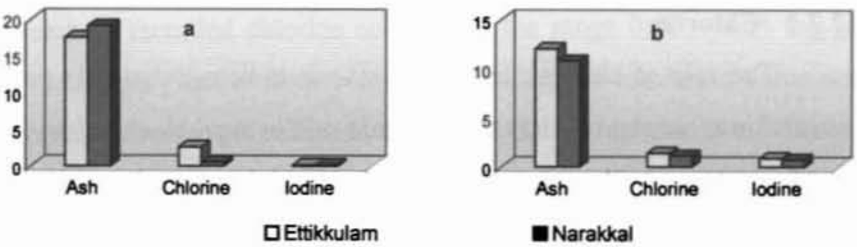

Fig. 4.8. Spatial variations in the ash (% dry weight), chlorine (mg g^{-1}) and iodine (mg g^{-1}) contents of a) *Enteromorpha intestinalis* in the summer monsoon and b) *Grateloupia filicina* in the retreating monsoon season.

contents of 14.49% at Ettikkulam and 17.74% at Narakkal. Rhodophyceae exhibited average ash contents of 12.32% at Ettikkulam and 16.30% at Narakkal. Considering individual species, *Chaetomorpha antennina* (Fig. 4.7) and *Enteromorpha intestinalis* (Fig. 4.8 a) were found to exhibit higher ash contents at Narakkal compared to that at Ettikkulam. *Centroceras clavulatum* (Fig. 4.9) showed a similar trend in the mid winter monsoon season, but a reverse trend was observed in the retreating monsoon season. *Grateloupia filicina* (Fig. 4.8 b) collected in the retreating monsoon season also showed higher ash content at Ettikkulam than at Narakkal.

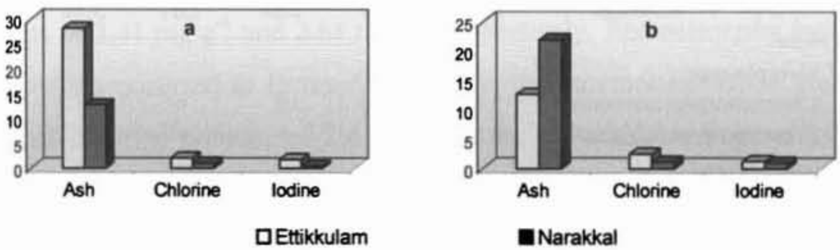

Fig. 4.9. Spatial variations in the ash (% dry weight), chlorine (mg g^{-1}) and iodine (mg g^{-1}) contents of *Centroceras clavulatum* in a) retreating monsoon and b) winter monsoon seasons.

4.2.2 Halogens

4.2.2.1 Chlorine

The role of chlorine in photosynthesis indicates that it is probably essential in most algae. It has an important role in algal biochemistry. In the present study, the chlorine contents of marine algae were observed to vary in the range 0.53 – 8.49 mg g^{-1} dry weight (Table 4.2). The minimum and maximum values recorded by the members of chlorophyceae collected from Narakkal in different periods. The minimum value was shown by *Entero-morpha intestinalis* in the summer monsoon season and maximum value by

Table 4.2. Chlorine contents (mg g^{-1} dry wt.) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	1.24	3.21	2.28	1.91	7.15	4.66
Ulva lactuca	3.19	3.22	4.81	1.13	1.55	2.19
Enteromorpha intestinalis	2.61	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	1.20	1.24	1.55	1.98	1.68	1.09
Centroceras clavulatum	*	2.11	*	2.46	*	4.64
Grateloupia filicina	*	1.37	*	*	*	*
Average	**2.06**	**2.23**	**2.88**	**1.87**	**3.46**	**3.14**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	3.66	8.49	*	*	*	*
Enteromorpha intestinalis	0.53	*	*	*	*	6.46
Rhodophyceae						
Centroceras clavulatum	1.48	1.00	1.31	1.08	1.24	0.96
Grateloupia filicina	1.00	1.11	1.15	*	3.10	0.86
Average	**1.67**	**3.53**	**1.23**	**1.08**	**2.17**	**2.76**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season

* Algal species were absent

Chaetomorpha antennina in the retreating monsoon season. The rhodophyceae members recorded chlorine contents in the range 0.86 – 4.64 mg g^{-1} with *Grateloupia filicina* from Narakkal showing the minimum value and *Centroceras clavulatum* collected from Ettikkulam in the same season showing the maximum value. At Narakkal, maximum value among rhodophyceae was shown by *Grateloupia filicina* in the mid hot season (3.10 mg g^{-1}), while the minimum chlorine content at Ettikkulam was shown by *Gracilaria corticata* in the late hot season (1.09 mg g^{-1}). The chlorophyceae members from Ettikkulam recorded chlorine contents in the range 1.13 – 4.81 mg g^{-1} with minimum and maximum values shown by the same species, *viz.*, *Ulva lactuca* in mid and early winter monsoon seasons respectively.

The chlorine content of algae exhibited species to species variability (Fig. 4.1). Among the rhodophyceae from Ettikkulam, *Centroceras clavulatum* recorded average chlorine content of 3.07 mg g^{-1}, which was about twice as high as the average value recorded by *Gracilaria corticata* (1.46 mg g^{-1}). *Grateloupia filicina*, which occurred only in the retreating monsoon season showed chlorine content of 1.37 mg g^{-1}. The chlorophyceae members *Chaetomorpha antennina* and *Ulva lactuca* showed average chlorine contents of 3.41 mg g^{-1} and 2.68 mg g^{-1} respectively. *Enteromorpha intestinalis*, which occurred at Ettikkulam only in the summer monsoon season recorded chlorine content of 2.61 mg g^{-1}. At Narakkal, average chlorine content of the rhodophyceae members *Centroceras clavulatum* and *Grateloupia filicina* showed only a slight variation. The values were 1.18 mg g^{-1} and 1.44 mg g^{-1} respectively. However, chlorophyceae members showed considerable variation. *Chaetomorpha antennina* recorded average chlorine content of 6.08 mg g^{-1}, while *Enteromorpha intestinalis* showed a value of 3.49 mg g^{-1}.

Chlorine contents of marine algae exhibited notable temporal variations. The average values for the whole algae collected from Ettikkulam ranged from 1.87 mg g^{-1} in the mid winter monsoon season to 3.46 mg g^{-1} in the mid hot season (Table 4.2). At Narakkal, values varied in the range 1.08 (mid winter monsoon) – 3.53 mg g^{-1} (retreating monsoon season). Chlorophyceae recorded average chlorine contents varying in the ranges 1.52 (mid winter monsoon) – 4.35 mg g^{-1} (mid hot season) at Ettikkulam and 2.09 (summer monsoon) – 8.49 mg g^{-1} (retreating monsoon season) at Narakkal (Fig. 4.2). The ranges of average chlorine contents in rhodophyceae were 1.20 (summer monsoon) – 2.86 mg g^{-1} (late hot season) at Ettikkulam and 0.91(late hot season) – 2.17 mg g^{-1} (mid hot season) at Narakkal. A classwise comparison showed that at both the locations, the average chlorine contents of chlorophyceae were higher than rhodophyceae in all the seasons except in the retreating monsoon season when rhodophyceae of Ettikkulam showed higher value for the average chlorine content than chlorophyceae (Fig. 4.2). The chlorine content averaged over the whole study period also showed a similar trend (Fig. 4.3). The values for chlorophyceae and rhodophyceae were 3.01 mg g^{-1} and 1.93 mg g^{-1} respectively at Ettikkulam and 4.78 mg g^{-1} and 1.30 mg g^{-1} respectively at Narakkal.

Considering the temporal variations in the chlorine contents of individual species, members of both chlorophyceae and rhodophyceae were observed to exhibit marked variations (Fig. 4.4). In most cases, maximum values were more than twice the minimum values. Among the chlorophyceae from Ettikkulam, *Chaetomorpha antennina* exhibited chlorine contents in the range 1.24 – 7.15 mg g^{-1} with minimum in the summer monsoon season and maximum in the mid hot season. The chlorine content of *Ulva lactuca* varied from 1.13 mg g^{-1} in mid winter monsoon season to 4.81mg g^{-1} in the early winter monsoon season. *Enteromorpha intestinalis* recorded a chlorine content of 2.61 mg g^{-1} in the summer monsoon season. It was absent from Ettikkulam in other seasons. Among the rhodophyceae, *Gracilaria corticata* did not show much variation in its chlorine content with respect to time. It recorded values varying in the range 1.09 – 1.98 mg g^{-1} with minimum in the late hot season and maximum in mid winter monsoon season. *Centroceras clavulatum* recorded chlorine contents varying from 2.11 mg g^{-1} in the retreating monsoon season to 4.64 mg g^{-1} in the late hot season. *Grateloupia filicina,* which was present at Ettikkulam only in the retreating monsoon season showed chlorine content of 1.37 mg g^{-1}. At Narakkal, the chlorophyte *Chaetomorpha antennina* showed chlorine contents of 3.66 mg g^{-1} in the summer monsoon season and 8.49 mg g^{-1} in the retreating monsoon season. *Enteromorpha intestinalis* recorded 0.53 mg g^{-1} in the summer monsoon season and 6.46 mg g^{-1} in the late hot season. These algal species were absent from Narakkal in other seasons. The rhodophyte *Centroceras clavulatum* exhibited chlorine contents varying in the range 0.96 – 1.48 mg g^{-1} with minimum in the late hot season and maximum in the summer monsoon season while *Grateloupia filicina* recorded values ranging from 0.86 mg g^{-1} in the late hot season to 3.10 mg g^{-1} in mid hot season.

The marine algae were observed to show spatial variations in their chlorine content. The average chlorine contents of algae from Ettikkulam were found to be higher than that from Narakkal in all the seasons except in the retreating monsoon season when reverse was true (Table 4.2). A class-wise comparison showed variable trend (Fig. 4.5). Chlorophyceae from Narakkal showed higher values compared to that from Ettikkulam in the late hot season and in the retreating monsoon season. However, a slightly higher value was observed at Ettikkulam in the summer monsoon season. In other seasons, chlorophyceae members were absent from Narakkal. Rhodophyceae of Narakkal recorded higher value for average chlorine content in the mid hot season and a value comparable to that of rhodophyceae from Ettikkulam in the summer monsoon season. In all the other seasons, rhodophyceae from Ettikkulam recorded higher values for average chlorine content compared to the rhodophyceae from Narakkal. When chlorine contents of marine algae were averaged over the whole study period (Fig. 4.6), chlorophyceae of Narakkal showed higher value (4.78 mg g^{-1}) than that from Ettikkulam (3.01 mg g^{-1}), while a reverse trend was observed with rhodophyceae (Narakkal: 1.30 mg g^{-1} and Ettikkulam: 1.93 mgg^{-1}). Considering individual species, the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* were found to exhibit higher chlorine contents at Ettikkulam than at Narakkal. The chlorophyte *Enteromorpha intestinalis* also showed a similar trend, while *Chaetomorpha antennina* exhibited a reverse trend (Figs. 4.7 – 4.9).

4.2.2.2 Iodine

Iodine appears to be essential for growth, morphogenesis and reproduction of some algae, and many marine algae are strong concentrators of iodine. The iodine contents of marine algae selected for the present study varied in the range 0.08 – 1.72 mg g^{-1} (Table 4.3). The minimum value was shown by the chlorophyte *Ulva lactuca* in the summer monsoon season and the maximum value by the rhodophyte *Centroceras clavulatum* in the retreating monsoon season, both collected from Ettikkulam. Among the chlorophyceae members, maximum iodine content was recorded by *Chaetomorpha antennina* from Ettikkulam in the mid hot season. *Gracilaria*

Table 4.3. Iodine contents (mg g^{-1} dry weight) of different species of marine algae in different periods.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	0.28	0.47	0.45	0.46	0.72	0.49
Ulva lactuca	0.08	0.21	0.20	0.23	0.34	0.18
Enteromorpha intestinalis	0.16	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	0.23	0.34	0.38	0.28	0.32	0.36
Centroceras clavulatum	*	1.72	*	1.22	*	*
Grateloupia filicina	*	0.89	*	*	*	*
Average	**0.19**	**0.73**	**0.34**	**0.55**	**0.46**	**0.34**
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	0.20	0.27	*	*	*	*
Enteromorpha intestinalis	0.17	*	*	*	*	0.21
Rhodophyceae						
Centroceras clavulatum	0.27	0.39	0.79	0.90	0.98	*
Grateloupia filicina	0.37	0.72	0.81	*	1.05	*
Average	**0.25**	**0.46**	**0.80**	**0.90**	**1.02**	**0.21**

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Algal species were absent / iodide content was not determined

corticata collected in the summer monsoon season from Ettikkulam exhibited the lowest iodine content (0.23 mg g^{-1}) among the rhodophyceae members. At Narakkal, iodine contents varied in the range 0.17-1.05 mg g^{-1}, with the chlorophyte *Enteromorpha intestinalis* showing the minimum value in the summer monsoon season and the rhodophyte *Grateloupia filicina* exhibiting the maximum value in the mid hot season. *Chaetomorpha antennina* collected in the retreating monsoon season recorded the highest iodine content (0.27 mg g^{-1}) among the chlorophyceae of Narakkal and *Centroceras clavulatum* collected in the summer monsoon season showed the lowest iodine content among the rhodophyceae. Thus, it can be seen that both chlorophyceae and rhodophyceae recorded lowest iodine contents in the summer monsoon season at both the locations. The highest values were recorded in the mid hot season or in the retreating monsoon season.

Interspecies variability was observed in the iodine content of marine algae during the present study (Fig. 4.1). Among the chlorophyceae, *Chaetomorpha antennina* from Ettikkulam showed average iodine content of 0.48 mg g^{-1} which was more than twice the value recorded by *Ulva lactuca* (0.21 mg g^{-1}). *Enteromorpha intestinalis* recorded iodine content of 0.16 mg g^{-1} in the summer monsoon season and this species was absent in other seasons. Among rhodophyceae of Ettikkulam, *Centroceras clavulatum* recorded average iodine content of 1.47 mg g^{-1} that is more than four times the value shown by *Gracilaria corticata* (0.32 mg g^{-1}). *Grateloupia filicina*, which occurred only in the retreating monsoon season, exhibited iodine content of 0.89 mg g^{-1}. The chlorophyceae members of Narakkal exhibited comparable values for the average iodine contents. *Chaetomorpha antennina* recorded a value of 0.23 mg g^{-1} while *Enteromorpha intestinalis* showed a value of 0.19 mg g^{-1}. The values for rhodophyceae members also did not vary much. *Centroceras clavulatum* recorded average iodine content of 0.67 mg g^{-1}, while *Grateloupia filicina* recorded a value of 0.74 mg g^{-1}.

Iodine contents of marine algae exhibited considerable temporal variations. At Ettikkulam, the average values for all the algal species taken together varied from 0.19 mg g^{-1} in the summer monsoon season to 0.73 mg g^{-1} in the retreating monsoon season and at Narakkal, it ranged from 0.21 mg g^{-1} in the late hot season to 1.02 mg g^{-1} in the mid hot season (Table 4.3). The chlorophyceae of Ettikkulam recorded average iodine contents in the range 0.17 – 0.53 mg g^{-1} with minimum in the summer monsoon season and maximum in the mid hot season (Fig. 4.2). Chlorophyceae, which was absent from Narakkal in the winter monsoon and most of the hot season recorded average iodine contents in the range 0.18 mg g^{-1} (summer monsoon) – 0.27 mg g^{-1} (retreating monsoon season). The rhodophyceae of Ettikkulam exhibited average iodine contents varying from 0.23 mg g^{-1} in the summer monsoon season to 0.98 mg g^{-1} in the retreating monsoon season, while that of Narakkal showed values in the range 0.32 (summer monsoon) – 1.02 mg g^{-1} (mid hot season). At both the locations, rhodophyceae recorded higher iodine contents than chlorophyceae except in the mid hot season when chlorophyceae of Ettikkulam showed higher iodine content than rhodophyceae (Fig. 4.2). The iodine content averaged over the whole study period also showed the same trend (Fig. 4.3). The value for chlorophyceae and rhodophyceae of Ettikkulam were 0.33 mg g^{-1} and 0.64 mg g^{-1} respectively and at Narakkal, values were 0.21 mg g^{-1} and 0.70 mg g^{-1} respectively.

Considering the temporal variation of iodine content in individual species, marked variations were observed in the chlorophyceae of Ettikkulam and rhodophyceae of Narakkal (Fig. 4.4). Rhodophyceae of Ettikkulam and chlorophyceae of Narakkal did not show much temporal variations in their iodine content. Among the chlorophyceae of Ettikkulam, *Chaetomorpha antennina* recorded iodine contents varying in the range 0.28 – 0.72 mg g^{-1} with minimum in the summer monsoon season and maximum in the mid hot season. *Ulva lactuca* showed values ranging from 0.08 – 0.34 mg g^{-1} with minimum and maximum values recorded in the same seasons as in the case of *C. antennina*. *Enteromorpha intestinalis*, which occurred only in the summer monsoon season showed iodine content of 0.16 mg g^{-1}. Rhodophyceae members showed only slight variations in the iodine content.

Gracilaria corticata recorded values ranging from 0.23 (summer monsoon) to 0.38 mg g^{-1} (early winter monsoon season) with most of values varying between 0.32 and 0.38 mg g^{-1}. *Centroceras clavulatum* showed iodine contents of 1.72 mg g^{-1} in the retreating monsoon season and 1.22 mg g^{-1} in the mid winter monsoon season. *Grateloupia filicina* occurred only in the retreating monsoon season with an iodine content of 0.89 mg g^{-1}. Among the rhodophyceae of Narakkal, *Centroceras clavulatum* showed iodine contents varying from 0.27 mg g^{-1} in the summer monsoon season to 0.98 mg g^{-1} in the mid hot season. *Grateloupia filicina* recorded values in the range 0.37 – 1.05 mg g^{-1} with minimum and maximum values in the same season as in the case of *C. clavulatum.* The chlorophyceae members were present at Narakkal only in the period from late hot season to retreating monsoon season and they showed only slight variations in the iodine content during this period. *Chaetomorpha antennina* recorded iodine contents of 0.20 mg g^{-1} in the summer monsoon season and 0.27 mg g^{-1} in the retreating monsoon season. *Enteromorpha intestinalis* recorded iodine contents of 0.21 mg g^{-1} in the late hot season and 0.17 mg g^{-1} in the summer monsoon season. It can be seen that most of the algal species selected for the present study recorded lowest iodine contents in the summer monsoon season and highest values in the mid hot season.

Iodine content of marine algae showed spatial variations in some cases. The average iodine contents of algae from Ettikkulam were found to be higher than that from Narakkal in the late hot season and in the retreating monsoon season, while reverse was true in other seasons (Table 4.3). A classwise comparison also showed variable trend (Fig. 4.5). Rhodophyceae showed higher values for average iodine content at Narakkal than at Ettikkulam except in the retreating monsoon season when reverse was true.

Chlorophyceae exhibited very little spatial variations in their average iodine content with slightly higher values at Ettikkulam in some seasons (late hot season and retreating monsoon season). When the iodine contents of algae were averaged over the whole study period, chlorophyceae as well as rhodophyceae recorded comparable values at the two locations (Fig. 4.6). Considering the individual species, the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* were observed to show higher iodine contents at Ettikkulam than at Narakkal. The chlorophyte *Chaetomorpha antennina* also showed the same trend, while *Enteromorpha intestinalis* recorded comparable values at both the locations in the summer monsoon season (Figs. 4.7 – 4.9).

4.2.3 Trace Metals

4.2.3.1 Iron

Iron is an essential element and all algae have an unquestioned requirement for it. It functions both as a structural component and as a cofactor for enzymatic reactions. Oxidation – reduction reactions are most commonly associated with iron containing systems. In the present study, Fe content of algae varied in the range 180 – 18590 $\mu g\ g^{-1}$ with the chlorophyte *Ulva lactuca* and the rhodophyte *Centroceras clavulatum*, both collected from Ettikkulam in the late hot season, showing the minimum and maximum values respectively (Tables 4.4 and 4.5). *Enteromorpha intestinalis* collected from Narakkal in the same season showed the highest Fe accumulation (7410 $\mu g\ g^{-1}$) among the chlorophytes. *Chaetomorpha antennina* collected in the late hot season showed the highest Fe content (6690 $\mu g\ g^{-1}$) among the chlorophytes of Ettikkulam. *Grateloupia filicina* collected from Narakkal in the retreating monsoon season recorded the least accumulation

Table 4.4. Trace metal content (μg g^{-1} dry wt.) of different species of marine algae from Ettikkulam in different periods.

	Season	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe (x 10^3)
Chlorophyceae												
Chaetomorpha antennina												
	SM	4.30	24.08	44.52	2.00	16.34	4.10	5.95	18.69	8.89	0.90	2.51
	RM	7.34	15.87	42.69	7.98	14.69	4.49	3.39	26.64	4.39	0.10	5.04
	WM1	7.78	20.24	52.04	6.98	19.74	2.99	6.78	31.11	6.78	ND	5.21
	WM2	5.63	12.26	44.31	ND	32.24	5.88	3.19	57.82	5.28	1.35	4.90
	HS1	7.56	18.91	28.85	16.41	22.84	5.87	1.29	44.72	11.93	1.34	5.08
	HS2	5.23	17.30	43.16	10.47	32.94	2.18	9.37	23.13	5.52	1.35	6.69
Ulva lactuca												
	SM	3.88	14.67	14.52	3.48	3.19	2.43	8.41	27.26	15.41	0.30	0.34
	RM	2.39	7.89	14.02	3.99	2.45	2.09	5.29	30.74	1.60	1.55	0.32
	WM1	6.32	15.18	15.53	9.45	3.04	3.34	2.14	19.77	3.19	ND	0.52
	WM2	7.83	12.41	29.26	4.00	ND	5.19	2.89	35.57	0.20	0.50	1.65
	HS1	7.51	12.83	65.53	9.45	ND	4.68	4.77	22.14	10.55	1.54	1.43
	HS2	5.87	14.03	19.11	11.94	ND	5.48	4.13	31.84	6.03	5.98	0.18
Enteromorpha intestinalis												
	SM	4.22	15.00	26.76	0.99	ND	1.29	8.24	26.86	15.04	0.55	1.58

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season ND: Non-detectable level.

Table 4.4. (*Continued*)

	Season	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe ($x 10^3$)
Rhodophyceae												
Gracilaria corticata												
	SM	3.84	12.40	202.30	ND	4.92	4.33	6.20	11.32	13.78	0.49	1.16
	RM	0.60	13.08	110.38	ND	2.88	2.04	4.13	20.93	0.60	6.06	2.03
	WM1	5.92	25.13	196.41	17.41	5.97	3.78	4.28	22.18	8.94	ND	1.48
	WM2	2.09	5.49	147.44	11.47	ND	4.09	5.03	30.66	20.43	0.80	1.41
	HS1	4.42	12.46	78.33	8.94	8.22	4.86	2.38	15.04	2.33	5.97	1.57
	HS2	3.77	16.70	166.33	13.88	2.17	6.69	5.79	11.49	2.38	0.64	0.86
Centroceras clavulatum												
	RM	11.82	22.20	71.86	10.99	18.28	2.84	4.49	10.73	12.82	1.49	11.87
	WM2	12.56	22.84	126.54	3.99	16.04	3.39	8.88	4.20	18.40	2.34	4.73
	HS2	8.99	35.95	145.94	14.98	8.59	2.25	9.11	5.99	14.11	1.69	18.59
Grateloupia filicina												
	RM	9.03	34.03	59.47	9.48	15.45	4.79	3.14	45.95	3.49	6.39	6.22

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season ND: Non-detectable level.

Table 4.5. Trace metal content ($\mu g\ g^{-1}$) of different species of algae from Narakkal in different periods.

	Season	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe ($x\ 10^3$)
Chlorophyceae												
Chaetomorpha antennina												
	SM	6.23	39.31	37.91	1.99	4.58	1.59	6.77	103.90	14.33	0.60	2.10
	RM	7.03	23.19	39.05	ND	27.22	1.10	0.8	26.93	11.87	1.90	4.78
Enteromorpha intestinalis												
	SM	4.89	20.71	86.82	ND	17.86	2.54	10.08	9.23	18.96	2.69	4.52
	HS2	9.23	27.27	37.48	12.91	8.59	0.84	3.62	27.45	10.03	1.25	7.41
Rhodophyceae												
Centroceras clavulatum												
	SM	9.08	33.91	94.76	1.99	13.02	2.59	7.68	11.97	13.86	1.20	4.20
	RM	6.43	29.62	81.04	1.99	17.11	2.79	3.34	33.20	8.78	1.00	7.46
	WM1	14.59	51.48	116.03	13.91	10.33	3.58	7.50	23.10	16.85	ND	10.57
	WM2	9.63	30.90	106.18	9.99	26.41	2.50	5.09	5.49	18.97	1.05	5.55
	HS1	11.84	35.83	114.64	7.00	37.18	4.09	6.39	33.44	8.48	2.10	5.08
	HS2	6.87	25.09	62.44	5.97	20.61	0.30	5.48	2.19	16.33	1.39	13.03
Grateloupia filicina												
	SM	4.87	83.88	38.14	3.98	4.72	2.83	5.72	69.63	8.80	1.74	1.69
	RM	3.45	62.06	26.46	ND	16.38	4.99	ND	44.18	2.90	0.10	0.65
	WM1	8.00	80.09	41.04	18.92	15.39	0.90	6.27	86.05	21.06	ND	2.95
	HS1	5.89	44.72	60.59	13.98	5.09	2.30	9.74	4.30	20.49	1.60	2.57
	HS2	3.20	35.36	39.76	17.98	2.80	2.80	9.19	61.54	1.20	1.40	2.90

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season ND: Non-detectable level.

of Fe (650 μg g^{-1}) among the rhodophyceae members. This was also the lowest Fe content in marine algae from Narakkal. The highest value at this location was shown by the rhodophyte *Centroceras clavulatum* collected in the late hot season (13030 μg g^{-1}). At Ettikkulam, the least accumulation of Fe among the rhodophyceae was shown by *Gracilaria corticata* collected in the late hot season (860 μg g^{-1}). The chlorophytes of Narakkal recorded Fe contents in the range 2100 – 7410 μg g^{-1} with *Chaetomorpha antennina* collected in the summer monsoon season showing the least accumulation of Fe.

Iron content of algae showed marked interspecies variability (Fig. 4.10). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded average Fe content of 4910 μg g^{-1}, while *Ulva lactuca* showed only 740 μg g^{-1}. *Enteromorpha intestinalis* (present only in the summer monsoon season) exhibited Fe content of 1580 μg g^{-1}. Among the rhodophytes, *Gracilaria corticata* recorded average Fe content of 1420 μg g^{-1}, while *Centroceras clavulatum* showed a value of 11730 μg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) recorded Fe content of 6220 μg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* showed average Fe contents of 3440 and 5960 μg g^{-1} respectively, whereas the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* recorded the values 7650 μg g^{-1} and 2150 μg g^{-1} respectively.

Temporal variations were observed in the Fe content of marine algae. Chlorophyceae of Ettikkulam showed average Fe contents varying in the range 1480 – 3440 μg g^{-1} while that from Narakkal recorded values in the range 3310 – 7410 μg g^{-1} (Fig. 4.11). The rhodophyceae of Ettikkulam exhibited average Fe contents in the range 1160 – 9730 μg g^{-1}, whereas that

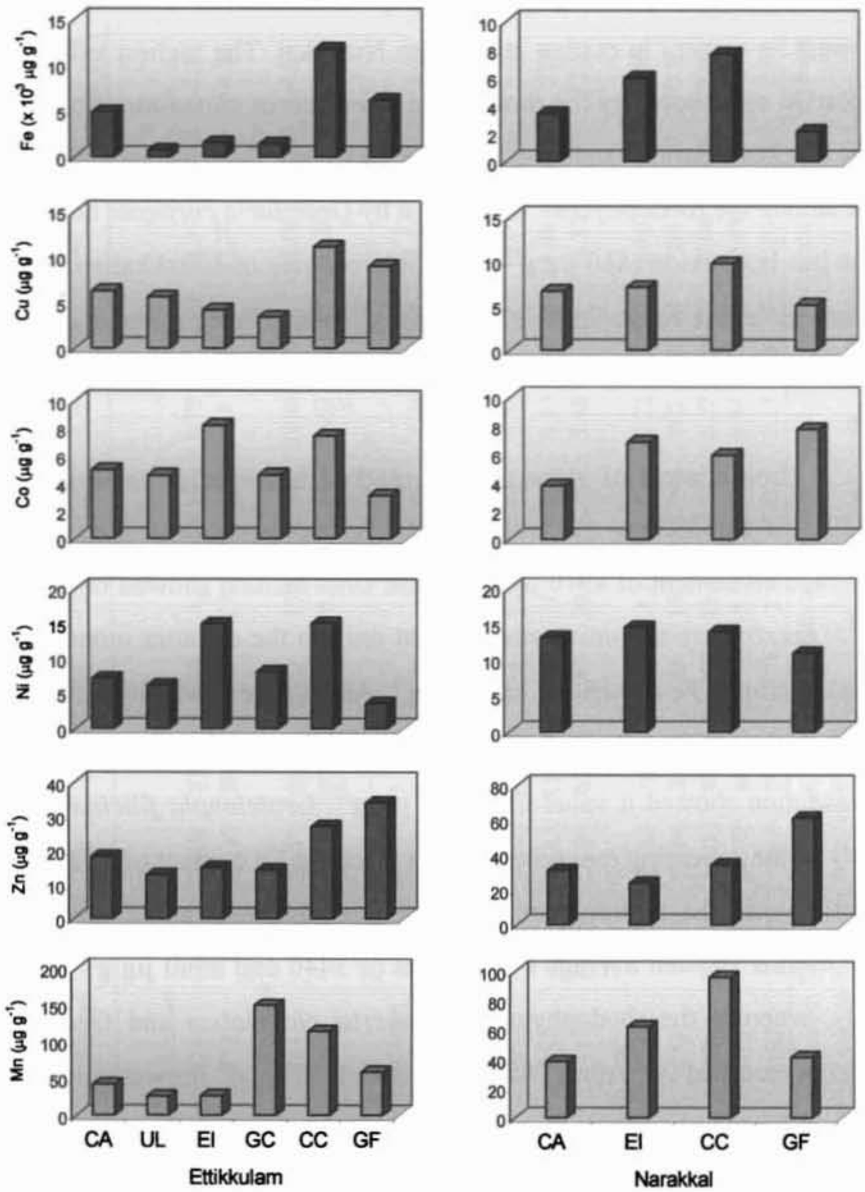

Fig. 4.10. Interspecies variations in the trace metal contents of marine algae (CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

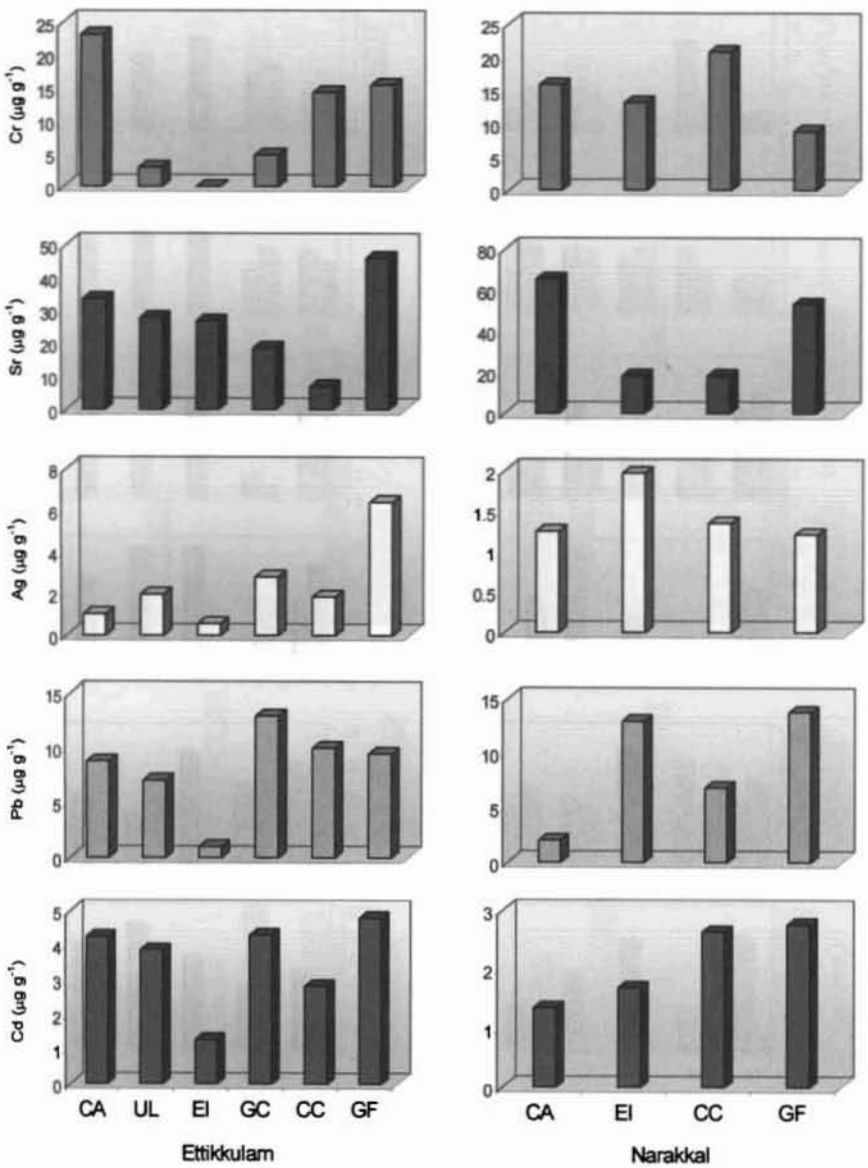

Fig. 4.10. (*Continued*)

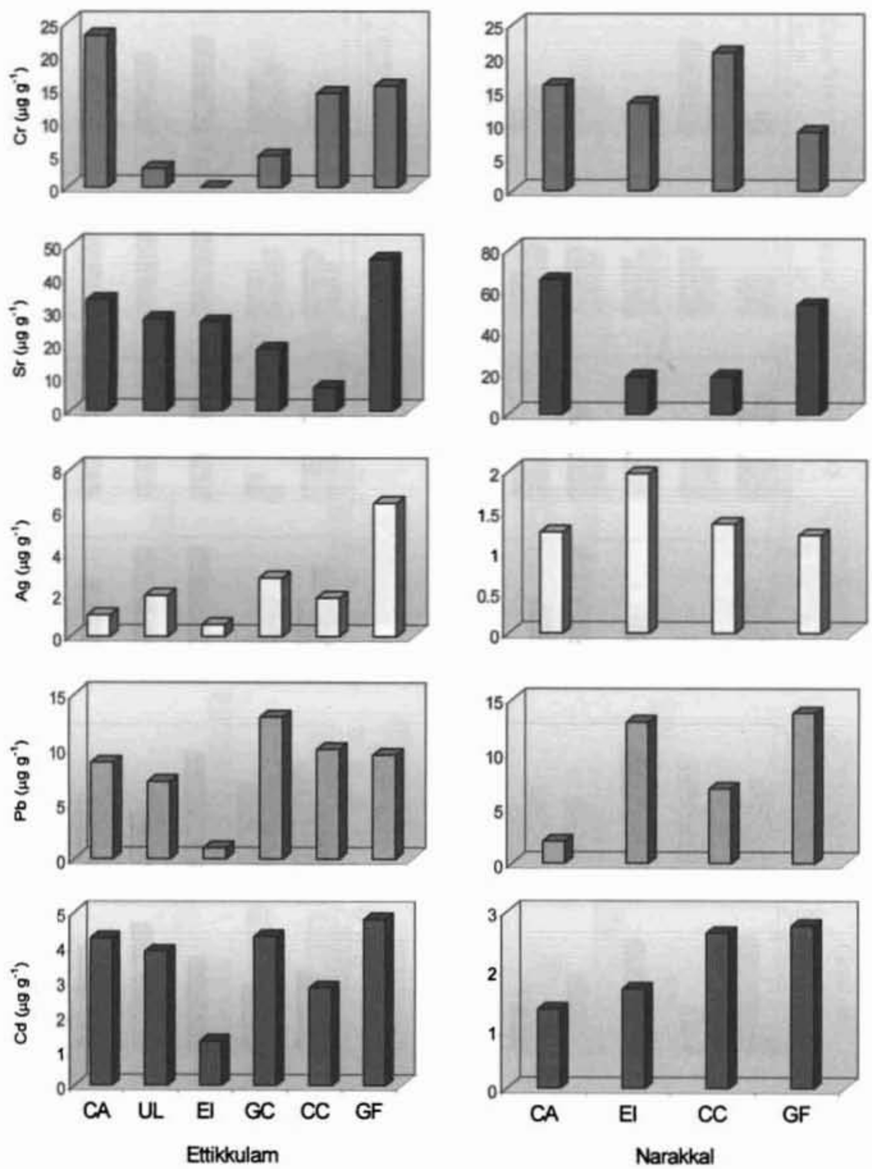

Fig. 4.10. (*Continued*)

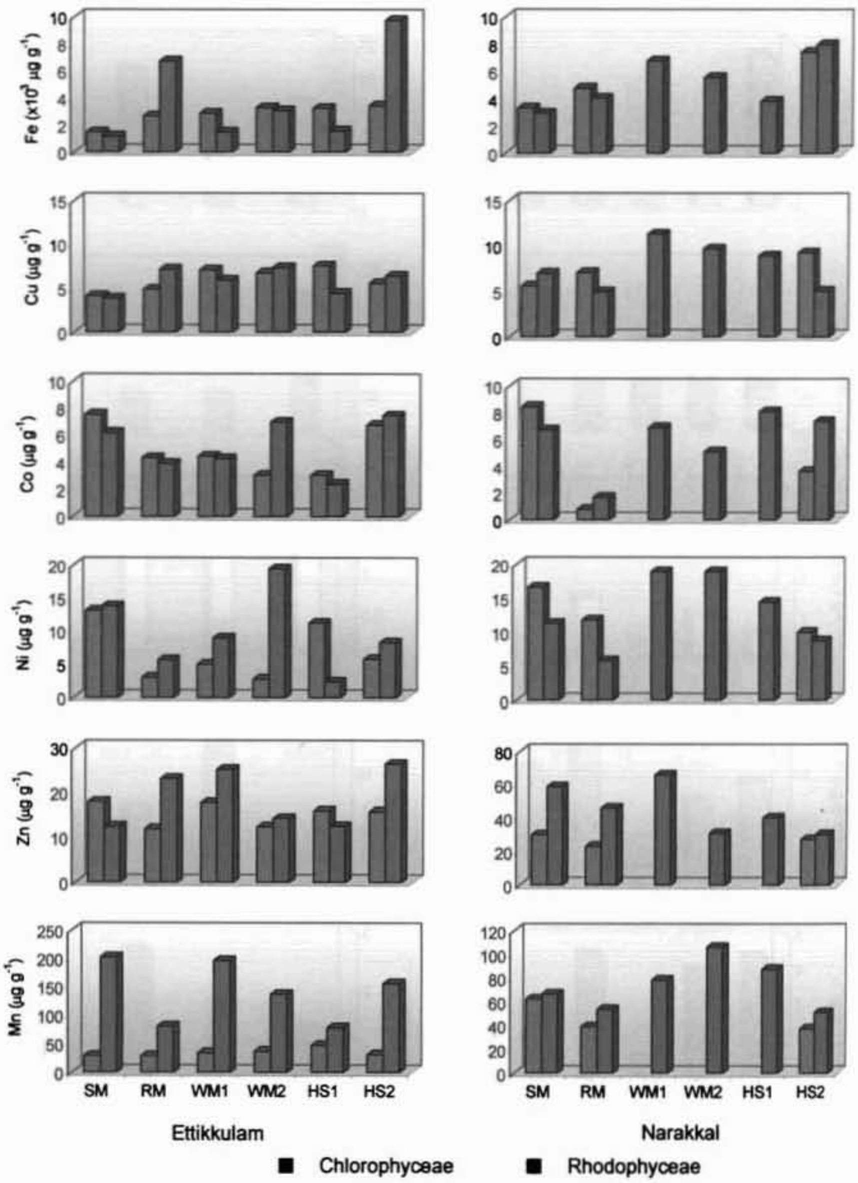

Fig. 4.11. Temporal variations in the trace metal contents of different classes of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season).

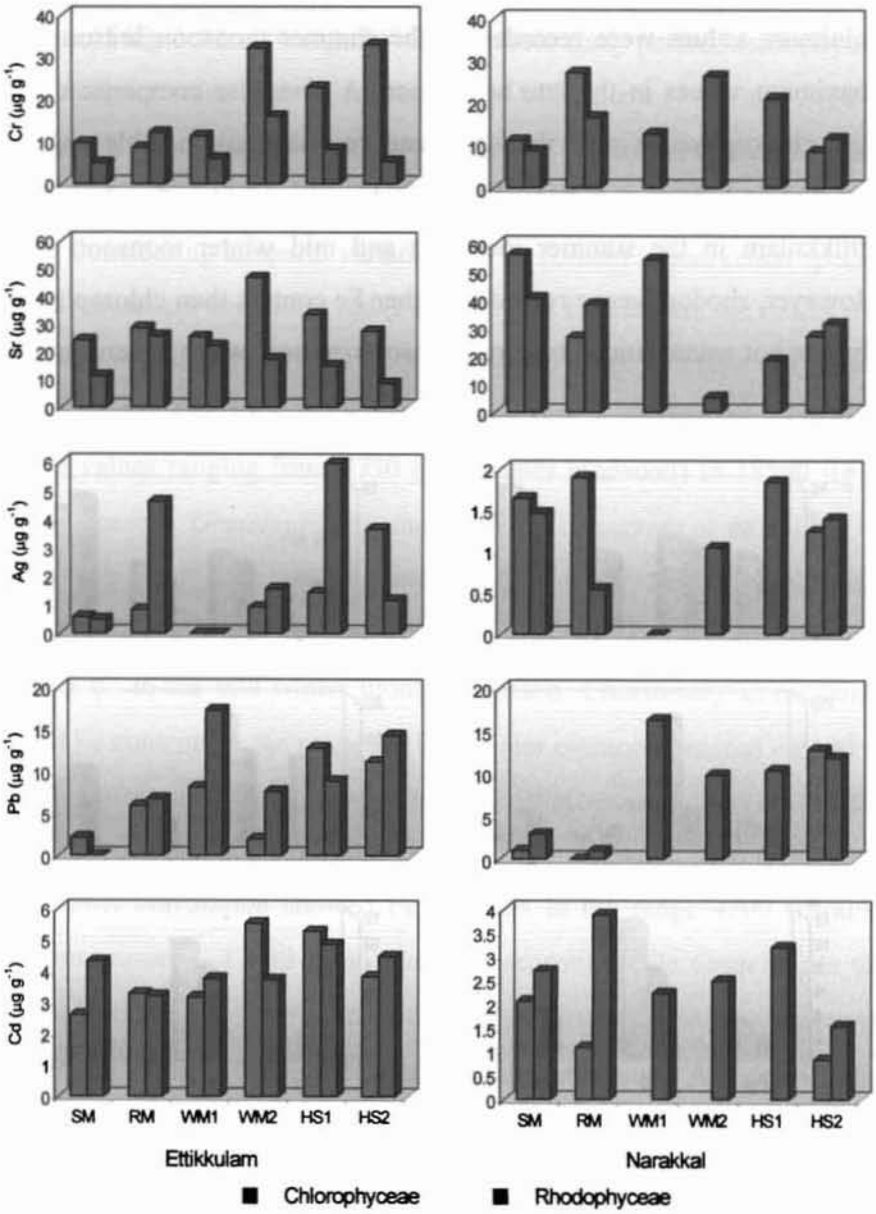

Fig. 4.11. (*Continued*)

from Narakkal showed values in the range 2940 – 7960 µg g^{-1}. In all cases, minimum values were recorded in the summer monsoon season and the maximum values in the late hot season. A classwise comparison showed that chlorophyceae and rhodophyceae recorded comparable values for average Fe content at Narakkal (Fig. 4.11). A similar trend was observed at Ettikkulam in the summer monsoon and mid winter monsoon seasons. However, rhodophyceae recorded higher Fe content than chlorophyceae in the late hot season and retreating monsoon season, while reverse trend was

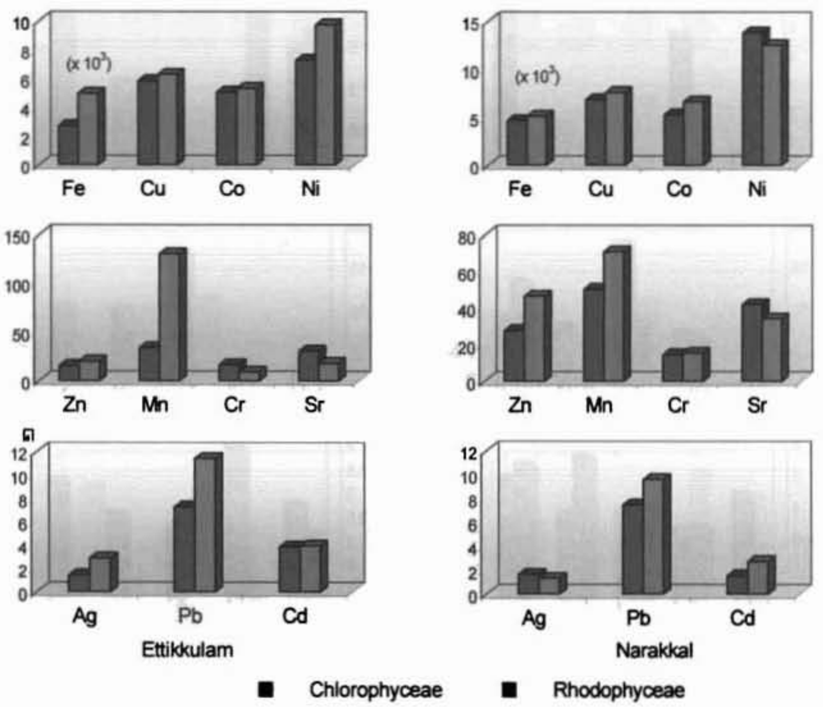

Fig. 4.12. Interclass variations in the average trace metal content (µg g^{-1}) of marine algae from Kerala coast.

observed in the early winter monsoon and mid hot season. When Fe contents of algae were averaged over the whole study period, rhodophyceae showed higher value than chlorophyceae at Ettikkulam (4990 and 2730 μg g^{-1} respectively) while at Narakkal, comparable values were recorded (chlorophyceae: 4700 μg g^{-1} and rhodophyceae: 5150 μg g^{-1}) (Fig. 4.12).

Considering temporal variations of Fe content in individual species, notable variations were observed (Fig. 4.13). At Ettikkulam, the rhodophyte *Gracilaria corticata* recorded Fe contents in the range 860 (late hot season) – 2030 μg g^{-1} (retreating monsoon season) while *Centroceras clavulatum* showed values ranging from 4730 (mid winter monsoon) to 18590 μg g^{-1} (late hot season). *Grateloupia filicina* exhibited Fe content of 6220 μg g^{-1} in the retreating monsoon season. Among the chlorophyceae members, *Ulva lactuca* recorded values varying from 180 μg g^{-1} in the late hot season to 1650 μg g^{-1} in the mid winter monsoon season. *Chaetomorpha antennina* showed Fe contents in the range 2510 (summer monsoon season) – 6690 μg g^{-1} (late hot season). *Enteromorpha intestinalis* recorded Fe content of 1580 μg g^{-1} in the summer monsoon season. At Narakkal, the rhodophyte *Centroceras clavulatum* showed Fe contents in the range 4200 (summer monsoon season) – 13030 μg g^{-1} (late hot season), while *Grateloupia filicina* recorded values ranging from 650 μg g^{-1} (retreating monsoon season) to 2950 μg g^{-1} (early winter monsoon and late hot season). Among the chlorophytes, *Chaetomorpha antennina* showed Fe contents of 2100 and 4780 μg g^{-1} in summer monsoon and retreating monsoon seasons respectively. *Enteromorpha intestinalis* recorded the values 4520 and 7410 μg g^{-1} in the summer monsoon and late hot season respectively.

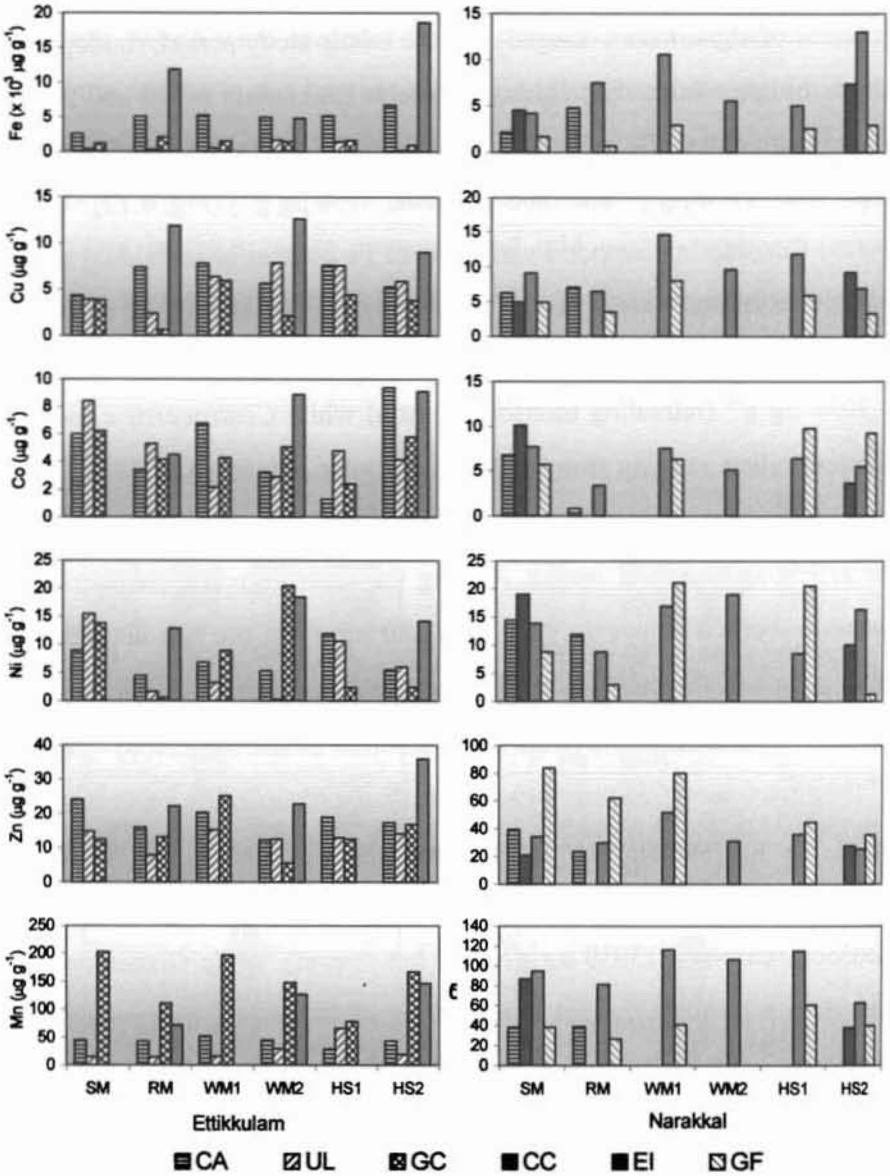

Fig. 4.13. Temporal variations in the trace metal contents of different species of marine algae (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

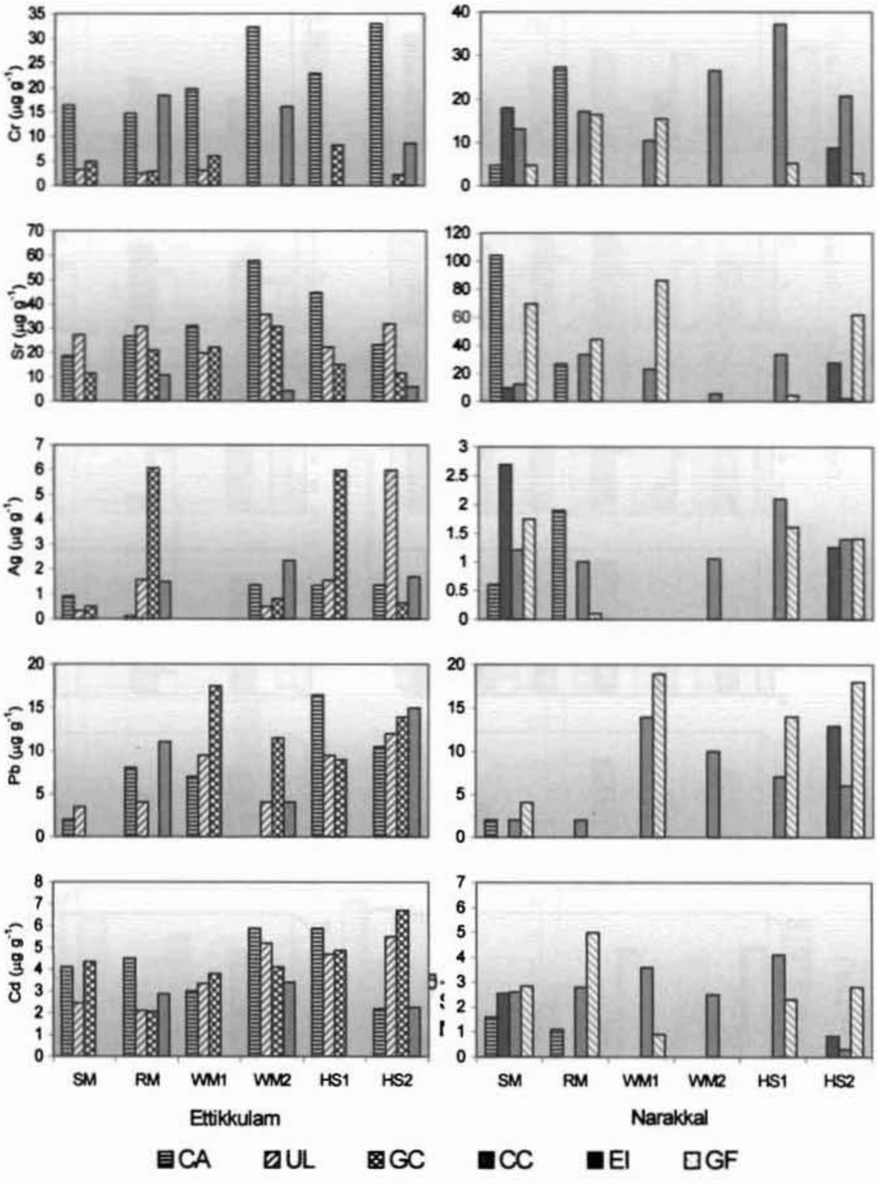

Fig. 4.13. *(Continued)*

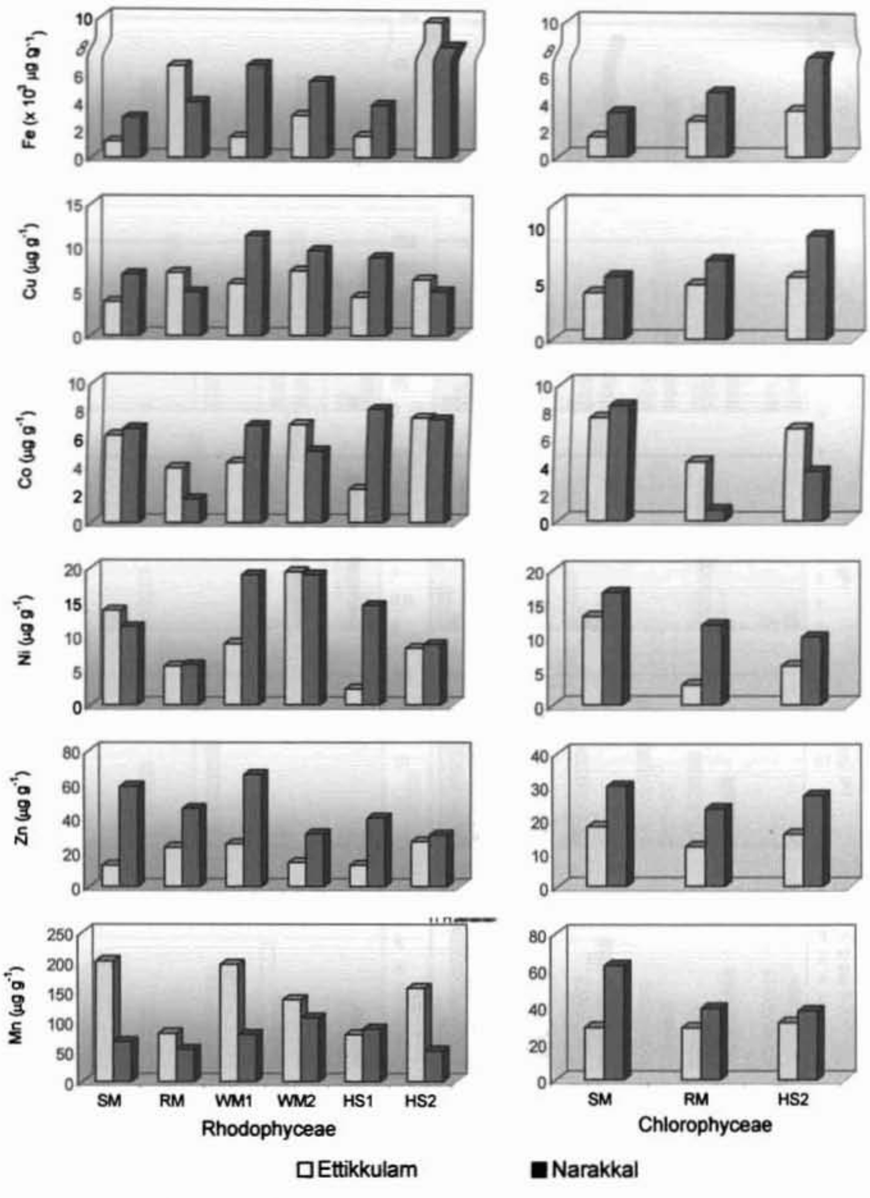

Fig. 4.14. Spatial variations in the trace metal contents of different classes marine algae in different periods (SM: Summer monsoon RM: Retreating monso WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot seas HS2: Late hot season).

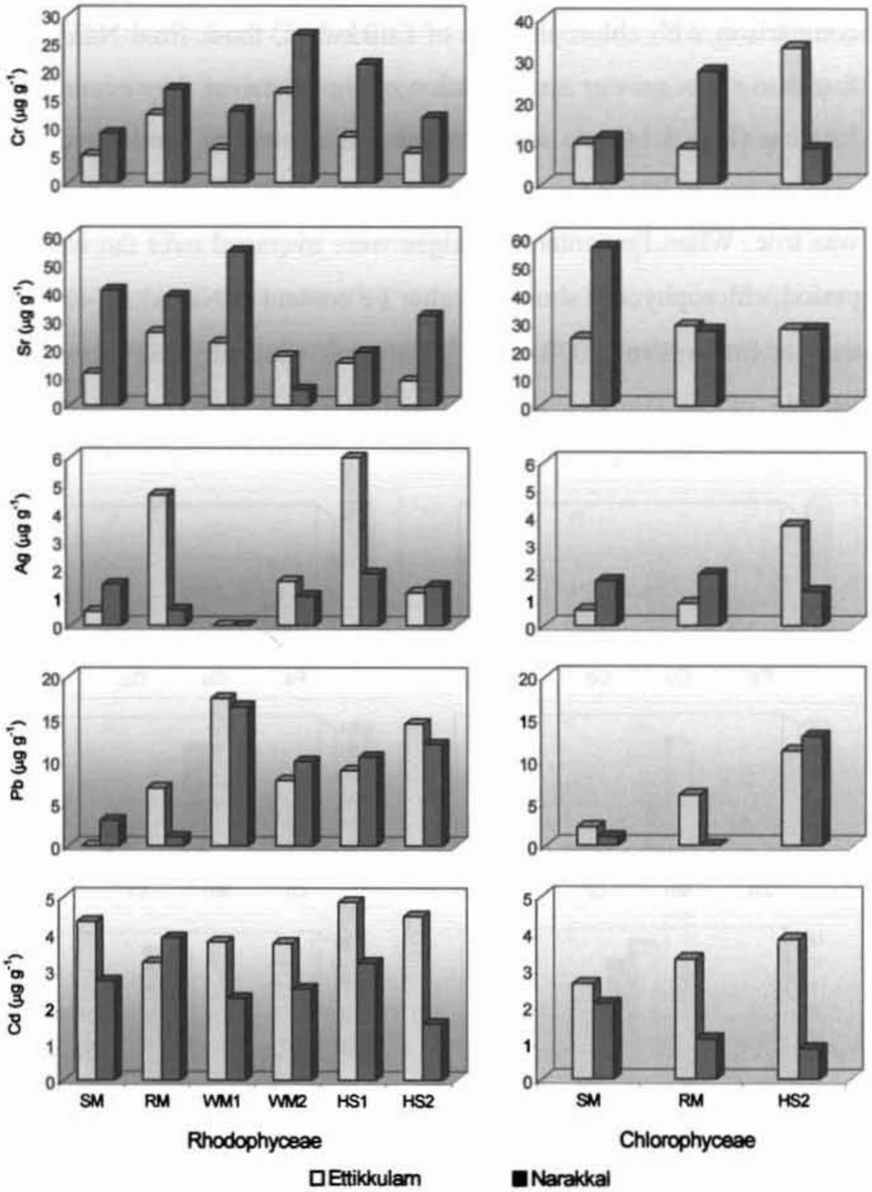

Fig. 4.14. (*Continued*)

Spatial variations were observed in the Fe content of algae. In comparison with chlorophyceae of Ettikkulam, those from Narakkal were found to show greater accumulation of Fe whenever they occurred at this location (Fig. 4.14). A similar trend was shown by rhodophyceae in all seasons except late hot season and retreating monsoon season when reverse was true. When Fe contents of algae were averaged over the whole study period, chlorophyceae showed higher Fe content at Narakkal (4700 μg g^{-1}) than at Ettikkulam (2370 μg g^{-1}), whereas rhodophyceae recorded comparable values (Ettikkulam: 4990 and Narakkal: 5150 μg g^{-1}) (Fig. 4.15).

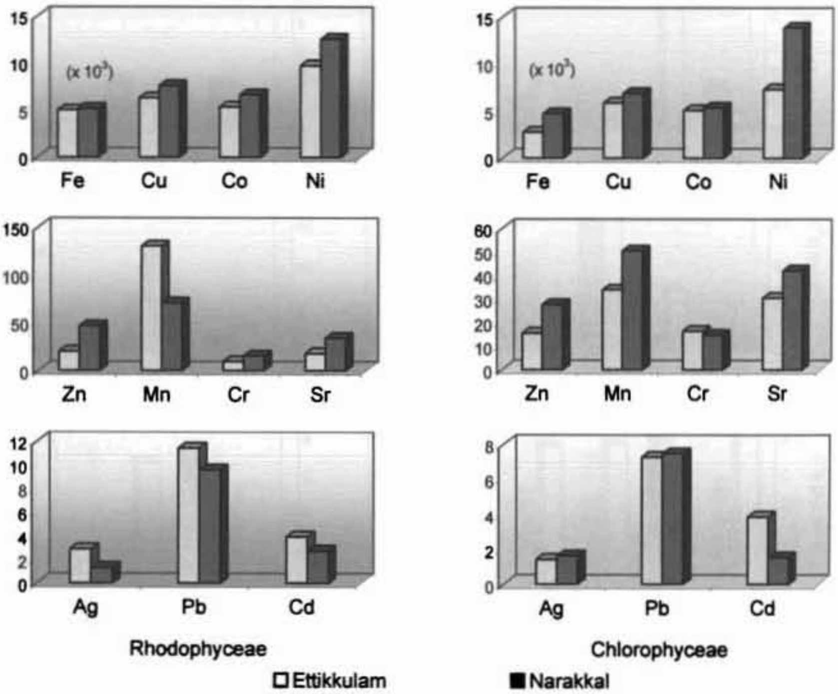

Fig. 4.15. Spatial variations in the average trace metal contents (μg g^{-1}) of different classes of marine algae from Kerala coast.

A consideration of individual species showed variable trend (Figs. 4.16 – 4.18). The rhodophyte *Centroceras clavulatum* recorded higher Fe content at Ettikkulam in the late hot season and retreating monsoon season, while reverse was true in the mid winter monsoon season. *Grateloupia filicina* showed higher value at Ettikkulam in the retreating monsoon season. The chlorophyte *Chaetomorpha antennina* showed comparable values in the summer monsoon and retreating monsoon seasons, while *Enteromorpha intestinalis* recorded higher Fe content at Narakkal in the summer monsoon season.

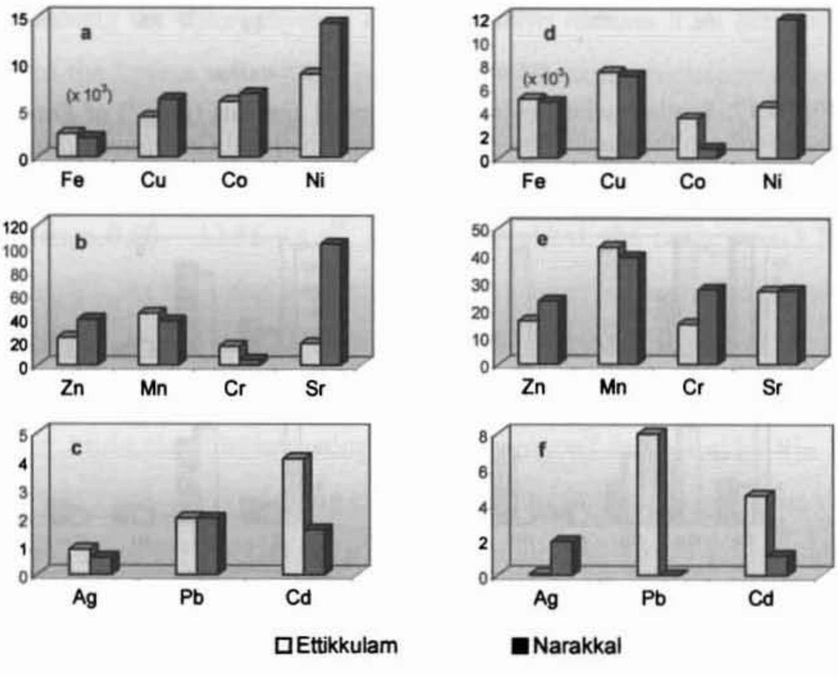

Fig. 4.16. Spatial variations in the trace metal contents (µg g^{-1}) of *Chaetomorpha antennina* in summer monsoon (a,b,c) and retreating monsoon (d,e,f) seasons.

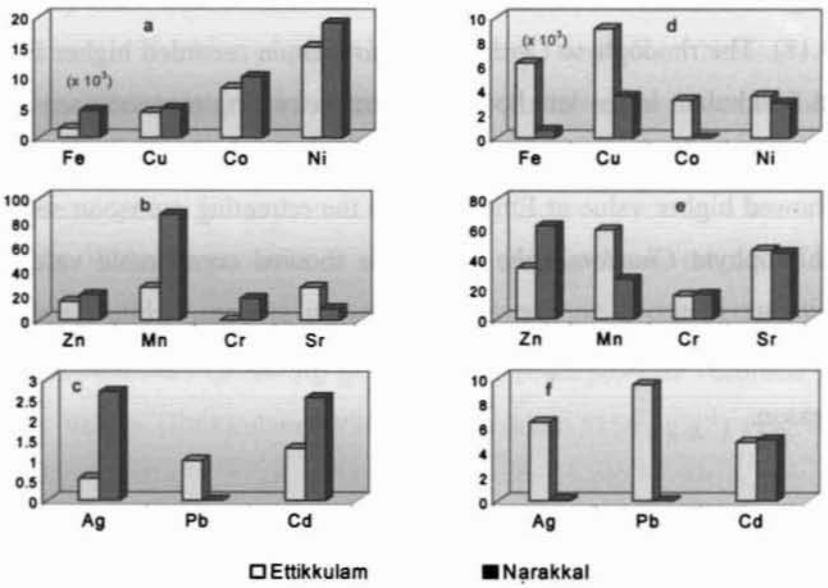

Fig. 4.17. Spatial variations in the trace metal contents (µg g^{-1}) of *Enteromorpha intestinalis* in the summer monsoon season (a,b,c) and *Grateloupia filicina* in the retreating monsoon season (d,e,f)

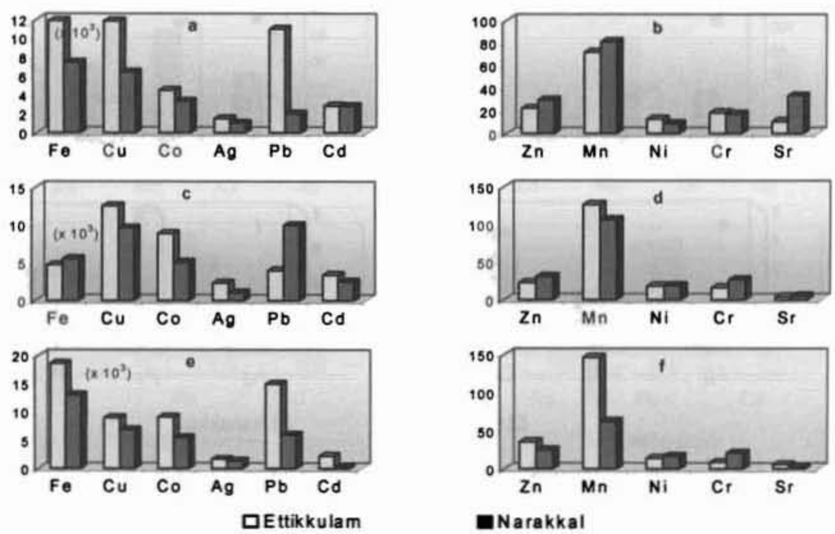

Fig. 4.18. Spatial variations in the trace metal contents (µg g^{-1}) of *Centroceras clavulatum* in retreating monsoon (a,b), winter monsoon (c,d) and late hot season (e,f)

4.2.3.2 Copper

Copper is an essential trace element, but is toxic at high levels. All algae have a micronutrient requirement for Cu. It is present in plastocyanin, one of the photosynthetic electron transfer molecules and is a cofactor of some enzymes. In the present study, the content of Cu in marine algae varied in the range 0.60 – 14.59 $\mu g\ g^{-1}$ (Tables 4.4 and 4.5). The minimum and maximum values were recorded by the members of rhodophyceae. *Gracilaria corticata* collected from Ettikkulam in the retreating monsoon season showed the minimum value and *Centroceras clavulatum* collected from Narakkal in the early winter monsoon season showed the maximum value. Among the chlorophyceae members, *Ulva lactuca* from Ettikkulam exhibited the lowest value (2.39 $\mu g\ g^{-1}$ in the retreating monsoon season), whereas *Enteromorpha intestinalis* from Narakkal recorded the highest value (9.23 $\mu g\ g^{-1}$ in the late hot season). At Ettikkulam, Cu contents varied in the range 0.60 – 12.56 $\mu g\ g^{-1}$ while at Narakkal, the range was 3.20 – 14.59 $\mu g\ g^{-1}$. At both the locations, highest values were recorded by the rhodophyte *Centroceras clavulatum* in the winter monsoon season. The rhodophytes *Gracilaria corticata* and *Grateloupia filicina* showed lowest values at Ettikkulam (in retreating monsoon season) and Narakkal (in late hot season) respectively. Among chlorophyceae members, Cu contents varied in the ranges 2.39 – 7.83 $\mu g\ g^{-1}$ at Ettikkulam and 4.89 – 9.23 $\mu g\ g^{-1}$ at Narakkal. The minimum and maximum values were recorded by the same species *Ulva lactuca* at Ettikkulam (retreating monsoon season and mid winter monsoon season respectively) and *Enteromorpha intestinalis* at Narakkal (summer monsoon season and late hot season respectively).

Interspecies variability in the Cu content was notable only in rhodophyceae members (Fig. 4.10). At Ettikkulam, *Gracilaria corticata* recorded an average Cu content of 3.44 μg g^{-1}, while *Centroceras clavulatum* showed a value of 11.12 μg g^{-1}. *Grateloupia filicina*, which occurred only in the retreating monsoon season, recorded Cu content of 9.03μg g^{-1}. At Narakkal, *Centroceras clavulatum* exhibited average Cu content of 9.74 μg g^{-1}, while *Grateloupia filicina* showed a value of 5.08 μg g^{-1}. Among the chlorophyceae from Ettikkulam, *Chaetomorpha antennina* and *Ulva lactuca* recorded comparable values for the average Cu content (6.31 and 5.63 μg g^{-1} respectively). *Enteromorpha intestinalis*, which occurred only in the summer monsoon season, showed Cu content of 4.22 μg g^{-1}. At Narakkal, *Chaetomorpha antennina* and *Enteromorpha intestinalis* exhibited comparable values (6.63 and 7.06 μg g^{-1} respectively).

The Cu contents of marine algae showed temporal variations. The chlorophyceae from Ettikkulam recorded average Cu contents in the range 4.13 (summer monsoon) – 7.54 μg g^{-1} (mid hot season) while that from Narakkal showed values in the range 5.56 (summer monsoon) – 9.23 μg g^{-1} (late hot season) (Fig. 4.11). The average Cu contents of rhodophyceae varied in the ranges 3.84 (summer monsoon) – 7.33 μg g^{-1} (mid winter monsoon) at Ettikkulam and 4.94 (retreating monsoon) – 11.30 μg g^{-1} (early winter monsoon) at Narakkal. A classwise comparison of Cu contents showed a variable trend (Fig. 4.11). However, when the Cu contents of algae were averaged over the whole study period, rhodophyceae showed slightly higher value than chlorophyceae at both the locations (Fig. 4.12). But, the differences were very small. The values were 5.84 μg g^{-1} for chlorophyceae and 6.30 μg g^{-1} for rhodophyceae at Ettikkulam while at Narakkal, the respective values were 6.85 and 7.62 μg g^{-1}.

Considering the individual species, most of the algae were observed to show temporal variations in their Cu contents (Fig. 4.13). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded Cu contents in the range 4.30 – 7.78 μg g^{-1} with minimum in summer monsoon season and maximum in the early winter monsoon season (Table 4.4). *Ulva lactuca* exhibited values ranging from 2.39 μg g^{-1} in the retreating monsoon season to 7.83 μg g^{-1} in the mid winter monsoon season. *Enteromorpha intestinalis* occurred only in the summer monsoon season with Cu content of 4.22 μg g^{-1}. Among the rhodophyceae members, *Gracilaria corticata* recorded Cu contents in the range 0.60 – 5.92 μg g^{-1} with minimum in the retreating monsoon season and maximum in the early winter monsoon season. *Centroceras clavulatum* accumulated Cu in the range 8.99 (late hot season) – 12.56 μg g^{-1} (mid winter monsoon). *Grateloupia filicina* exhibited a value of 9.03 μg g^{-1} in the retreating monsoon season. This species was absent from Ettikkulam in other seasons. At Narakkal, the chlorophyte *Chaetomorpha antennina* showed Cu contents of 6.23 μg g^{-1} in the summer monsoon season and 7.03 μg g^{-1} in the retreating monsoon season (Table 4.5). *Enteromorpha intestinalis* recorded the values 4.89 μg g^{-1} in the summer monsoon season and 9.23 μg g^{-1} in the late hot season. These species were absent from Narakkal in other seasons. Among the rhodophyceae members, *Centroceras clavulatum* accumulated Cu in the range 6.43 (retreating monsoon season) – 14.59 μg g^{-1} (early winter monsoon). The Cu content of *Grateloupia filicina* varied from 3.20 μg g^{-1} in the late hot season to 8.00 μg g^{-1} in the early winter monsoon season. Thus, it can be seen that most of the algal species showed maximum accumulation of Cu in the winter monsoon season.

In the present study, considerable spatial variations were observed in the Cu content of marine algae. The chlorophyceae from Narakkal showed higher values for the average Cu content than that from Ettikkulam. Rhodophyceae also showed a similar trend except in the late hot season and retreating monsoon season when reverse was true (Fig. 4.14). When the Cu contents were averaged over the whole study period (Fig. 4.15), both the classes of algae showed slightly higher values at Narakkal (For chlorophyceae, Narakkal: 6.85 and Ettikkulam: 5.84 µg g^{-1}; for rhodophyceae, Narakkal: 7.62 and Ettikkulam: 6.30 µg g^{-1}). However, when spatial variations in Cu contents of individual species in different seasons were considered, higher values were observed at Ettikkulam in some cases (Figs. 4.16 - 4.18). The rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* showed greater accumulation of Cu whenever they occurred at Ettikkulam in comparison with the same species from Narakkal. *Enteromorpha intestinalis* showed comparable levels of Cu accumulation at both the locations in the summer monsoon season. A similar case was observed with *Chaetomorpha antennina* in the retreating monsoon season, whereas a higher value was observed at Narakkal in the summer monsoon season.

4.2.3.3 Cobalt

Cobalt, which constitutes 4% of the weight of Vitamin B_{12}, is an essential element. It is also involved in photosynthesis. The cobalt content of marine algae selected for the present study varied from non-detectable level shown by the rhodophyte *Grateloupia filicina* collected from Narakkal in the retreating monsoon season to 10.08 µg g^{-1} recorded by the chlorophyte *Enteromorpha intestinalis* collected from the same location in the summer monsoon season (Tables 4.4 and 4.5). *Grateloupia filicina* collected from Narakkal in the mid hot season showed the highest accumulation of Co (9.74 μg g^{-1}) among the rhodophyceae members. *Chaetomorpha antennina* collected from the same location in the retreating monsoon season exhibited the lowest Co content (0.08 μg g^{-1}) among the chlorophyceae. At Ettikkulam, the Co content of algae varied in the range 1.29 – 9.37 μg g^{-1}. The minimum value was shown by the chlorophyte *Chaetomorpha antennina* in the mid hot season, while the same species collected in the late hot season showed the maximum Co content. Among the rhodophyceae of Ettikkulam, *Gracilaria corticata* collected in the mid hot season recorded the lowest Co content (2.38 μg g^{-1}), while *Centroceras clavulatum* collected in the late hot season showed the maximum Co accumulation (9.11 μg g^{-1}).

Algae were found to show species to species variability in the accumulation of Co (Fig. 4.10). At Ettikkulam, the rhodophyte *Gracilaria corticata* showed average Co content of 4.64 μg g^{-1}, while *Centroceras clavulatum* recorded a value of 7.49 μg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon) showed a Co content of 3.14 μg g^{-1}. Among the chlorophyceae members, *Chaetcmorpha antennina* and *Ulva lactuca* did not show any considerable variation in their average Co content. The values were 5.00 μg g^{-1} and 4.61 μg g^{-1} respectively. *Enteromorpha intestinalis*, which was present only in the summer monsoon season, recorded Co content of 8.24 μg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* showed average Co contents of 3.79 μg g^{-1} and 6.85 μg g^{-1} respectively. The rhodophyte *Centroceras clavulatum* recorded average value of 5.91 μg g^{-1}, while *Grateloupia filicina* showed a value of 7.73 μg g^{-1}.

Temporal variations were also observed in the Co content of marine algae. Chlorophyceae of Ettikkulam showed average Co contents varying from 3.03 μg g^{-1} (mid winter monsoon and mid hot season) to 7.53 μg g^{-1} (summer monsoon) while that from Narakkal recorded values in the range 0.80 (retreating monsoon) – 8.43 μg g^{-1} (summer monsoon) (Fig. 4.11). Rhodophyceae from Ettikkulam exhibited average values ranging from 2.38 (mid hot season) to 7.45 μg g^{-1} (late hot season), whereas that from Narakkal recorded values in the range 1.67 (retreating monsoon) - 8.07 μg g^{-1} (mid hot season). A classwise comparison showed variable trend (Fig. 4.11). At Ettikkulam, chlorophyceae accumulated lower amounts of Co compared to rhodophyceae in the late hot season and mid winter monsoon season while in the other seasons reverse trend was observed. At Narakkal, chlorophyceae recorded lower Co contents in the late hot season and retreating monsoon season while in the summer monsoon, rhodophyceae showed the lower values. When Co contents of algae were averaged over the whole study period, chlorophyceae and rhodophyceae showed comparable values at Ettikkulam (5.06 and 5.34 μgg^{-1} respectively) whereas the latter showed slightly higher value at Narakkal (5.32 and 6.64 μg g^{-1} respectively) (Fig. 4.12).

Considering temporal variations of Co content in individual species (Fig. 4.13), the chlorophyte *Chaetomorpha antennina* from Ettikkulam were found to show values in the range 1.29 (mid hot season) – 9.37 μg g^{-1} (late hot season). *Ulva lactuca* recorded Co contents in the range 2.14 (early winter monsoon) – 8.41 μg g^{-1} (summer monsoon). *Enteromorpha intestinalis* (present only in the summer monsoon) showed a value of 8.24 µg g^{-1}. Among the rhodophyceae members, *Gracilaria corticata* showed Co contents varying from 2.38 (mid hot season) to 6.20 µg g^{-1} (summer monsoon) while *Centroceras clavulatum* recorded values ranging from 4.49 (retreating monsoon) – 9.11 µg g^{-1} (late hot season). *Grateloupia filicina* (present only in the retreating monsoon) showed a value of 3.14 µg g^{-1}. At Narakkal, the chlorophyte *Chaetomorpha antennina* recorded Co contents of 0.80 µg g^{-1} in the retreating monsoon season and 6.77 µg g^{-1} in the summer monsoon season. *Enteromorpha intestinalis* recorded the values of 3.62 and 10.08 µg g^{-1} in the late hot season and summer monsoon respectively. Among the rhodophyceae members, *Centroceras clavulatum* showed values in the range 3.34 (retreating monsoon) – 7.68 µg g^{-1} (summer monsoon), while *Grateloupia filicina* showed values ranging from non-detectable level observed in the retreating monsoon season to 9.74 µg g^{-1} recorded in the mid hot season.

In the present study, marine algae showed a variable trend in the spatial variation of Co content. Chlorophyceae of Narakkal showed lower accumulation of Co than that from Ettikkulam in the late hot season and retreating monsoon season while a reverse trend was observed in the summer monsoon season (Fig. 4.14). Similarly, rhodophyceae from Narakkal recorded lower Co contents compared to that from Ettikkulam in the late hot season, retreating monsoon and mid winter monsoon whereas a reverse trend was observed in other seasons. When Co contents of algae were averaged over the whole study period, chlorophyceae showed comparable values at the two locations (Ettikkulam: 5.06 µg g^{-1} and Narakkal: 5.32 µg g^{-1}), while rhodophyceae showed slightly higher value at Narakkal

(Ettikkulam: 5.34 μg g^{-1} and Narakkal: 6.64 μg g^{-1}) (Fig. 4.15). Considering individual species, it was found that the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* showed higher accumulation of Co at Ettikkulam whenever these species occurred at this location (Figs. 4.16 – 4.18). The chlorophytes *Enteromorpha intestinalis* and *Chaetomorpha antennina* showed higher Co content at Narakkal in the summer monsoon season while the latter showed a reverse trend in the retreating monsoon season.

4.2.3.4 Nickel

Nickel is an essential element and is present in some enzymes. In the present study, marine algae accumulated Ni in the range 0.20 – 21.06 μg g^{-1} (Tables 4.4 and 4.5) with the chlorophyte *Ulva lactuca* collected from Ettikkulam in the mid winter monsoon season showing the lowest Ni content and the rhodophyte *Grateloupia filicina* collected from Narakkal in the early winter monsoon season showing the highest value. The lowest Ni content among rhodophyceae members (0.60 μg g^{-1}) was recorded by *Gracilaria corticata* from Ettikkulam in the retreating monsoon season. *Enteromorpha intestinalis* collected from Narakkal in the summer monsoon season showed the highest Ni content (18.96 μg g^{-1}) among chlorophyceae members. At Ettikkulam, the Ni content of chlorophyceae members varied in the range 0.20 – 15.41 μg g^{-1} with *Ulva lactuca* showing the lowest value in the mid winter monsoon season and highest value in the summer monsoon season. Rhodophyceae members recorded values in the range 0.60 - 20.43 μg g^{-1} with *Gracilaria corticata* showing minimum and maximum values in different periods (retreating monsoon and mid winter monsoon season respectively). At Narakkal, the algae showed Ni accumulation in the range 1.20 – 21.06 µg g^{-1}. The rhodophyte *Grateloupia filicina* recorded the minimum and maximum values in the late hot season and early winter monsoon season respectively. Among chlorophyceae of Narakkal, values varied in the range 10.03 – 18.96 µg g^{-1} with *Enteromorpha intestinalis* showing the minimum and maximum values in different seasons (late hot season and summer monsoon season respectively).

The Ni content of algae showed interspecies variability in some cases (Fig. 4.10). At Ettikkulam, the rhodophyte *Gracilaria corticata* showed average Ni content of 8.08 µg g^{-1}, while *Centroceras clavulatum* exhibited a value of 15.11 µg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) recorded Ni content of 3.49 µg g^{-1}. The chlorophytes *Chaetomorpha antennina* and *Ulva lactuca* did not show much variation in the average Ni content (7.13 and 6.16 µg g^{-1} respectively). *Enteromorpha intestinalis* (present only in the summer monsoon season) recorded Ni content of 15.04 µg g^{-1}. At Narakkal, the rhodophyte *Centroceras clavulatum* recorded average Ni content of 13.88 µg g^{-1} while *Grateloupia filicina* showed a value of 10.89 µg g^{-1}. The chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* recorded the values 13.10 and 14.50 µg g^{-1} respectively.

Temporal variations were observed in the Ni content of algae. The chlorophyceae from Ettikkulam showed average Ni contents varying in the range 2.74 (mid winter monsoon season) – 13.11 µg g^{-1} (summer monsoon season) while that from Narakkal recorded values in the range 10.03 (late hot season) – 16.65 µg g^{-1} (summer monsoon) (Fig. 4.11). Rhodophyceae from Ettikkulam exhibited average Ni contents varying from 2.33 µg g^{-1} in the mid hot season to 19.42 µg g^{-1} in the mid winter monsoon season whereas that from Narakkal showed values ranging from 5.84 $\mu g\ g^{-1}$ (retreating monsoon season) to 18.97 $\mu g\ g^{-1}$ (winter monsoon season). A classwise comparison showed that rhodophyceae accumulated more Ni than chlorophyceae at Ettikkulam in all the seasons except in the mid hot season when reverse was true (Fig. 4.11). At Narakkal, chlorophyceae showed greater accumulation of Ni than rhodophyceae. When Ni contents of algae were averaged over the whole study period (Fig. 4.12), rhodophyceae showed slightly higher value than chlorophyceae at Ettikkulam (9.73 and 7.29 $\mu g\ g^{-1}$ respectively). At Narakkal, the values were comparable (chlorophyceae: 13.80 $\mu g\ g^{-1}$ and rhodophyceae: 12.52 $\mu g\ g^{-1}$).

Considering the temporal variation of Ni content in individual species (Fig. 4.13), the chlorophyte *Ulva lactuca* from Ettikkulam was observed to show Ni contents varying from 0.20 $\mu g\ g^{-1}$ in the mid winter monsoon season to 15.41 $\mu g\ g^{-1}$ in the summer monsoon season. *Chaetomorpha antennina* showed values in the range 4.39 (retreating monsoon) – 11.93 $\mu g\ g^{-1}$ (mid hot season). *Enteromorpha intestinalis* recorded Ni content of 15.04 $\mu g\ g^{-1}$ in the summer monsoon season. Among rhodophyceae, *Gracilaria corticata* exhibited Ni contents varying from 0.60 $\mu g\ g^{-1}$ (retreating monsoon) to 20.43 $\mu g\ g^{-1}$ (mid winter monsoon), while *Centroceras clavulatum* showed values in the range 12.82 (retreating monsoon) – 18.40 $\mu g\ g^{-1}$ (mid winter monsoon). *Grateloupia filicina* recorded Ni content of 3.49 $\mu g\ g^{-1}$ in the retreating monsoon season. At Narakkal, the chlorophyte *Chaetomorpha antennina* showed Ni content of 11.87 $\mu g\ g^{-1}$ in the retreating monsoon season and 14.33 $\mu g\ g^{-1}$ in the summer monsoon season. *Enteromorpha intestinalis* recorded the values 10.03 and 18.96 $\mu g\ g^{-1}$ in late hot season and summer monsoon season respectively (These species were absent in other seasons). Among rhodophyceae members, *Centroceras clavulatum* showed Ni accumulation in the range 8.48 (mid hot season) – 18.97 $\mu g\ g^{-1}$ (mid winter monsoon season), while *Grateloupia filicina* recorded values in the range 1.20 (late hot season) – 21.06 $\mu g\ g^{-1}$ (early winter monsoon season).

Spatial variations in Ni accumulation were observed in some cases. Chlorophyceae recorded higher Ni contents at Narakkal (whenever they occurred at this location) than at Ettikkulam (Fig. 4.14). A similar trend was shown by rhodophyceae in the early winter monsoon and mid hot seasons whereas comparable values were recorded at the two locations in other seasons. When Ni contents of algae were averaged over the whole study period, both chlorophyceae and rhodophyceae showed higher values at Narakkal (chlorophyceae, Narakkal: 13.80 $\mu g\ g^{-1}$ and Ettikkulam: 7.29 $\mu g\ g^{-1}$; rhodophyceae, Narakkal: 12.52 $\mu g\ g^{-1}$ and Ettikkulam: 9.73 $\mu g\ g^{-1}$) (Fig. 4.15). A consideration of spatial variation of Ni in individual species showed that the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* showed higher values at Narakkal (Figs. 4.16 – 4.18). The rhodophyte *Grateloupia filicina* recorded comparable values at the two locations in the retreating monsoon season while *Centroceras clavulatum* showed variable trend.

4.2.3.5 Zinc

Zinc is an essential element and it is an activator of several important enzymes including many dehydrogenases. The marine algae selected for the present study recorded Zn contents in the range 5.49 – 83.88 $\mu g\ g^{-1}$ (Tables 4.4 and 4.5). The minimum and maximum values were shown by the members of rhodophyceae from different locations in different periods. *Gracilaria corticata* collected from Ettikkulam in mid winter monsoon season showed the lowest value while *Grateloupia filicina* from Narakkal showed highest value in the summer monsoon season. The chlorophyceae members accumulated Zn in a narrower range of 7.89 - 39.31 $\mu g\ g^{-1}$. *Ulva lactuca* from Ettikkulam showed the minimum Zn content in the retreating monsoon season and *Chaetomorpha antennina* from Narakkal exhibited the maximum value in the summer monsoon season. Marine algae from Ettikkulam showed Zn contents varying in the range 5.49 – 35.95 $\mu g\ g^{-1}$ with maximum accumulation in the rhodophyte *Centroceras clavulatum* in the late hot season. Chlorophyceae members recorded values varying from 7.89 to 24.08 $\mu g\ g^{-1}$ with maximum Zn content in *Chaetomorpha antennina* collected in the summer monsoon season. At Narakkal, Zn content of algae ranged from 20.71 $\mu g\ g^{-1}$ to 83.88 $\mu g\ g^{-1}$. The minimum and maximum values were recorded by the chlorophyte *Enteromorpha intestinalis* and the rhodophyte *Grateloupia filicina* respectively, both collected in the summer monsoon season. Among chlorophyceae members, *Chaetomorpha antennina* recorded the maximum value in the summer monsoon season (39.31 $\mu g\ g^{-1}$). The least accumulation of Zn in rhodophyceae was shown by *Centroceras clavulatum* collected in the late hot season (25.09 $\mu g\ g^{-1}$).

The algae were observed to show marked interspecies variability in the accumulation of Zn at both the locations (Fig. 4.10). Among chlorophyceae from Ettikkulam, *Chaetomorpha antennina* recorded average Zn content of 18.11 $\mu g\ g^{-1}$ while *Ulva lactuca* showed a value of 12.84 $\mu g\ g^{-1}$. *Enteromorpha intestinalis*, (present only in the summer monsoon season) recorded Zn content of 15.00 μg g^{-1}. The rhodophyte *Gracilaria corticata* exhibited average Zn content of 14.21 μg g^{-1} and *Centroceras clavulatum* showed a value of 27.00 μg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) recorded Zn content of 34.03 μg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* recorded average Zn contents of 31.25 μg g^{-1} and 23.99 μg g^{-1} respectively. Among rhodophyceae members, *Centroceras clavulatum* showed an average value of 34.47 μg g^{-1}, while *Grateloupia filicina* recorded average Zn content of 61.22 μg g^{-1}.

Temporal variations were observed in the Zn contents of marine algae. Variations in the average Zn contents were more pronounced in rhodophyceae than in chlorophyceae. In this case, maximum values were twice as high as the minimum values. The ranges of values were 12.40 (summer monsoon) – 26.33 μg g^{-1} (late hot season) at Ettikkulam and 30.23 (late hot season) – 65.79 μg g^{-1} (early winter monsoon) at Narakkal (Fig. 4.11). Chlorophyceae exhibited narrow ranges such as 11.88 (retreating monsoon season) – 17.92 μg g^{-1} (summer monsoon) at Ettikkulam and 23.19 (retreating monsoon season) – 30.01 μg g^{-1} (summer monsoon) at Narakkal. A classwise comparison showed that rhodophyceae accumulated more Zn than chlorophyceae in all seasons except summer monsoon and early hot season when chlorophyceae of Ettikkulam showed higher Zn content than rhodophyceae from that location (Fig. 4.11). A similar trend was observed when Zn contents were averaged over the whole study period (Ettikkulam, chlorophyceae: 15.44 μg g^{-1} and rhodophyceae: 20.03 μg g^{-1}; Narakkal, chlorophyceae: 27.62 μg g^{-1} and rhodophyceae: 46.63 μg g^{-1}) (Fig. 4.12).

Considering the temporal variations of Zn content in individual species, it was observed that in most cases, maximum values were about two times the minimum values (Fig. 4.13). Among the chlorophyceae of Ettikkulam, *Chaetomorpha antennina* accumulated Zn in the range 12.26 – 24.08 μg g^{-1}, with minimum in the mid winter monsoon season and maximum in the summer monsoon season. *Ulva lactuca* recorded values varying from 7.89 μg g^{-1} in the retreating monsoon season to 15.18 μg g^{-1} in the early winter monsoon season. *Enteromorpha intestinalis* (present only in the summer monsoon season) showed Zn content of 15.00 μg g^{-1}. The rhodophyte *Gracilaria corticata* exhibited values ranging from 5.49 μg g^{-1} in the mid winter monsoon season to 25.13 μg g^{-1} in the early winter monsoon season. The Zn content of *Centroceras clavulatum* ranged from 22.20 μg g^{-1} (retreating monsoon season) to 35.95 μg g^{-1} (late hot season). *Grateloupia filicina* showed a value of 34.03 μg g^{-1} in the retreating monsoon season (This species was absent in other seasons). At Narakkal, the chlorophyte *Chaetomorpha antennina* recorded Zn contents of 23.19 μg g^{-1} in the retreating monsoon season and 39.31 μg g^{-1} in the summer monsoon season. *Enteromorpha intestinalis* showed the values 20.71 μg g^{-1} in the summer monsoon season and 27.27 μg g^{-1} in the late hot season (These species were absent in other seasons). Among the rhodophyceae of Narakkal, *Centroceras clavulatum* accumulated Zn in concentrations varying in the range 25.09 – 51.48 μg g^{-1} with minimum in the late hot season and maximum in the early winter monsoon season. The Zn content of *Grateloupia filicina* varied in the range 35.36 μg g^{-1} (late hot season) – 83.88 μg g^{-1} (summer monsoon season).

Spatial variations were observed in the Zn content of marine algae. Both the chlorophyceae and rhodophyceae of Narakkal recorded higher Zn contents than those from Ettikkulam in all the seasons (Fig. 4.14). A similar trend was observed when Zn contents of algae were averaged over the whole study period (rhodophyceae, Ettikkulam: 20.03 $\mu g\ g^{-1}$ and Narakkal: 46.63 $\mu g\ g^{-1}$; chlorophyceae, Ettikkulam: 15.44 $\mu g\ g^{-1}$ and Narakkal: 27.62 $\mu g\ g^{-1}$) (Fig. 4.15). Considering the spatial variation of Zn content in individual species in different seasons (Figs. 4.16 – 4.18), it was observed that all the species showed higher Zn accumulation at Narakkal than at Ettikkulam in all the seasons except the rhodophyte *Centroceras clavulatum* collected in the late hot season which showed higher Zn content at Ettikkulam.

4.2.3.6 Manganese

Manganese is an essential element. In algae, it plays a vital role in the oxygen – evolving system of photosynthesis and is a cofactor in several enzymes. In the present study, marine algae were found to accumulate Mn in the range 14.02 – 202.30 $\mu g\ g^{-1}$ (Tables 4.4 and 4.5). The minimum was shown by the chlorophyte *Ulva lactuca* in the retreating monsoon season and the maximum by the rhodophyte *Gracilaria corticata* in the summer monsoon season, both collected from Ettikkulam. Among the chlorophytes, maximum Mn accumulation was observed in *Enteromorpha intestinalis* collected from Narakkal in the summer monsoon season (86.82 $\mu g\ g^{-1}$). *Grateloupia filicina* collected from Narakkal in the retreating monsoon season exhibited the lowest Mn content among the rhodophyceae members. Thus, both the chlorophyceae and rhodophyceae recorded lowest Mn content in the retreating monsoon season and highest in the summer monsoon season. At Narakkal, Mn content of algae varied in the range 26.46 – 116.03 µg g^{-1} with minimum and maximum values shown by the members of rhodophyceae, *viz.*, *Grateloupia filicina* in the retreating monsoon season and *Centroceras clavulatum* in the early winter monsoon season respectively. At Ettikkulam, the range was 14.02 – 202.30 µg g^{-1}. Chlorophyceae members of Narakkal recorded Mn contents in the range 37.48 – 86.82 µg g^{-1} with *Enteromorpha intestinalis* showing the minimum and maximum values in different seasons (minimum in the late hot season and maximum in the summer monsoon season). The same algal class from Ettikkulam exhibited values in the range 14.02 – 65.53 µg g^{-1} with *Ulva lactuca* showing minimum and maximum values in different periods (minimum in the retreating monsoon season and maximum in the mid hot season). The rhodophytes of Ettikkulam recorded Mn contents varying from 59.47 µg g^{-1} shown by *Grateloupia filicina* in the retreating monsoon season to 202.30 µg g^{-1} shown by *Gracilaria corticata* in the summer monsoon season.

The Mn accumulation in the marine algae showed considerable variations among different species (Fig. 4.10). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* showed average Mn content of 42.60 µg g^{-1} while *Ulva lactuca* showed a value of 26.33 µg g^{-1}. *Enteromorpha intestinalis* (present only in the summer monsoon season) recorded Mn content of 26.76 µg g^{-1}. Among the rhodophyceae members, *Gracilaria corticata* recorded average Mn content of 150.20 µg g^{-1}, whereas *Centroceras clavulatum* showed a value of 114.78 µg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) showed Mn content of 59.47µg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha* *intestinalis* exhibited average Mn contents of 38.48 and 62.15 μg g^{-1} respectively. The values recorded by the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* were 95.85 and 41.20 μg g^{-1} respectively.

The accumulation of Mn by marine algae was observed to show notable temporal variations. The chlorophyceae from Ettikkulam exhibited average Mn contents in the range 28.36 (retreating monsoon) – 47.19 μg g^{-1} (mid hot season), while that from Narakkal recorded values in the range 37.48 (late hot season) – 62.37 μg g^{-1} (summer monsoon) (Fig. 4.11). The average Mn contents of rhodophyceae varied in wider ranges compared to those in chlorophyceae. The ranges were 78.33 (mid hot season) – 202.30 μg g^{-1} (summer monsoon) at Ettikkulam and 51.10 (late hot season) – 106.18 μg g^{-1} (mid winter monsoon) at Narakkal. A classwise comparison showed that rhodophyceae accumulated more Mn than chlorophyceae in all the seasons at both the locations (Fig. 4.11). A similar trend was observed when the Mn contents of marine algae were averaged over the whole study period (Fig. 4.12). At Ettikkulam, the values were 33.87 μg g^{-1} and 130.50 μg g^{-1} for chlorophyceae and rhodophyceae respectively, while at Narakkal, the respective values were 50.32 μg g^{-1} and 71.01 μg g^{-1}.

Considering the temporal variations of Mn content in individual species, it was observed that in many cases, maximum values were more than twice the minimum values (Fig. 4.13). At Ettikkulam, the rhodophyte *Gracilaria corticata* showed Mn accumulation in the range 78.33 (mid hot season) – 202.30 μg g^{-1} (summer monsoon) while *Centroceras clavulatum* recorded values in the range 71.86 (retreating monsoon) – 145.94 μg g^{-1} (late hot season). *Grateloupia filicina* (present only in the retreating monsoon season) recorded Mn content of 59.47 μg g^{-1}. Among the members of chlorophyceae, *Ulva lactuca* showed Mn contents varying from 14.02 µg g^{-1} in the retreating monsoon season to 65.53 µg g^{-1} in the mid hot season. *Chaetomorpha antennina* recorded values in the range 28.85 (mid hot season) – 52.04 µg g^{-1} (early winter monsoon). *Enteromorpha intestinalis* (present only in the summer monsoon season) exhibited Mn content of 26.76 µg g^{-1}. At Narakkal, the chlorophyte *Chaetomorpha antennina* exhibited Mn accumulation of 37.91 µg g^{-1} in the summer monsoon season and 39.05 µg g^{-1} in the retreating monsoon season, while *Enteromorpha intestinalis* showed the values of 37.48 µg g^{-1} in the late hot season and 86.82 µg g^{-1} in the summer monsoon season. These species were absent in other seasons. Among the rhodophyceae members, *Centroceras clavulatum* exhibited Mn contents in the range 62.44 (late hot season) – 116.03 µg g^{-1} (early winter monsoon), while *Grateloupia filicina* recorded values in the range 26.46 (retreating monsoon season) – 60.59 µg g^{-1} (mid hot season).

Spatial variations were observed in the accumulation of Mn by marine algae. Chlorophyceae showed higher accumulation of Mn at Narakkal, whereas rhodophyceae showed higher values at Ettikkulam in all the seasons except in the mid hot season when rhodophyceae of Narakkal showed higher Mn contents (Fig. 4.14). A similar trend was observed when Mn contents of algae were averaged over the whole study period (Fig. 4.15). The values for chlorophyceae were 33.87 µg g^{-1} at Ettikkulam and 50.32 µg g^{-1} at Narakkal and the values for rhodophyceae were 130.50 µg g^{-1} at Ettikkulam and 71.01 µg g^{-1} at Narakkal.

4.2.3.7 Chromium

Chromium is an essential element. In the present study, Cr content of algae varied from non-detectable level to 37.18 µg g^{-1} (Tables 4.4 and 4.5). The rhodophyte *Centroceras clavulatum* collected from Narakkal in the mid hot season recorded the maximum value. *Gracilaria corticata* collected from Ettikkulam in the mid winter monsoon season showed Cr content below detectable level. Among chlorophyceae, *Enteromorpha intestinalis* collected from Ettikkulam in the summer monsoon season and *Ulva lactuca* collected in the hot season and mid winter monsoon showed non-detectable levels of Cr. *Chaetomorpha antennina* collected from Ettikkulam in the late hot season showed the highest Cr content (32.94 µg g^{-1}) among chlorophyceae members. This was also the highest Cr content of algae from Ettikkulam. The highest Cr content in rhodophyceae from Ettikkulam was recorded by *Centroceras clavulatum* in the retreating monsoon season (18.28 µg g^{-1}). At Narakkal, the Cr content of algae varied in the range 2.80 – 37.18 µg g^{-1} with *Grateloupia filicina* collected in the late hot season showing the least accumulation of Cr. The chlorophyceae members of Narakkal recorded Cr contents in the range 4.58 – 27.22 µg g^{-1} with *Chaetomorpha antennina* exhibiting the lowest value in the summer monsoon season and the highest value in the retreating monsoon season.

The Cr contents of marine algae were found to show considerable interspecies variability (Fig. 4.10). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded average Cr content of 23.13 µg g^{-1} while *Ulva lactuca* recorded a value of 2.89 µg g^{-1}. *Enteromorpha intestinalis*, which was present only in the summer monsoon season showed Cr content below detectable level. Among the rhodophyceae, *Gracilaria corticata* showed average Cr content of 4.83 µg g^{-1} whereas *Centroceras clavulatum* recorded average value of 14.30 µg g^{-1}. *Grateloupia filicina*, which occurred only in the retreating monsoon season, exhibited Cr content of 15.45 µg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* recorded average Cr contents of 15.90 and 13.23 µg g^{-1} respectively. Among rhodophyceae members, *Centroceras clavulatum* showed a value of 20.78 µg g^{-1} while *Grateloupia filicina* recorded average Cr content of 8.88 µg g^{-1}.

Considerable temporal variations were observed in the Cr accumulation by marine algae. The chlorophyceae from Ettikkulam showed average Cr contents varying in the range 8.57 (retreating monsoon) – 32.94 µg g^{-1} (late hot season) while that from Narakkal recorded values in the range 8.59 (late hot season) – 27.22 µg g^{-1} (retreating monsoon) (Fig. 4.11). Rhodophyceae from Ettikkulam showed average Cr contents ranging from 4.92 (summer monsoon season) to 16.04 µg g^{-1} (mid winter monsoon) whereas that from Narakkal exhibited values varying in the range 8.87 (summer monsoon) – 26.41 µg g^{-1} (mid winter monsoon). A classwise comparison showed that chlorophyceae of Ettikkulam accumulated more Cr than rhodophyceae in all the seasons except in the retreating monsoon season (Fig. 4.11). A similar trend was observed at Narakkal in the summer monsoon and retreating monsoon seasons whereas a reverse case was observed in the late hot season. In other seasons, chlorophyceae members were absent from Narakkal. When Cr contents of algae were averaged over the whole study period, chlorophyceae of Ettikkulam recorded higher Cr content than rhodophyceae (16.38 µg g^{-1} and 9.17 µg g^{-1} respectively) while at Narakkal, the values were comparable (chlorophyceae: 14.56 μg g^{-1} and rhodophyceae: 15.37 μg g^{-1}) (Fig. 4.12).

A consideration of temporal variation of Cr content in individual species revealed notable variations (Fig. 4.13). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded Cr contents varying in the range 14.69 (retreating monsoon) – 32.94 μg g^{-1} (late hot season). *Ulva lactuca* showed low levels of Cr accumulation ranging from non-detectable level observed in the mid winter monsoon and hot season to 3.19 μg g^{-1} recorded in the summer monsoon season. *Enteromorpha intestinalis*, which occurred only in the summer monsoon season, showed Cr content below detectable level. Among the rhodophyceae members, *Gracilaria corticata* recorded Cr contents varying from the non-detectable level observed in the mid winter monsoon season to 8.22 μg g^{-1} in the mid hot season. *Centroceras clavulatum* showed values in the range 8.59 (late hot season) – 18.28 μg g^{-1} (retreating monsoon season). *Grateloupia filicina* (present only in the retreating monsoon season) recorded Cr content of 15.45 μg g^{-1}. At Narakkal, the chlorophyte *Chaetomorpha antennina* showed Cr contents of 4.58 μg g^{-1} in the summer monsoon season and 27.22 μg g^{-1} in the retreating monsoon season. *Enteromorpha intestinalis* recorded the values 8.59 and 17.86 μg g^{-1} in the late hot season and summer monsoon season respectively. Among the rhodophyceae members, *Centroceras clavulatum* exhibited values varying in the range 10.33 (early winter monsoon season) – 37.18 μg g^{-1} (mid hot season) while *Grateloupia filicina* showed Cr contents ranging from 2.80 μg g^{-1} (late hot season) to 16.38 μg g^{-1} (retreating monsoon season).

The Cr contents of algae showed spatial variations. Rhodophyceae showed higher accumulation of Cr at Narakkal than at Ettikkulam in all the seasons (Fig. 4.14). Chlorophyceae also showed a similar trend in the summer monsoon and retreating monsoon seasons while a reverse case was observed in the late hot season. In other seasons, chlorophyceae were absent from Narakkal. When Cr contents of algae were averaged over the whole study period, rhodophyceae showed higher value at Narakkal (Narakkal: 15.37 $\mu g\ g^{-1}$ and Ettikkulam: 9.17 $\mu g\ g^{-1}$) whereas chlorophyceae showed slightly higher value at Ettikkulam (Narakkal: 14.56 $\mu g\ g^{-1}$ and Ettikkulam: 16.38 $\mu g\ g^{-1}$) (Fig. 4.15).

4.2.3.8 Strontium

Strontium is a non-essential element. In the present study, Sr content of algae varied in the range 2.19 – 103.90 $\mu g\ g^{-1}$ (Tables 4.4 and 4.5). The minimum value was recorded by the rhodophyte *Centroceras clavulatum* collected from Narakkal in the late hot season, while the maximum value was shown by the chlorophyte *Chaetomorpha antennina* collected from the same location in the summer monsoon season. *Enteromorpha intestinalis* collected in the summer monsoon season showed the least accumulation of Sr among the chlorophyceae (9.23 $\mu g\ g^{-1}$). The highest Sr level among rhodophyceae was recorded by *Grateloupia filicina* collected from Narakkal in the early winter monsoon season (86.05 $\mu g\ g^{-1}$). At Ettikkulam, Sr content of algae showed values in the range 4.20 – 57.82 $\mu g\ g^{-1}$ with the rhodophyte *Centroceras clavulatum* and the chlorophyte *Chaetomorpha antennina*, both collected in the mid winter monsoon season showing the minimum and maximum values respectively. *Chaetomorpha antennina* collected in the summer monsoon season showed the lowest Sr content (18.69 μg g^{-1}) among the chlorophyceae of Ettikkulam. The maximum value of Sr accumulation in rhodophyceae members of Ettikkulam was 45.95 μg g^{-1} recorded by *Grateloupia filicina* in the retreating monsoon season.

In the present study, marine algae showed considerable interspecies variability in the accumulation of Sr (Fig. 4.10). At Narakkal, the chlorophyte *Chaetomorpha antennina* showed average Sr content of 65.42 μg g^{-1}, while *Enteromorpha intestinalis* recorded a value of 18.34 μg g^{-1}. The rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* exhibited average Sr contents of 18.23 μg g^{-1} and 53.14 μg g^{-1} respectively. At Ettikkulam, the rhodophyte *Gracilaria corticata* showed average Sr content of 18.60 μg g^{-1}, while *Centroceras clavulatum* recorded a value of 6.97 μg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) exhibited Sr content of 45.95 μg g^{-1}. The chlorophyceae members of Ettikkulam did not show much variation in the average Sr content. *Chaetomorpha antennina* recorded a value of 33.69 μg g^{-1}, whereas *Ulva lactuca* recorded a value of 27.89 μg g^{-1}. *Enteromorpha intestinalis* showed Sr content of 26.86 μg g^{-1} in the summer monsoon season (it was absent in other seasons).

Considerable temporal variations were observed in the Sr content of marine algae. Chlorophyceae of Ettikkulam showed average Sr contents in the range 24.27 (summer monsoon) – 46.70 μg g^{-1} (mid winter monsoon), while that from Narakkal showed values in the range 26.93 (retreating monsoon season) – 56.57 μg g^{-1} (summer monsoon season) (Fig. 4.11). Rhodophyceae from Ettikkulam recorded average Sr contents ranging from 8.74 μg g^{-1} in the late hot season to 25.87 μg g^{-1} in the retreating monsoon season, whereas that from Narakkal exhibited values varying in the range 5.49 (mid winter monsoon) – 54.58 μg g^{-1} (early winter monsoon). A classwise comparison showed that chlorophyceae of Ettikkulam showed higher Sr contents than rhodophyceae from the same location in all the seasons (Fig. 4.11). At Narakkal, rhodophyceae recorded higher Sr content than chlorophyceae in the late hot season and retreating monsoon season, while reverse was true in the summer monsoon season. When Sr contents of algae were averaged over the whole study period, chlorophyceae showed higher values than rhodophyceae at both the locations with the difference being more pronounced at Ettikkulam (Fig. 4.12). The average values for chlorophyceae and rhodophyceae were 30.48 and 17.85 μg g^{-1} respectively at Ettikkulam and 41.88 and 34.10 μg g^{-1} respectively at Narakkal.

Considering the temporal variation of Sr contents in individual species (Fig. 4.13), the chlorophytes *Chaetomorpha antennina* and *Ulva lactuca* from Ettikkulam were found to show values in the ranges 18.69 (summer monsoon) – 57.82 μg g^{-1} (mid winter monsoon) and 19.77 (early winter monsoon) – 35.57 μg g^{-1} (mid winter monsoon) respectively. *Enteromorpha intestinalis* recorded a value of 26.86 μg g^{-1} in the summer monsoon season. Among the rhodophytes, *Gracilaria corticata* showed Sr accumulation in the range 11.32 (summer monsoon) – 30.66 μg g^{-1} (mid winter monsoon), while *Centroceras clavulatum* recorded values varying from 4.20 μg g^{-1} in the mid winter monsoon season to 10.73 μg g^{-1} in the retreating monsoon season. *Grateloupia filicina* exhibited Sr content of 45.95 μg g^{-1} in the retreating monsoon season. At Narakkal, the chlorophyte *Chaetomorpha antennina* showed Sr contents of 26.93 and 103.90 μg g^{-1} in the retreating monsoon and summer monsoon seasons respectively. *Enteromorpha inte-*

stinalis recorded the values of 9.23 and 27.45 μg g^{-1} in the summer monsoon and late hot season respectively. Among the rhodophyceae members, *Centroceras clavulatum* showed Sr contents varying from 2.19 μg g^{-1} in the late hot season to 33.44 μg g^{-1} in the mid hot season. *Grateloupia filicina* showed Sr accumulations in the range 4.30 (mid hot season) – 86.05 μg g^{-1} (early winter monsoon).

Considerable spatial variations were observed in the Sr content of marine algae. Rhodophyceae showed higher accumulation of Sr at Narakkal than at Ettikkulam in all the seasons except in the mid winter monsoon season when reverse was true (Fig. 4.14). Sr content of chlorophyceae showed variable trend in the spatial variation. When Sr contents of algae were averaged over the whole study period, both chlorophyceae and rhodophyceae were observed to show higher accumulation of Sr at Narakkal than at Ettikkulam, the difference being more pronounced in the case of rhodophyceae (Fig. 4.15). The values for rhodophyceae were 17.85 μg g^{-1} at Ettikkulam and 34.10 μg g^{-1} at Narakkal. Chlorophyceae showed the values 30.48 μg g^{-1} at Ettikkulam and 41.88 μg g^{-1} at Narakkal. A consideration of spatial variations of Sr content in individual species revealed a variable trend in different seasons (Figs. 4.16 – 4.18).

4.2.3.9 Silver

Silver is a non-essential element. In the present study, the silver contents of marine algae were observed to vary from the non-detectable level to 6.39 μg g^{-1} (Tables 4.4 and 4.5). The rhodophyte *Grateloupia filicina* collected from Ettikkulam in the retreating monsoon season recorded the maximum value, whereas that collected from Narakkal in the early winter monsoon season showed Ag content below the detectable level. The rhodophyte *Centroceras clavulatum* collected from Narakkal in the early winter monsoon season also showed non-detectable level of Ag, while that collected in the mid hot season showed the highest Ag content (2.10 µg g^{-1}) among the rhodophyceae members of Narakkal. The algae such as the rhodophyte *Gracilaria corticata* and the chlorophytes *Chaetomorpha antennina* and *Ulva lactuca*, all collected from Ettikkulam in the early winter monsoon season showed Ag contents below non-detectable level. *Ulva lactuca* collected from Ettikkulam in the late hot season showed the maximum Ag accumulation (5.98 µg g^{-1}) among the chlorophyceae. At Narakkal, the chlorophyte *Enteromorpha intestinalis* collected in the summer monsoon season showed the maximum Ag content (2.69 µg g^{-1}). *Chaetomorpha antennina* collected in the same period showed the lowest Ag accumulation (0.60 µg g^{-1}) among the chlorophyceae members.

Specieswise variations were observed in the Ag content of algae in some cases (Fig. 4.10). At Ettikkulam, the rhodophyte *Gracilaria corticata* showed average Ag content of 2.79 µg g^{-1}, while *Centroceras clavulatum* recorded a value of 1.84 µg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) exhibited Ag content of 6.39 µg g^{-1}. The chlorophytes *Chaetomorpha antennina* and *Ulva lactuca* recorded average Ag contents of 1.01 and 1.97 µg g^{-1} respectively. *Enteromorpha intestinalis* (present only in the summer monsoon season) showed Ag content of 0.55 µg g^{-1}. At Narakkal, different species of algae did not show much variation in the average Ag content. The rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* recorded the values 1.35 and 1.21 µg g^{-1} respectively, whereas the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* showed the values 1.25 and 1.97 μg g^{-1} respectively.

Temporal variations were observed in the Ag accumulation by marine algae. The rhodophyceae of Ettikkulam showed average Ag contents varying from non-detectable level observed in the early winter monsoon season to 5.97 μg g^{-1} in the mid hot season (Fig. 4.11). The same algal class from Narakkal also showed Ag content below detectable level in the early winter monsoon season and maximum value in the mid hot season (1.85 μg g^{-1}). The chlorophyceae from Ettikkulam recorded average Ag contents ranging from non-detectable level in the early winter monsoon season to 3.67 μg g^{-1} in the late hot season. This algal class was present at Narakkal only in the period from late hot season to retreating monsoon season with average Ag contents varying from 1.25 (late hot season) to 1.90 μg g^{-1} (retreating monsoon season). A classwise comparison showed variable trend in different seasons (Fig. 4.11). At Ettikkulam, rhodophyceae showed greater accumulation of Ag than chlorophyceae in the retreating monsoon, mid winter monsoon and mid hot seasons, while a reverse trend was observed in the late hot season. In the summer monsoon and early winter monsoon seasons, comparable values were shown by the two classes of algae. At Narakkal, chlorophyceae showed higher Ag content in the retreating monsoon season, while comparable values were recorded by the two algal classes in the late hot season and summer monsoon season. When Ag contents of algae were averaged over the whole study period, rhodophyceae recorded a higher value (2.88 μg g^{-1}) than chlorophyceae (1.41 μg g^{-1}) at Ettikkulam while at Narakkal, the two algal classes showed comparable values (chlorophyceae: 1.61 µg $g^{-1}$and rhodophyceae: 1.29 µg g^{-1}) with chlorophyceae showing slightly higher value (Fig. 4.12).

Considering the temporal variations in the Ag content of individual species, it was observed that all the algal species present in the early winter monsoon season at both the locations showed Ag contents below detectable level (Fig. 4.13). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded Ag contents varying from non-detectable level (early winter monsoon) to 1.35 µg g^{-1} (mid winter monsoon and hot seasons). *Ulva lactuca* showed values ranging from non-detectable level (early winter monsoon) to 5.98 µg g^{-1} (late hot season). *Enteromorpha intestinalis* recorded Ag content of 0.55 µg g^{-1} in the summer monsoon season. Among rhodophyceae members, *Gracilaria corticata* exhibited values varying from non-detectable level (early winter monsoon) to 6.06 µg g^{-1} (retreating monsoon). *Centroceras clavulatum*, which occurred only in the late hot season, retreating monsoon and mid winter monsoon seasons, recorded average Ag contents of 1.69, 1.49 and 2.34 µg g^{-1} respectively. *Grateloupia filicina* recorded Ag content of 6.39 µg g^{-1} in the retreating monsoon season. At Narakkal, the rhodophyte *Centroceras clavulatum* showed Ag contents varying from non-detectable level (early winter monsoon) to 2.10 µg g^{-1} (mid hot season), while *Grateloupia filicina* recorded values ranging from non-detectable level (early winter monsoon) to 1.74 µg g^{-1} (summer monsoon season). The chlorophyte *Chaetomorpha antennina* recorded Ag contents of 0.60 and 1.90 µg g^{-1} in the summer monsoon and retreating monsoon seasons respectively whereas *Enteromorpha intestinalis* showed the values of 1.25 and 2.69 µg g^{-1} in the late hot season and summer monsoon season respectively.

Spatial variations in the Ag accumulation by algae were observed to show variable trend. Rhodophyceae of Ettikkulam showed greater accumulation of Ag than those from Narakkal in the retreating monsoon, mid winter monsoon and mid hot seasons, whereas reverse was true in the late hot season and summer monsoon season (Fig. 4.14). In the early winter monsoon season, Ag contents were below detectable level at both the locations. Chlorophyceae showed higher Ag contents at Narakkal in the summer monsoon and retreating monsoon seasons, while reverse was true in the late hot season. In other seasons, chlorophyceae were absent from Narakkal. When Ag contents of algae were averaged over the whole study period, rhodophyceae showed higher Ag content at Ettikkulam (2.88 $\mu g\ g^{-1}$) than at Narakkal (1.29 $\mu g\ g^{-1}$) whereas chlorophyceae recorded comparable values (Ettikkulam: 1.41 $\mu g\ g^{-1}$ and Narakkal: 1.16 $\mu g\ g^{-1}$) (Fig. 4.15). Considering individual species, the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* were found to show higher accumulation of Ag at Ettikkulam whenever they occurred at this location (Figs. 4.16 – 4.18). The chlorophyte *Enteromorpha intestinalis* accumulated more Ag at Narakkal than at Ettikkulam in the summer monsoon season. *Chaetomorpha antennina* showed greater accumulation of Ag at Narakkal in the retreating monsoon season, while a slightly higher Ag content at Ettikkulam in the summer monsoon season.

4.2.3.10 Lead

Lead is a non-essential element and is toxic for most organisms. The lead contents of marine algae selected for the present study were observed to vary from the non-detectable level to 18.92 $\mu g\ g^{-1}$ (Tables 4.4 and 4.5). The rhodophyte *Grateloupia filicina* collected from Narakkal in the early winter monsoon season recorded the maximum value, whereas that collected in the retreating monsoon season showed Pb content below the detection limit. The rhodophyte *Gracilaria corticata* collected from Ettikkulam also showed Pb contents below detectable level in the summer monsoon and retreating monsoon seasons. However, the same species collected in the early winter monsoon season showed highest Pb accumulation at Ettikkulam (17.41 µg g^{-1}). Among the chlorophyceae, *Chaetomorpha antennina* collected from Ettikkulam in the mid winter monsoon season and that collected from Narakkal in the retreating monsoon season showed non-detectable levels of Pb. However, the same species collected from Ettikkulam in the mid hot season recorded the highest Pb content among the chlorophyceae (16.41 µg g^{-1}). *Enteromorpha intestinalis* collected from Narakkal in the summer monsoon season recorded Pb content below the detection limit while that collected in the late hot season showed the maximum Pb content (12.91 µg g^{-1}) among the chlorophyceae of Narakkal.

The accumulation of Pb by algae showed some species to species variability (Fig. 4.10). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded average Pb content of 8.77 µg g^{-1} and *Ulva lactuca* showed a value of 7.05 µg g^{-1}. *Enteromorpha intestinalis*, which occurred only in the summer monsoon season, exhibited Pb content of 0.99 µg g^{-1}. Among the rhodophyceae members, *Gracilaria corticata* recorded average Pb content of 12.93 µg g^{-1}, while *Centroceras clavulatum* showed a value of 9.99 µg g^{-1}. *Grateloupia filicina* (present only in the retreating monsoon season) showed Pb content of 9.48 µg g^{-1}. At Narakkal, the rhodophyte *Centroceras clavulatum* exhibited average Pb content of 6.81 µg g^{-1}, whereas *Grateloupia filicina* showed a value of 13.72 µg g^{-1}. Among chlorophyceae members, *Chaetomorpha antennina* showed non-detectable level of Pb in the retreating monsoon season and 1.99 $\mu g\ g^{-1}$ in the summer monsoon season, whereas *Enteromorpha intestinalis* showed non-detectable level of Pb in the summer monsoon season and 12.91 $\mu g\ g^{-1}$ in the late hot season (these species were absent in other seasons).

Marine algae were observed to show marked temporal variations in the accumulation of Pb. The chlorophyceae from Ettikkulam showed average Pb contents varying in the range 2.00 (mid winter monsoon) – 12.93 $\mu g\ g^{-1}$ (mid hot season), while that from Narakkal recorded values varying from non-detectable level (retreating monsoon) to 12.91 $\mu g\ g^{-1}$ (late hot season) (Fig. 4.11). Rhodophyceae from Ettikkulam exhibited average Pb contents ranging from non-detectable level (summer monsoon season) to 17.41 $\mu g\ g^{-1}$ (early winter monsoon season), whereas that from Narakkal showed values varying in the range 1.00 (retreating monsoon) – 16.42 $\mu g\ g^{-1}$ (early winter monsoon). A classwise comparison showed that rhodophyceae accumulated more Pb than chlorophyceae in most part of the year with a few exceptions (Fig. 4.11). A similar trend was observed when Pb contents of algae were averaged over the whole study period (Fig. 4.12). The values for chlorophyceae and rhodophyceae were 7.26 and 11.39 $\mu g\ g^{-1}$ respectively at Ettikkulam and 7.45 and 9.57 $\mu g\ g^{-1}$ respectively at Narakkal.

Considering the temporal variations in the Pb accumulation in individual species, wide variations were observed (Fig. 4.13). At Ettikkulam, the chlorophyte *Chaetomorpha antennina* recorded Pb contents varying from non-detectable level (mid winter monsoon) to 16.41 $\mu g\ g^{-1}$ (mid hot season). *Ulva lactuca* showed values in the range 3.48 (summer monsoon) – 11.94 $\mu g\ g^{-1}$ (late hot season). *Enteromorpha intestinalis* (present only in summer monsoon season) showed a value of 0.99 µg g^{-1}. Among rhodophyceae, *Gracilaria corticata* recorded Pb contents ranging from non-detectable level (summer monsoon and retreating monsoon seasons) to 17.41 µg g^{-1} (early winter monsoon), while *Centroceras clavulatum* recorded values in the range 3.99 (mid winter monsoon) – 14.98 µg g^{-1} (late hot season). *Grateloupia filicina* (present only in the retreating monsoon season) showed a value of 9.48 µg g^{-1}. At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* showed Pb contents below detectable level in the retreating monsoon and summer monsoon seasons respectively. *Chaetomorpha antennina* recorded Pb content of 1.99 µg g^{-1} in the summer monsoon season and *Enteromorpha intestinalis* showed a value of 12.91 µg g^{-1} in the late hot season. Among the rhodophyceae, *Centroceras clavulatum* exhibited Pb contents in the range 1.99 (summer monsoon and retreating monsoon seasons) – 13.19 µg g^{-1} (early winter monsoon season). *Grateloupia filicina* recorded values ranging from non-detectable level (retreating monsoon season) to 18.92 µg g^{-1} (early winter monsoon season).

Spatial variations were observed in the Pb content of marine algae. Chlorophyceae showed higher accumulation of Pb at Ettikkulam than at Narakkal in the summer monsoon and retreating monsoon seasons, while reverse was true in the late hot season (Fig. 4.14). In other seasons, chlorophyceae members were absent from Narakkal. Rhodophyceae showed higher values of Pb at Ettikkulam in the late hot season, retreating monsoon and early winter monsoon seasons, whereas higher values were observed at Narakkal in other seasons. When Pb contents of algae were averaged over the whole study period, chlorophyceae showed comparable values at the two locations (7.26 μg g^{-1} at Ettikkulam and 7.45 μg g^{-1} at Narakkal), while rhodophyceae showed slightly higher value at Ettikkulam (9.57 μg g^{-1} at Narakkal and 11.39 μg g^{-1} at Ettikkulam) (Fig. 4.15). Considering spatial variation of Pb content in individual species, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* were observed to show higher Pb accumulation at Ettikkulam than at Narakkal (Figs. 4.16 – 4.18). The rhodophyte *Grateloupia filicina* also showed higher value at Ettikkulam. *Centroceras clavulatum* showed higher value at Ettikkulam in the late hot season and retreating monsoon season, while reverse case was observed in the mid winter monsoon season.

4.2.3.11 Cadmium

Cadmium is a non-essential element and also one of the most toxic elements. In the present study, Cd content of marine algae varied in the range 0.30 – 6.69 μg g^{-1} (Tables 4.4 and 4.5). The minimum and maximum values were shown by the members of rhodophyceae collected from different locations in the same period. *Centroceras clavulatum* from Narakkal recorded the minimum value, while *Gracilaria corticata* from Ettikkulam showed the maximum value, both in the late hot season. The Cd content of chlorophyceae members ranged from 0.84 to 5.88 μg g^{-1} with *Enteromorpha intestinalis* collected from Narakkal in the late hot season showing the minimum value and *Chaetomorpha antennina* from Ettikkulam showing the maximum value in mid winter monsoon and mid hot seasons. At Ettikkulam, lowest Cd content was recorded by the chlorophyte *Enteromorpha intestinalis* in the summer monsoon season (1.29 μg g^{-1}). Among rhodophyceae of Ettikkulam, least accumulation of Cd was observed in *Gracilaria corticata* in the retreating monsoon season (2.04 μg g^{-1}), while the same species showed the maximum Cd content in the late hot season (6.69 μg g^{-1}). At Narakkal, highest Cd accumulation (4.99 μg g^{-1}) was shown by the rhodophyte *Grateloupia filicina* in the retreating monsoon season. Among chlorophyceae, *Enteromorpha intestinalis* collected in the summer monsoon season recorded the highest Cd content (2.54 μg g^{-1}).

Species to species variability in the accumulation of Cd was observed to be very small (Fig. 4.10). At Narakkal, the chlorophytes *Chaetomorpha antennina* and *Enteromorpha intestinalis* recorded average Cd contents of 1.35 and 1.69 μg g^{-1} respectively, while the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina* showed the values of 2.64 and 2.76 μg g^{-1} respectively. At Ettikkulam, the chlorophytes *Chaetomorpha antennina* and *Ulva lactuca* recorded average Cd contents of 4.25 and 3.87 μg g^{-1} respectively. *Enteromorpha intestinalis* (present only in the summer monsoon season) showed Cd content of 1.29 μg g^{-1}. The rhodophytes *Gracilaria corticata* and *Centroceras clavulatum* exhibited average Cd contents of 4.30 and 2.83 μg g^{-1} respectively. *Grateloupia filicina* (present only in the retreating monsoon season) recorded a value of 4.79 μg g^{-1}.

Marine algae were found to exhibit temporal variations in their Cd contents. The chlorophyceae from Ettikkulam recorded average Cd contents in the range 2.61 (summer monsoon) – 5.54 μg g^{-1} (mid winter monsoon) while that from Narakkal showed values ranging from 0.84 (late hot season) to 2.07 μg g^{-1} (summer monsoon) (Fig. 4.11). The rhodophyceae from Ettikkulam exhibited average Cd contents varying in the range 3.22 (retreating monsoon) – 4.86 μg g^{-1} (mid hot season), whereas that from Narakkal recorded values in the range 1.55 (late hot season) – 3.89 μg g^{-1} (retreating monsoon). A classwise comparison showed that chlorophyceae recorded lower Cd content than rhodophyceae whenever they occurred at Narakkal (Fig. 4.11). A similar trend was observed at Ettikkulam in the late hot season, summer monsoon and early winter monsoon, whereas reverse was true in the other seasons. When Cd contents of algae were averaged over the whole study period, chlorophyceae showed lower Cd accumulation at Narakkal compared to rhodophyceae (1.52 and 2.70 μg g^{-1} respectively) whereas comparable values were observed at Ettikkulam (chlorophyceae: 3.85 μg g^{-1} and rhodophyceae: 3.91 μg g^{-1}) (Fig. 4.12).

Considering the temporal variation of Cd accumulation in individual species of algae (Fig. 4.13), the chlorophyte *Chaetomorpha antennina* from Ettikkulam was found to show Cd contents in the range 2.18 (late hot season) – 5.88 μg g^{-1} (mid winter monsoon and mid hot season). *Ulva lactuca* recorded values ranging from 2.09 (retreating monsoon) – 5.48 μg g^{-1} (late hot season). *Enteromorpha intestinalis* showed Cd content of 1.29 μg g^{-1} in the summer monsoon season. Among the rhodophyceae members, *Gracilaria corticata* exhibited Cd contents varying from 2.04 μg g^{-1} in the retreating monsoon season to 6.69 μg g^{-1} in the late hot season. *Centroceras clavulatum* showed values in the range 2.25 (late hot season) – 3.39 μg g^{-1} (mid winter monsoon). *Grateloupia filicina* recorded Cd content of 4.79 μg g^{-1} in the retreating monsoon season. At Narakkal, the chlorophyte *Chaetomorpha antennina* exhibited Cd contents of 1.59 μg g^{-1} in the summer monsoon season and 1.10 μg g^{-1} in the retreating monsoon season, while *Enteromorpha intestinalis* showed Cd contents of 0.84 μg g^{-1} in the late hot season and 2.54 μg g^{-1} in the summer monsoon season. Among the rhodophyceae members, *Centroceras clavulatum* recorded Cd contents in

Table 4.6. The mean values of ash, chlorine and iodine contents of marine algae from Kerala coast

	Ash (%)	Chlorine (mg g^{-1})	Iodine (mg g^{-1})
Chaetomorpha antennina	13.67	4.07	0.42
Ulva lactuca	15.03	2.68	0.21
Enteromorpha intestinalis	19.91	3.20	0.18
Gracilaria corticata	7.78	1.46	0.32
Centroceras clavulatum	19.44	1.81	0.90
Grateloupia filicina	12.85	1.43	0.77

Table 4.7. The average trace metal contents (μg g^{-1}) of marine algae from Kerala coast

	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe (x 10^3)
C. antennina	6.39	21.40	41.57	7.64	21.32	3.53	4.70	41.62	8.62	1.08	4.54
U. lactuca	5.63	12.84	26.33	7.05	2.89	3.87	4.61	27.89	6.16	1.97	0.74
E. intestinalis	6.11	20.99	50.35	6.95	13.23	1.56	7.31	21.18	14.68	1.50	4.50
G. corticata	3.44	14.21	150.20	12.93	4.83	4.30	4.64	18.60	8.08	2.79	1.42
C. clavulatum	10.20	31.98	102.16	7.87	18.62	2.70	6.44	14.48	14.29	1.53	9.01
G. filicina	5.74	56.69	44.25	12.87	9.98	3.10	6.81	51.94	9.66	2.25	2.83

the range 0.30 (late hot season) – 4.09 μg g^{-1} (mid hot season), while *Grateloupia filicina* showed values in the range 0.90 (early winter monsoon season) – 4.99 μg g^{-1} (retreating monsoon season).

Spatial variations were observed in the Cd accumulation by marine algae. Chlorophyceae showed higher Cd contents at Ettikkulam than at Narakkal (Fig. 4.14). Rhodophyceae also showed a similar trend except in the retreating monsoon season when reverse was true. A similar trend was observed when Cd contents of algae were averaged over the whole study period (Fig. 4.15). Chlorophyceae showed the average values 3.85 and 1.52 $\mu g g^{-1}$ at Ettikkulam and Narakkal respectively, while for rhodophyceae, the respective values were 3.91 and 2.70 $\mu g\ g^{-1}$. Considering spatial variations of Cd content in individual species, the chlorophyte *Chaetomorpha antennina* and the rhodophyte *Centroceras clavulatum* were found to show higher Cd contents at Ettikkulam (Figs. 4.16 – 4.18). The chlorophyte *Enteromorpha intestinalis* and the rhodophyte *Grateloupia filicina*, which occurred at both the locations only in one season, showed higher Cd accumulation at Narakkal than at Ettikkulam.

The mean values for ash, halogens and trace metals in different species of marine algae from Kerala coast are given in Tables 4.6 and 4.7.

4.2.4 Statistical Analysis

The significance of the observed variations in the inorganic constituents was tested statistically. Interspecies variations were tested by one-way ANOVA, while interclass and spatial comparisons were made by Student's t-test. The analysis showed that the interspecies variations in the ash content of marine algae were statistically significant ($P< 0.05$) at Ettikkulam only (Tables 4.8 and 4.9). The variations were found to be significant for the algal combinations *Chaetomorpha antennina – Centroceras clavulatum*, *Ulva lactuca – Gracilaria corticata* and *C. clavulatum – G. corticata* (Table 4.10). The chlorophytes from this location did not show any significant variation in the ash content among themselves ($P> 0.05$). Although chlorophyceae showed slightly higher ash content than rhodophyceae at both the locations, these differences were not statistically significant ($P> 0.05$) (Tables 4.11 and 4.12). The ash content of rhodophyceae showed

Table. 4.8. One-way ANOVA results for interspecies variations in ash, halogens and trace metal contents of marine algae from Ettikkulam.

		Sum of Squares	df	Mean Square	F	P
Ash	Between Groups	420.234	5	84.047	6.813	0.001
	Within Groups	209.704	17	12.336		
	Total	629.938	22			
Chloride	Between Groups	13.863	5	2.773	1.269	0.322
	Within Groups	37.141	17	2.185		
	Total	51.003	22			
Iodide	Between Groups	2.822	5	0.564	33.023	0.000
	Within Groups	.273	16	0.017		
	Total	3.096	21			
Cu	Between Groups	131.752	5	26.350	7.819	0.001
	Within Groups	57.290	17	3.370		
	Total	189.042	22			
Zn	Between Groups	747.697	5	149.539	5.710	0.003
	Within Groups	445.190	17	26.188		
	Total	1192.887	22			
Mn	Between Groups	61740.534	5	12348.107	12.269	0.000
	Within Groups	17109.981	17	1006.469		
	Total	78850.515	22			
Pb	Between Groups	151.831	5	30.366	1.525	0.245
	Within Groups	278.688	14	19.906		
	Total	430.519	19			

Cr	Between Groups	823.364	5	164.673	2.382	0.001
	Within Groups	829.658	12	69.138		
	Total	1653.023	17			
Cd	Between Groups	12.743	5	2.549	1.280	0.318
	Within Groups	33.846	17	1.991		
	Total	46.589	22			
Co	Between Groups	33.550	5	6.710	1.268	0.323
	Within Groups	89.979	17	5.293		
	Total	123.530	22			
Sr	Between Groups	2165.319	5	433.064	4.655	0.007
	Within Groups	1581.575	17	93.034		
	Total	3746.894	22			
Ni	Between Groups	243.512	5	48.702	1.550	0.227
	Within Groups	534.093	17	31.417		
	Total	777.605	22			
Ag	Between Groups	29.423	5	5.885	1.429	0.274
	Within Groups	57.644	14	4.117		
	Total	87.067	19			
Fe	Between Groups	296.707	5	59.341	9.350	0.000
	Within Groups	107.896	17	6.347		
	Total	404.603	22			

Table. 4.9. One-way ANOVA results for interspecies variations in ash, halogens and trace metal contents of marine algae from Narakkal.

		Sum of Squares	df	Mean Square	F	P
Ash	Between Groups	99.607	3	33.202	1.868	0.213
	Within Groups	142.220	8	17.777		
	Total	241.827	11			
Chloride	Between Groups	42.543	3	14.181	4.740	0.023
	Within Groups	32.907	11	2.992		
	Total	75.450	14			
Iodide	Between Groups	0.669	3	0.223	3.114	0.081
	Within Groups	0.644	9	0.072		
	Total	1.313	12			
Cu	Between Groups	61.133	3	20.378	3.082	0.072
	Within Groups	72.729	11	6.612		
	Total	133.862	14			
Zn	Between Groups	3064.440	3	1021.480	4.724	0.024
	Within Groups	2378.447	11	216.222		
	Total	5442.887	14			
Mn	Between Groups	9961.668	3	3320.556	9.072	0.003
	Within Groups	4026.328	11	366.030		
	Total	13987.996	14			
Pb	Between Groups	181.606	3	60.535	1.954	0.200
	Within Groups	247.852	8	30.981		
	Total	429.457	11			

Cr	Between Groups	395.308	3	131.769	1.526	0.263
	Within Groups	950.092	11	86.372		
	Total	1345.400	14			
Cd	Between Groups	4.243	3	1.414	0.831	0.504
	Within Groups	18.722	11	1.702		
	Total	22.964	14			
Co	Between Groups	22.312	3	7.437	1.155	0.374
	Within Groups	64.383	10	6.438		
	Total	86.695	13			
Sr	Between Groups	5716.965	3	1905.655	2.634	0.102
	Within Groups	7957.289	11	723.390		
	Total	13674.254	14			
Ni	Between Groups	31.088	3	10.363	0.230	0.874
	Within Groups	496.493	11	45.136		
	Total	527.581	14			
Ag	Between Groups	0.850	3	0.283	0.582	0.642
	Within Groups	4.382	9	0.487		
	Total	5.232	12			
Fe	Between Groups	89.333	3	29.778	4.548	0.026
	Within Groups	72.018	11	6.547		
	Total	161.350	14			

Table. 4.10. Results of post hoc test (Tukey's HSD) for parameters (ash, halogens and trace metals) showing significant interspecies variations in one-way ANOVA

Ettikkulam					Narakkal				
Parameter	Species	CA	UL	GC	Parameter	Species	CA	EI	CC
Ash	UL	NS			Cl	EI	NS		
	GC	NS	SIG			CC	SIG	NS	
	CC	SIG	NS	SIG		GF	SIG	NS	NS
I	UL	SIG			Zn	EI	NS		
	GC	NS	NS			CC	NS	NS	
	CC	SIG	SIG	SIG		GF	NS	SIG	NS
Cu	UL	NS			Mn	EI	NS		
	GC	NS	NS			CC	SIG	NS	
	CC	SIG	SIG	SIG		GF	NS	NS	SIG
Zn	UL	NS			Fe	EI	NS		
	GC	NS	NS			CC	NS	NS	
	CC	NS	SIG	SIG		GF	NS	NS	SIG
Mn	UL	NS							
	GC	SIG	SIG						
	CC	SIG	SIG	NS					
Cr	UL	SIG							
	GC	SIG	NS						
	CC	NS	SIG	SIG					
Sr	UL	NS							
	GC	NS	NS						
	CC	SIG	SIG	NS					
Fe	UL	SIG							
	GC	NS	NS						
	CC	SIG	SIG	SIG					

CA: *Chaetomorpha antennina* UL: *Ulva lactuca* EI: *Enteromorpha intestinalis*
GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*
SIG: Significant at 0.05 level NS: Not significant

Table 4.11. Results of t-test for classwise comparisons of ash, halogens and trace metal contents of marine algae from Ettikkulam

	Class	N	Mean	Standard Deviation	t	df	P
Ash	Chlorophyceae	6	14.5683	1.9085	2.081	10	0.064
	Rhodophyceae	6	10.9133	3.8553			
Cl	Chlorophyceae	6	3.0650	0.9922	2.578	10	0.027
	Rhodophyceae	6	1.8467	0.5961			
I	Chlorophyceae	6	0.3417	0.1143	-1.256	10	0.238
	Rhodophyceae	6	0.5033	0.2940			
Cu	Chlorophyceae	6	5.9783	1.3402	0.173	10	0.866
	Rhodophyceae	6	5.8400	1.4318			
Zn	Chlorophyceae	6	15.2317	2.5909	-1.278	10	0.230
	Rhodophyceae	6	18.9317	6.5992			
Mn	Chlorophyceae	6	34.3117	7.0763	-4.823	10	0.001
	Rhodophyceae	6	141.7900	54.1273			
Pb	Chlorophyceae	6	7.0850	4.5559	-1.435	9	0.185
	Rhodophyceae	5	11.0660	4.6163			
Cr	Chlorophyceae	6	32.2233	43.6292	-0.358	10	0.728
	Rhodophyceae	6	47.6283	95.9685			
Cd	Chlorophyceae	6	3.9533	1.1959	-0.208	10	0.840
	Rhodophyceae	6	4.0667	0.5945			
Co	Chlorophyceae	6	4.8583	1.8864	-0.305	10	0.767
	Rhodophyceae	6	5.1983	1.9798			
Sr	Chlorophyceae	6	31.0033	8.3217	3.308	10	0.008
	Rhodophyceae	6	16.7633	6.4760			
Ni	Chlorophyceae	6	6.8100	4.3536	-0.956	10	0.362
	Rhodophyceae	6	9.7267	6.0762			
Ag	Chlorophyceae	5	1.4900	1.2582	-1.057	8	0.321
	Rhodophyceae	5	2.7700	2.3965			
Fe	Chlorophyceae	6	2.8350	0.7219	-0.766	10	0.461
	Rhodophyceae	6	3.9537	3.5025			

Table 4.11. Results of t-test for classwise comparisons of ash, halogens and trace metal contents of marine algae from Ettikkulam

	Class	N	Mean	Standard Deviation	t	df	P
Ash	Chlorophyceae	6	14.5683	1.9085	2.081	10	0.064
	Rhodophyceae	6	10.9133	3.8553			
Cl	Chlorophyceae	6	3.0650	0.9922	2.578	10	0.027
	Rhodophyceae	6	1.8467	0.5961			
I	Chlorophyceae	6	0.3417	0.1143	-1.256	10	0.238
	Rhodophyceae	6	0.5033	0.2940			
Cu	Chlorophyceae	6	5.9783	1.3402	0.173	10	0.866
	Rhodophyceae	6	5.8400	1.4318			
Zn	Chlorophyceae	6	15.2317	2.5909	-1.278	10	0.230
	Rhodophyceae	6	18.9317	6.5992			
Mn	Chlorophyceae	6	34.3117	7.0763	-4.823	10	0.001
	Rhodophyceae	6	141.7900	54.1273			
Pb	Chlorophyceae	6	7.0850	4.5559	-1.435	9	0.185
	Rhodophyceae	5	11.0660	4.6163			
Cr	Chlorophyceae	6	32.2233	43.6292	-0.358	10	0.728
	Rhodophyceae	6	47.6283	95.9685			
Cd	Chlorophyceae	6	3.9533	1.1959	-0.208	10	0.840
	Rhodophyceae	6	4.0667	0.5945			
Co	Chlorophyceae	6	4.8583	1.8864	-0.305	10	0.767
	Rhodophyceae	6	5.1983	1.9798			
Sr	Chlorophyceae	6	31.0033	8.3217	3.308	10	0.008
	Rhodophyceae	6	16.7633	6.4760			
Ni	Chlorophyceae	6	6.8100	4.3536	-0.956	10	0.362
	Rhodophyceae	6	9.7267	6.0762			
Ag	Chlorophyceae	5	1.4900	1.2582	-1.057	8	0.321
	Rhodophyceae	5	2.7700	2.3965			
Fe	Chlorophyceae	6	2.8350	0.7219	-0.766	10	0.461
	Rhodophyceae	6	3.9537	3.5025			

Table 4.12. Results of t-test for classwise comparisons of ash, halogens and trace metal contents of marine algae from Narakkal.

Parameter	Class	N	Mean	Standard Deviation	t	df	P
Ash	Chlorophyceae	3	18.4667	4.1029	0.363	6	0.729
	Rhodophyceae	5	17.2620	4.7463			
Cl	Chlorophyceae	3	5.6800	3.2705	3.477	7	0.010
	Rhodophyceae	6	1.2800	0.4530			
I	Chlorophyceae	3	0.2200	0.0458	-2.944	6	0.026
	Rhodophyceae	5	0.7180	0.2818			
Cu	Chlorophyceae	3	7.2733	1.8471	-0.307	7	0.767
	Rhodophyceae	6	7.7933	2.5773			
Zn	Chlorophyceae	3	26.8233	3.4319	-2.098	7	0.074
	Rhodophyceae	6	45.3233	14.5928			
Mn	Chlorophyceae	3	46.3000	13.9391	-2.020	7	0.083
	Rhodophyceae	6	73.9400	21.1335			
Pb	Chlorophyceae	2	6.9550	8.4216	-0.361	6	0.730
	Rhodophyceae	6	8.8117	5.7800			
Cr	Chlorophyceae	3	64.1833	49.4374	2.509	7	0.040
	Rhodophyceae	6	16.2900	6.5413			
Cd	Chlorophyceae	3	1.3367	0.6483	-2.492	7	0.041
	Rhodophyceae	6	2.6817	0.8048			
Co	Chlorophyceae	3	4.2833	3.8580	-0.833	7	0.432
	Rhodophyceae	6	5.9600	2.3208			
Sr	Chlorophyceae	3	36.9833	16.9645	0.432	7	0.679
	Rhodophyceae	6	31.7167	17.3595			
Ni	Chlorophyceae	3	12.8500	3.4171	-0.061	7	0.953
	Rhodophyceae	6	13.0600	5.3909			
Ag	Chlorophyceae	3	1.6000	0.3279	1.040	6	0.339
	Rhodophyceae	5	1.2640	0.4899			
Fe	Chlorophyceae	3	5.1671	2.0759	-0.009	7	0.993
	Rhodophyceae	6	5.1794	1.9237			

Table 4.13. Results of t-test for spatial comparisons of ash, halogens and trace metal contents of marine algae from Kerala coast.

Parameter	Location	N	Mean	Standard Deviation	t	df	P
Chlorophyceae							
Ash	Ettikkulam	6	14.5683	1.9085	-2.025	7	0.083
	Narakkal	3	18.4667	4.1029			
Cl	Ettikkulam	6	3.0650	0.9922	-1.907	7	0.098
	Narakkal	3	5.6800	3.2705			
I	Ettikkulam	6	0.3417	0.1143	1.727	7	0.128
	Narakkal	3	0.2200	0.0458			
Cu	Ettikkulam	6	5.9783	1.3402	-1.219	7	0.262
	Narakkal	3	7.2733	1.8471			
Zn	Ettikkulam	6	15.2317	2.5909	-5.739	7	0.001
	Narakkal	3	26.8233	3.4319			
Mn	Ettikkulam	6	34.3117	7.0763	-1.775	7	0.119
	Narakkal	3	46.3000	13.9391			
Pb	Ettikkulam	6	7.0850	4.5559	0.030	6	0.977
	Narakkal	2	6.9550	8.4216			
Cr	Ettikkulam	6	32.2233	43.6292	-0.996	7	0.352
	Narakkal	3	64.1833	49.4374			
Cd	Ettikkulam	6	3.9533	1.1959	3.463	7	0.010
	Narakkal	3	1.3367	0.6483			
Co	Ettikkulam	6	4.8583	1.8864	0.312	7	0.764
	Narakkal	3	4.2833	3.8580			
Sr	Ettikkulam	6	31.0033	8.3217	-0.737	7	0.485
	Narakkal	3	36.9833	16.9645			
Ni	Ettikkulam	6	6.8100	4.3536	-2.079	7	0.076
	Narakkal	3	12.8500	3.4171			
Ag	Ettikkulam	5	1.4900	1.2582	-0.144	6	0.890
	Narakkal	3	1.6000	0.3279			
Fe	Ettikkulam	6	2.8350	0.7219	-2.604	7	0.035
	Narakkal	3	5.1671	2.0759			

Table 4.13. (*Continued*)

Parameter	Location	N	Mean	Standard Deviation	t	df	P
Rhodophyceae							
Ash	Ettikkulam	6	10.9133	3.8553	-2.453	9	0.037
	Narakkal	5	17.2620	4.7463			
Cl	Ettikkulam	6	1.8467	0.5961	1.854	10	0.093
	Narakkal	6	1.2800	0.4530			
I	Ettikkulam	6	0.5033	0.2940	-1.228	9	0.251
	Narakkal	5	0.7180	0.2818			
Cu	Ettikkulam	6	5.8400	1.4318	-1.623	10	0.136
	Narakkal	6	7.7933	2.5773			
Zn	Ettikkulam	6	18.9317	6.5992	-4.036	10	0.002
	Narakkal	6	45.3233	14.5928			
Mn	Ettikkulam	6	141.7900	54.1273	2.860	10	0.017
	Narakkal	6	73.9400	21.1335			
Pb	Ettikkulam	5	11.0660	4.6163	0.703	9	0.500
	Narakkal	6	8.8117	5.7800			
Cr	Ettikkulam	6	47.6283	95.9685	0.798	10	0.443
	Narakkal	6	16.2900	6.5413			
Cd	Ettikkulam	6	4.0667	0.5945	3.391	10	0.007
	Narakkal	6	2.6817	0.8048			
Co	Ettikkulam	6	5.1983	1.9798	-0.612	10	0.554
	Narakkal	6	5.9600	2.3208			
Sr	Ettikkulam	6	16.7633	6.4760	-1.977	10	0.076
	Narakkal	6	31.7167	17.3595			
Ni	Ettikkulam	6	9.7267	6.0762	-1.005	10	0.339
	Narakkal	6	13.0600	5.3909			
Ag	Ettikkulam	5	2.7700	2.3965	1.377	8	0.206
	Narakkal	5	1.2640	0.4899			
Fe	Ettikkulam	6	3.9537	3.5025	-0.751	10	0.470
	Narakkal	6	5.1794	1.9237			

Table 4.13. (*Continued*)

Parameter	Location	N	Mean	Standard Deviation	t	df	P
Whole algae							
Ash	Ettikkulam	23	13.5443	5.3510	-1.768	33	0.086
	Narakkal	12	16.7800	4.6887			
Cl	Ettikkulam	23	2.5413	1.5226	0.504	36	0.617
	Narakkal	15	2.2277	2.3215			
I	Ettikkulam	22	0.4552	0.3840	-0.727	33	0.472
	Narakkal	13	0.5482	0.3308			
Cu	Ettikkulam	23	6.0391	2.9313	-1.385	36	0.175
	Narakkal	15	7.4153	3.0922			
Zn	Ettikkulam	23	17.4326	7.3636	-5.355	36	0.000
	Narakkal	15	41.5613	19.7174			
Mn	Ettikkulam	23	75.8826	59.8675	0.617	36	0.541
	Narakkal	15	65.4893	31.6092			
Pb	Ettikkulam	20	8.9140	4.7601	-0.155	30	0.878
	Narakkal	12	9.2175	6.2483			
Cr	Ettikkulam	18	13.3328	9.8609	-0.529	31	0.600
	Narakkal	15	15.1527	9.8031			
Cd	Ettikkulam	23	3.8726	1.4552	3.230	36	0.003
	Narakkal	15	2.3827	1.2808			
Co	Ettikkulam	23	5.1857	2.3696	-1.296	35	0.204
	Narakkal	14	6.2621	2.5824			
Sr	Ettikkulam	23	24.9904	13.0504	-1.532	36	0.134
	Narakkal	15	36.1733	31.2527			
Ni	Ettikkulam	23	8.3517	5.9452	-2.256	36	0.030
	Narakkal	15	12.8607	6.1388			
Ag	Ettikkulam	20	2.0665	2.1407	1.107	31	0.277
	Narakkal	13	1.3862	0.6603			
Fe	Ettikkulam	23	3.7121	4.2885	-1.001	36	0.324
	Narakkal	15	5.0292	3.3949			

significant differences ($P<0.05$) between the two locations, whereas that of chlorophyceae was statistically indistinguishable ($P>0.05$) (Table 4.13). At Ettikkulam, iodine contents showed highly significant interspecies variations ($P<0.001$) while chlorine contents were not significantly different (Table 4.8). A reverse case was observed at Narakkal (Table 4.9). Here, iodine contents of different species were statistically indistinguishable ($P>0.05$), whereas chlorine contents showed significant specieswise variation ($P<0.05$). Interspecies variations in iodine contents were significant for the algal combinations *Chaetomorpha antennina* – *Ulva lactuca*, *C. antennina* – *Centroceras clavulatum*, *U. lactuca* – *C. clavulatum* and *Gracilaria corticata* – *C. clavulatum*, whereas those for chlorine contents were *C. antennina* – *C. clavulatum* and *C. antennina* – *Grateloupia filicina* (Table 4.10). Both iodine and chlorine exhibited significant interclass variations ($P<0.05$) at Narakkal (Table 4.12), while differences were significant only for chlorine at Ettikkulam (Table 4.11). The spatial variations observed in the halogen contents were not statistically significant (Table 4.13).

Among the trace metals, Zn, Mn and Fe showed significant differences among algal species at both the locations (Tables 4.8 and 4.9). Cu, Cr and Sr showed significant variations at Ettikkulam. Other metals did not show any significant interspecies variation. Comparison of different species of algae on the basis of ANOVA and Tukey's post hoc test are given in Table 4.10 (multiple comparison tables are given as appendices). Interclass variations were significant only for Mn and Sr at Ettikkulam (Table 4.11) and for Cr and Cd at Narakkal (Table 4.12). Taking all the algal species together, significant spatial variations were observed only for Zn, Cd and Ni (Table 4.13). Both chlorophyceae and rhodophyceae showed significant spatial variations in Zn and Cd contents. In addition, chlorophyceae exhibited

Table 4.14. Correlation matrix and Pearson correlation coefficients between different inorganic and organic constituents of marine algae from Ettikkulam

Variables	Ash	Cl	I	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe
Proteins	-0.245	-0.055	-0.235	-0.020	-0.093	-0.281	-0.323	0.090	0.210	-0.313	0.264	-0.192	-0.153	-0.347
Lipids	0.068	0.096	0.160	0.129	-0.349	-0.285	-0.028	0.673[a]	0.433[b]	-0.480[b]	0.468[b]	-0.063	0.056	-0.096
TC	-0.317	-0.463[b]	0.012	-0.208	0.012	0.623[a]	0.129	-0.606[a]	-0.204	0.114	-0.484[b]	0.193	0.289	-0.223
LMWC	-0.650[a]	-0.353	-0.298	-0.530[a]	-0.237	0.768[a]	0.505[b]	-0.501[b]	0.174	0.010	-0.228	0.020	-0.015	-0.344
PS	-0.208	-0.437[b]	0.080	-0.113	0.066	0.522[b]	0.031	-0.566[b]	-0.266	0.125	-0.488[b]	0.211	0.322	-0.172
Fibres	-0.042	0.338	0.133	0.105	0.141	-0.225	0.051	0.605[a]	0.148	-0.024	0.297	-0.075	-0.269	0.243
CV	-0.490[b]	-0.519[b]	-0.096	-0.205	-0.148	0.421[b]	-0.112	-0.424	0.035	-0.216	-0.231	0.069	0.241	-0.503[b]
Ash		0.436[b]	0.505[b]	0.587[a]	0.280	-0.395	0.064	0.297	-0.220	0.062	0.052	0.207	-0.163	0.643[a]
Chlorine			0.067	0.180	0.132	-0.355	0.284	0.036	-0.190	0.028	0.110	0.165	-0.220	0.335
Iodine				0.770[a]	0.545[a]	0.107	0.191	0.316	-0.008	-0.012	-0.180	0.237	0.101	0.862[a]
Cu					0.596[a]	-0.059	0.119	0.363	0.094	0.034	-0.131	0.220	-0.021	0.582[a]
Zn						0.191	0.273	0.037	-0.099	0.225	-0.195	0.113	0.190	0.693[a]
Mn							0.542[b]	-0.321	0.102	0.200	-0.509[b]	0.307	-0.044	0.130
Pb								-0.080	0.403	-0.301	-0.023	-0.006	0.216	0.321
Cr									0.178	0.052	0.372	0.031	-0.002	0.288
Cd										-0.597[a]	0.334	-0.249	0.073	-0.234
Co											-0.473[b]	0.500[b]	-0.338	0.279
Sr												-0.267	0.086	-0.201
Ni													-0.395	0.225
Ag														-0.037

TC: Total carbohydrates LMWC: Low-molecular-weight carbohydrates PS: Polysaccharides CV: Calorific value

[a] Significant at 0.01 level ($P<0.01$)

[b] Significant at 0.05 level ($P<0.05$)

Table 4.15. Correlation matrix and Pearson correlation coefficients between different inorganic and organic constituents of marine algae from Narakkal

Variables	Ash	Cl	I	Cu	Zn	Mn	Pb	Cr	Cd	Co	Sr	Ni	Ag	Fe
Proteins	0.167	-0.221	0.465	0.663[a]	0.280	0.468	0.195	-0.163	0.073	0.209	0.139	0.302	-0.198	0.152
Lipids	0.488	0.278	0.567[b]	0.284	-0.204	0.277	0.013	0.426	0.172	-0.149	-0.227	0.167	0.012	-0.164
TC	-0.199	-0.548[b]	0.040	-0.224	0.312	0.207	-0.272	-0.028	0.587[b]	0.466	-0.103	0.028	0.244	-0.335
LMWC	0.785[a]	0.225	0.286	0.449	0.122	0.031	0.682[b]	-0.045	-0.167	-0.071	0.035	0.163	0.041	0.076
PS	-0.257	-0.557[b]	0.014	-0.258	0.296	0.200	-0.351	-0.023	0.590[b]	0.464	-0.104	0.013	0.236	-0.335
Fibres	-0.279	0.552	-0.217	-0.086	-0.146	-0.348	-0.345	-0.009	-0.370	-0.292	0.523	0.096	-0.233	-0.280
CV	0.087	-0.548[b]	0.585[b]	0.379	0.417	0.585[b]	-0.056	-0.048	0.576[b]	0.474	-0.033	0.290	0.090	-0.201
Ash		0.149	0.317	0.667[b]	-0.439	0.513	0.708[b]	0.468	-0.153	-0.029	-0.479	0.326	0.501	0.595[b]
Chlorine			-0.365	0.063	-0.309	-0.394	0.076	0.029	-0.466	-0.597[b]	-0.003	-0.016	0.039	-0.009
Iodine				0.340	0.324	0.343	0.590	0.284	0.404	0.267	-0.190	0.229	-0.075	-0.002
Cu					-0.092	0.708[a]	0.058	0.372	0.031	-0.166	-0.268	0.364	0.173	0.541[b]
Zn						-0.324	0.240	-0.338	0.241	0.135	0.573'	-0.031	-0.276	-0.452
Mn							-0.183	0.475	0.322	0.220	-0.549[b]	0.349	0.374	0.454
Pb								-0.178	-0.117	0.370	0.036	0.142	0.278	-0.059
Cr									0.111	-0.406	-0.384	0.082	0.276	0.261
Cd										0.387	-0.030	-0.460	-0.184	-0.340
Co											-0.027	0.206	0.246	-0.268
Sr												-0.273	-0.347	-0.523[b]
Ni													0.331	0.255
Ag														0.164

TC: Total carbohydrates LMWC: Low molecular weight carbohydrates PS: Polysaccharides CV: Calorific value

[a] Significant at 0.01 level ($P<0.01$)

[b] Significant at 0.05 level ($P<0.05$)

significant variation for Fe and rhodophyceae for Mn. It can be seen that, most of the trace metals studied showed statistically indistinguishable variations.

Parameter Relationships

The correlation matrix and Pearson correlation coefficients between different inorganic and organic constituents are given in Tables 4.14 and 4.15. The ash content of algae from Ettikkulam showed a strong negative correlation ($P<0.01$) with low molecular weight carbohydrates (LMWC), whereas a strong positive correlation ($P<0.01$) was observed in algae of Narakkal. A significant negative correlation ($P<0.05$) was observed with calorific value of algae and this is in accordance with the fact that calorific value of algae decrease with increase in ash content. Ash contents of algae were observed to be positively intercorrelated with Fe and Cu at both the locations ($P<0.01$ at Ettikkulam and $P<0.05$ at Narakkal). The halogens chlorine and iodine also showed positive correlations with ash content at Ettikkulam ($P<0.05$). The chlorine content was negatively correlated with carbohydrates especially polysaccharides at both the locations ($P<0.05$). As a result, calorific value was also negatively correlated with chlorine content ($P<0.05$). At Narakkal, chlorine showed significant negative correlation with Co also ($P<0.05$). Iodine content of algae from Ettikkulam showed strong positive correlations with Fe, Cu and Zn ($P<0.01$). At Narakkal, it exhibited positive correlation with lipid content ($P<0.05$) and hence with calorific value.

Among the trace metals, only Cu and Fe showed significant correlations (positive) with ash content. At Narakkal, Pb content also showed significant positive correlation ($P<0.05$). Other metals did not significantly relate to ash content. At Ettikkulam, Mn content of algae exhibited strong positive correlation (P<0.01) with carbohydrates (LMWC, polysaccharides and total carbohydrates), while Cr and Sr showed significant negative correlations (P<0.05). Cr was positively correlated with fibre content (P<0.01). Pb showed significant positive correlation with LMWC (P<0.05), while Cu showed a strong negative correlation (P<0.01). Cr, Cd and Sr showed positive correlations with lipids, whereas Co showed significant negative correlation (P<0.05). Co also showed negative correlation with Cd and Sr and positive correlation with Ni. Zn contents were closely intercorrelated (positively) with Cu and Fe contents (P<0.01). Mn showed significant positive correlation with Pb and negative correlation with Sr (P<0.05). Ag did not show any significant correlation with other inorganic constituents at both the locations. At Narakkal, Ni and Cr also showed no significant correlations with other trace minerals. Here, Sr showed positive correlation with Zn (P<0.05). It also showed a negative correlation with Fe and Mn (P<0.05), while Cu showed a positive correlation with these metals. Cd exhibited positive correlation with carbohydrates especially polysaccharides. Pb showed a positive correlation with LMWC (P<0.05).

4.3 Discussion

In the present study, the total mineral contents (total inorganic constituents) of marine algae, represented by their ash contents, were found to show values that fit well within the wide range, from 6 to 83% of algal dry weight, reported earlier for marine algae from different parts of the world (Paine and Vadas, 1969; Joshi and Gowda, 1975; Mehta and Baxi, 1976; Parekh *et al.*, 1977; Sumitra *et al.*, 1980; Whyte and Englar, 1980a; McQuaid, 1985; Ito and Hori, 1989; Indergaard and Knutsen, 1990; Thomas and Subbaramaiah, 1991; Qari and Qasim, 1993; Ruperez, 2002). Rhodophyceae members showed ash contents varying in the range 6.36 – 28.13%, while chlorophyceae members recorded values in the range 8.99 – 23.16 %. Paine and Vadas (1969) reported ash contents of seventy marine macro algae, varying in the range 9 – 83%. Joshi and Gowda (1975) studied seasonal variation in the ash content of algae from Ratnagiri coast and obtained values in the range 27.93 – 35.48% with an average value of 31.14%. Mehta and Baxi (1976) determined the ash content of marine algae from Okha coast and Krusedai islands and obtained values varying in the range 24 - 40%. Parekh *et al.* (1977) studied the ash content of 27 species of marine algae from Saurashtra and reported values in the range 12 – 70%. Sumitra *et al.* (1980) recorded ash contents varying in the range 20 – 35% in marine algae from Goa coast. Indergaard and Knutsen (1990) obtained ash contents in the range 23.3 – 33.6% in marine red algae. Qari and Qasim (1993) reported ash contents varying in the ranges 22.30 – 41.90% for chlorophyceae and 15.30 – 40.85% for rhodophyceae from Karachi coast.

In the present study, most of the algal species recorded lower values of ash contents compared to marine algae from other coastal regions. *Enteromorpha intestinalis* recorded average ash content of 19.91%, while *Ulva lactuca* showed a value of 15.03% (Table 4.6). Paine and Vadas (1969) recorded ash contents of 36 and 21% in *Enteromorpha* sp. and *Ulva lactuca* respectively. They obtained a value of 19% for *Grateloupia doryphora*, while in the present study, *Grateloupia filicina* showed average ash content of 12.85%. Parekh *et al.* (1977) observed mean values of 43.50% and 23.50% for the ash contents of *Enteromorpha* and *Ulva* from Saurashtra coast. Qari and Qasim (1993) reported ash contents of 26.10% and 27.65% in *Ulva fasciata* from two locations along the Karachi coast. They obtained the values 22.30% and 31.60% for *Chaetomorpha antennina* from Buleji and Manora coasts respectively. In the present study, *Chaetomorpha antennina* recorded average ash content of 13,67%. Sumitra *et al.* (1980) obtained ash contents varying in the range 20 – 35% in *Gracilaria corticata* from Goa coast while this species recorded low values varying in the range 6.36 - 9.30% (mean = 7.78%) during the present study. McQuaid (1985) reported ash contents of *Centroceras clavulatum* from Cape of Good Hope varying in the range 20 – 40%, whereas in the present study, the values for the same species ranged from 12.57 to 28.13% (mean = 19.44%). He obtained ash contents varying in the range 15 – 30% for *Ulva* sp.

During the present investigation, the rhodophyte *Centroceras clavulatum* recorded the highest ash content at both the locations. This species belongs to the order Ceramiales and it has been found that algae of this order are good concentrators of many inorganic elements (Saenko *et al.*, 1976 & 1978). Among the chlorophyceae members, *Enteromorpha intestinalis* showed the highest ash content (23.16%). Several authors have reported higher amounts of inorganic constituents in the genus *Enteromorpha* compared to *Ulva* and *Chaetomorpha* (Parekh *et al.*, 1977; Agadi *et al.*, 1978; Rao *et al.*, 1995; Villares *et al.*, 2001 & 2002). The rhodophyte *Gracilaria corticata* recorded ash contents below 10% with an average value of 7.78%, whereas all other species recorded average ash contents above 12%. The low ash content of this species may be attributed to its high carbohydrate content (34%). High carbohydrate content is accompanied by a high level of dry solid, which results in low level of inorganic constituents determined on the dry weight basis. However, *Enteromorpha intestinalis* showed fairly high ash contents when carbohydrate levels were high (over 30%). This may be due to the fact that this species is susceptible to contamination by fine particulate materials adhering to the thallus, which is favoured by its morphology (Bryan *et al.*, 1985; Villares *et al.*, 2001).

Considering the spatial variations in the ash content, higher values were observed in the algae from Narakkal than those from Ettikkulam. This may be attributed to a possibly higher level of inorganic constituents in seawater at Narakkal due to its proximity to the Cochin barmouth through which the Cochin backwaters open out into the Arabian Sea. This backwater is intensely polluted due to the inputs from several major and minor industries situated in the upstream region. Several rivers, irrigation channels and sewers open into this backwater making it polluted. The inorganic pollutants delivered to the coastal waters through barmouth may have an influence on the ash content of marine algae present at Narakkal.

Marine algae have been found to show temporal variations in their ash content. Black (1950) observed maximum ash content in marine algae at the beginning of spring (March - April) and minimum in autumn (September – October). Joshi and Gowda (1975) studying the seasonal variation in the chemical composition of algae from Ratnagiri coast observed that inorganic constituents were minimum in the monsoon season. Whyte and Englar (1980a) studied seasonal variation in the ash content of algae from Canada and found maximum in April. McQuaid (1985) reported seasonal variation in the ash content of marine algae from Cape of Good Hope with maximum in spring declining through summer to a minimum in winter. Indergaard and Knutsen (1990) studying the seasonal differences in the ash content of algae from Norway obtained maximum value in March and minimum in June. Villares *et al.* (2002) studied the seasonal variation of inorganic constituents such as metals and they obtained values rising from March to a peak in May. The concentrations then fell during the summer with minimum values being found between June and September and the levels finally increased again during winter. In the present study, algae were found to record low values for ash contents in the summer monsoon season. In the retreating monsoon season, most of them showed low values with a few exceptions. In most cases values were higher than those in the summer monsoon season. The algae recorded variable ash contents in the early winter monsoon season with some showing moderate values while others high values. In the period from mid winter monsoon to hot season, algae recorded comparatively high values for ash contents. The ash contents showed moderate values in the late hot season.

There may be different reasons for the seasonal differences found, including the environmental factors such as variations in the concentration of inorganic constituents in the ambient seawater, interactions between different inorganic constituents, salinity, pH etc. and the metabolic factors such as dilution of inorganic constituents due to growth or they may be due to interactions between both kinds of factors. The idea that the inorganic constituents such as metals decrease in macroalgae during periods of growth and increase during dormant periods has long been considered (Fuge and James, 1974). The low values of ash contents observed in the summer monsoon season during the present study may be attributed to the environmental factors as well as the growth effect. The heavy rains during the wet summer monsoon season cause the dilution of seawater, which brings down the concentration of inorganic constituents in algae (Joshi and Gowda, 1975). Even if the increased fluvial input and land drainage during the rainy monsoon season cause an increase in the amount of inorganic constituents in seawater, the decrease in salinity produced by these inputs can lead to a decrease in the ash content of marine algae (Munda and

Kremer, 1977; Lobban and Harrison, 1997). Since the environmental conditions of the summer monsoon season are favourable for a high growth rate of algae, the low level of ash content observed in this season may also be attributed to the dilution of inorganic constituents in algae due to their active growth.

The higher ash content observed in most algae in the retreating monsoon season compared to that in the summer monsoon season may be due to the changes in the environmental conditions and growth rate of algae. The cessation of monsoon rains decreases the freshwater input from land, which causes an increase in the salinity of ambient seawater. This change in salinity is closely followed by the internal solute concentrations of algae (Dickson *et al.*, 1982) resulting in an increase in the ash content. A decrease in the growth rate of algae due to a possible switch from vegetative growth to reproductive development (Chapter 3) under the prevailing environmental conditions may also promote an increase in the ash content in the retreating monsoon season. The comparatively higher levels of ash contents observed in the early winter monsoon season may be due to the existence of higher rates of photosynthesis and respiration during this season, which would favour the assimilation of inorganic constituents such as metals (Catsiki and Papathanassiou, 1993). Although algae is expected to have a high growth rate in this season, its effect on ash content may be lower than that in the summer monsoon season because of the higher salinity of ambient seawater during this season compared to that in the summer monsoon season.

The high ash content observed in the period from mid winter monsoon season to hot season may be due to the interplay of environmental as well as metabolic factors. This period is characterized by progressively increasing temperature and light conditions. This increases the rates of photosynthesis and respiration, which promote the uptake by algae of inorganic elements such as metals. The increasing temperature and the low input of freshwater from land during this period, result in an increase in the salinity of seawater, which causes increased assimilation of inorganic ions by algae (Edwards *et al.*, 1987; Lobban and Harrison, 1997). A decrease in growth rate of algae due to increased respiration may also have an effect on the ash content of algae. Lower ash contents observed in some cases during the late hot season compared to that in early hot season may be due to the effect of emersion (Chapter 3) that causes a decrease in the uptake rate of inorganic elements from seawater.

Among the various inorganic constituents of algae, the halogens chlorine and iodine were found to show values varying in the ranges 0.53 – 8.49 mg g^{-1} (average = 2.42 mg g^{-1}) and 0.08 – 1.72 mg g^{-1} (average = 0.49 mg g^{-1}) respectively during the present study. These were in accordance with the earlier reports from different coastal regions. Rao (1992) carried out a compilation of data obtained by several authors on the elemental composition of marine algae from Indian coast and showed that the mean values for chlorine contents vary in the range 1.9 – 157.1 mg g^{-1} and that for iodine contents in the range 0.02 – 7.40 mg g^{-1}. Pillai (1956) reported chlorine contents in the range 2.5 – 27.5 mg g^{-1} and iodine in the range 0.90 – 5.00 mg g^{-1} in marine algae from Mandapam coast. Kappanna and Rao (1962) showed that iodine content of algae from Okha coast vary in the range 0.05 - 1.05 mg g^{-1}. Rao and Tipnis (1967) reported chlorine contents in the range 2.7 - 156.3 mg g^{-1} in marine algae from Gujarat coast. They recorded iodine contents in the range 0.05 – 0.65 mg g^{-1}, whereas Dave *et al.* (1969) obtained values in the range 0.04 – 5.57 mg g^{-1}. High values of chlorine contents were reported by Joshi and Gowda (1975) in marine algae from

Ratnagiri (68.1 – 98.9 mg g^{-1}) and by Mehta and Baxi (1976) in algae from Okha coast and Krusedai Island (14.65 – 99.22 mg g^{-1}). Solimabi and Das (1977) obtained iodine contents varying in the range 0.14 – 7.40 mg g^{-1} in algae from Goa coast. Saenko *et al.* (1978) reported iodine contents of marine algae from Sea of Japan and Okhotsk Sea varying in the ranges 0.02 - 7.50 mg g^{-1} and 0.02 – 4.50 mg g^{-1} respectively. Whyte and Englar (1980 b) reported high values of chlorine content in alga *Nereocystis* from Canada (151 mg g^{-1} in frond and 193 mg g^{-1} in stipe). They obtained iodine contents varying in the range 0.53 – 1.42 mg g^{-1} in fronds and 0.83 – 1.55 mg g^{-1} in stipe. Solimabi *et al.* (1981) studied the iodine content of algae from Andaman Sea and obtained values varying in the range 0.03 – 0.12 mg g^{-1}. Oza *et al.* (1983) recorded chlorine content of 21.2 mg g^{-1} in the alga *Monostroma* belonging to the order Ulvales. Thomas *et al.* (1987) estimated the iodine content of the rhodophyte *Asparagopsis* from Mandapam and obtained values ranging from 1.3 mg g^{-1} to 3.8 mg g^{-1}. Rao and Indusekhar (1989) reported the chlorine and iodine contents of marine algae from Saurashtra coast, which varied in the ranges 5.1 – 74.7 mg g^{-1} and 0.03 - 0.63 mg g^{-1} respectively. Thomas and Subbaramaiah (1991) recorded iodine contents varying from 0.3 to 3.5 mg g^{-1} in marine algae from Gulf of Mannar. Rao and Singbal (1995) reported high values of chlorine content (20 – 70 mg g^{-1}) in algae from Gujarat coast. They obtained iodine contents ranging from 0.04 to 0.34 mg g^{-1}. Devi *et al.* (1996) studied the abundance of iodine in marine algae from Cape Comorin and observed that the chlorophyte *Ulva fasciata* contained no iodine. They recorded a maximum iodine content of 0.71 mg g^{-1} in the rhodophyte *Sarconema*.

In the present study, the chlorophyte *Chaetomorpha antennina* recorded the average values 0.42 mg g^{-1} for iodine and 4.07 mg g^{-1} for chlorine (Table 4.6). The same species recorded lower iodine content (0.23 mg g^{-1}) at Cape Comorin (Devi *et al.*, 1996) and higher value (1.12 mg g^{-1}) at Goa (Solimabi and Das, 1977). This species showed a lower iodine content (0.07 mg g^{-1}) and a much higher chlorine content (63.93 mg g^{-1}) at Saurashtra coast (Rao and Indusekhar, 1989). *Chaetomorpha linum* from Mandapam showed iodine content of 0.72 mg g^{-1} and chlorine contents in the range 5.0 – 18.5 mg g^{-1} (Pillai, 1956). *Chaetomorpha moniligera* from Sea of Japan recorded low iodine content of 0.02 mg g^{-1} (Saenko *et al.*, 1978). During the present study, *Ulva lactuca* showed average iodine content of 0.21 mg $g^{-1}$and average chlorine content of 2.68 mg g^{-1}. The same species collected from Gujarat coast (Dave *et al.*, 1969) and Andaman Sea (Solimabi *et al.*, 1981) recorded lower iodine contents (0.05 and 0.12 mg g^{-1} respectively). This species recorded higher chlorine content (7.9 mg g^{-1}) at Gujarat coast (Rao and Tipnis, 1967). However, *U. rigida* from the same location showed chlorine content (2.7 mg g^{-1}) comparable to that of *U. lactuca* in the present study. This species recorded iodine content of 0.05 mg g^{-1}. *U. fasciata* recorded iodine content of 0.07 mg g^{-1} at Gujarat coast (Dave *et al.*, 1969) and a much higher value of 1.19 mg g^{-1} at Goa (Solimabi and Das, 1977). Devi *et al.* (1996) observed no iodine content in *U. fasciata* from Cape Comorin, while Rao and Indusekhar (1989) recorded a value of 0.03 mg g^{-1} in the same species from Saurashtra coast. At this location, the chlorine content of the alga was 17 mg g^{-1}. *U. reticulata* from Andaman Sea (Solimabi *et al.*, 1981) exhibited iodine content of 0.11 mg g^{-1}. Iodine contents of 0.13 and 0.04 mg g^{-1} were recorded by *U. fenestrata* collected from Sea of Japan and Okhotsk Sea respectively (Saenko *et al.*, 1978). In the present study, *Enteromorpha intestinalis* recorded average values of 0.18 mg g^{-1} for iodine content and 3.20 mg g^{-1} for chlorine content. Pillai

(1956) obtained higher values for iodine (0.58 mg g^{-1}) and chlorine (in the range 7.5 – 14 mg g^{-1}) contents in the same species collected from Mandapam. This species showed a lower iodine content (0.04 mg g^{-1}) and a higher chlorine content (15.73 mg g^{-1}) at Saurashtra coast (Rao and Indusekhar, 1989). *E. linza* collected from Cape Comorin recorded higher iodine content of 0.39 mg g^{-1} (Devi *et al.*, 1996). *Enteromorpha* spp. exhibited a lower iodine content (0.04 mg g^{-1}) at Gujarat coast (Dave *et al.*, 1969) and a higher value (1.18 mg g^{-1}) at Goa coast (Solimabi and Das, 1977). *E. clathrata* from the Sea of Japan recorded iodine content of 0.02 mg g^{-1} (Saenko *et al.*, 1978). The rhodophyte *Gracilaria corticata* recorded average chlorine content of 1.46 mg g^{-1} and average iodine content of 0.32 mg g^{-1} during the present investigation. The same species from Cape Comorin (Devi *et al.*, 1996) showed lower iodine content of 0.10 mg g^{-1}, while that from Goa (Solimabi and Das, 1977) recorded higher value (1.36 mg g^{-1}). *G. corticata* collected from Gujarat coast (Dave *et al.*, 1969) and Andaman Sea (Solimabi *et al.*, 1981) also recorded low values of iodine content (0.18 and 0.09 mg g^{-1} respectively). The same species from Saurashtra coast exhibited iodine content of 0.13 mg $g^{-1}$and chlorine content of 33.33 mg g^{-1} (Rao and Indusekhar, 1989). *G. lichenoides* from Mandapam recorded chlorine contents in the range 7.5 – 25.5 mg g^{-1} and average iodine content of 2.08 mg g^{-1} (Pillai, 1956). Kappanna and Rao (1963) reported chlorine content of 12.6 mg g^{-1} in this species. *G. verrucosa* and *G.canaliculata* from Andaman Sea (Solimabi *et al.*, 1981) and *G. folii-fera* from Gujarat coast (Dave *et al.*, 1969) recorded low values of iodine content (0.03, 0.05 and 0.08 mg g^{-1} respectively). During the present study, *Centroceras clavulatum* showed average iodine content of 0.90 mg g^{-1} and average chlorine content of 1.81 mg g^{-1}. The same species from Gujarat coast recorded lower iodine content of 0.21 mg g^{-1} (Dave *et al.*, 1969). In the present study, the rhodophyte *Grateloupia filicina* exhibited the average values of 0.77 mg g^{-1} for iodine and 1.43 mg g^{-1} for chlorine contents. This species recorded an iodine content of 0.60 mg g^{-1} at Cape Comorin (Devi *et al.*, 1996). *G. turuturu* collected from the Sea of Japan showed low iodine content of 0.07 mg g^{-1} (Saenko *et al.*, 1978).

From the ongoing discussion, it can be seen that the halogen contents of algae show variations not only from species to species, but also in the same species collected from different places. Although such variations were observed between the locations selected for the present study also, these variations were not statistically significant. The variations in the halogen contents of algae from different coastal regions may probably be due to different reasons including the difference in the relative uptake rates of different halogens, species specificity, environmental conditions and different phenological stages of algae characterized by differential rate of metabolic activities.

Algae were also found to show interclass variations in their halogen contents. At both Ettikkulam and Narakkal, chlorophyceae were found to record higher chlorine contents than rhodophyceae in all the seasons except in the retreating monsoon season. A similar result was obtained by Rao and Indusekhar (1989) for marine algae from Saurashtra coast. Classwise variations in the iodine contents were observed to show a trend reverse to that of chlorine contents during the present study. At both the locations, rhodophyceae recorded higher iodine contents than chlorophyceae in all the seasons except in the mid hot season. Similar results have been reported for marine algae from different coastal regions including Saurashtra coast (Kappanna and Rao, 1962; Rao and Indusekhar, 1989), Goa (Solimabi and

Das, 1977), Cape Comorin (Devi *et al.*, 1996) and Japan (Saenko *et al.*, 1978). A reverse case was reported by Solimabi *et al.* (1981) for algae from Andaman Sea.

The marine algae selected for the present study were found to show temporal variations in their halogen contents. All the algal species recorded lowest iodine contents in the summer monsoon season and most of them showed highest values in the mid hot season. Intermediate values were obtained in other seasons. Such a general trend was not observed in the chlorine content of algae. The period of minimum and maximum chlorine contents were found to differ from one species to another and also between locations. The seasonal variations in the halogen content may be attributed to the different stages of growth cycles of algae and to the different environmental parameters such as salinity, temperature, wave action etc., which affect the accumulation of halogens in algae. The changes in the concentration of halogens in seawater can also have an influence on their absorption and accumulation by algae. Although high values of iodine have been reported during the period of early growth of algae (Thomas *et al.*, 1987; Rao and Singbal, 1995), low values were obtained in the growing period of summer monsoon season during the present study. This may be due to the heavy monsoon rain in the season, which causes the dilution of seawater and brings down the concentration of inorganic constituents including iodine in seawater. This is reflected in the algal constitution. The higher iodine contents observed during the early retreating monsoon season may be due to the increase in salinity (Lobban and Harrison, 1997) resulted from the cessation of monsoon rain and increase in temperature. Algae did not show much variation in the iodine content in the winter monsoon season compared to that in the retreating monsoon season. This may be due to the fact that algae show an increase in the iodine content as well as the dry solid content during their active growth in this period. The accumulation of iodine may be occurring at such a rate that the net increase in its concentration on dry weight basis is negligible. Most of the algae were found to show highest iodine content in the mid hot season. This may partly be due to comparatively higher salinity of seawater in this season. Lower values of iodine content reported during the late hot season may be due to effect of emersion. Slight damages of cells caused by drying or warmth during the period of emersion may result in the liberation of cellular iodine. This is due to the phenomenon of 'iodovolatilization' in which the iodide present in the algae is oxidized to I_2 by the enzyme iodide oxidase at the surface of the algae and is evaporated to the atmosphere (Shaw, 1962).

It was found during the present study that chlorine contents of algae were higher than iodine contents in all the seasons. This is in agreement with earlier reports (Rao and Singbal, 1995). The I:Cl ratios were always less than unity and varied from 0.02 to 0.84 (Table 4.16). This variation shows that the internal concentrations of chlorine and iodine in algae are not interrelated, whatever be the mechanism of their uptake and the accumulation of one halogen is probably independent of the other halogens present in the tissues (Rao and Indusekhar, 1989). The I:Cl ratios were found to be higher in rhodophyceae than in chlorophyceae. This is due to the higher iodine and lower chlorine contents of rhodophyceae compared to chlorophyceae.

It has been found that marine algae preferentially accumulate iodine over chlorine throughout their phenological stages (Rao and Indusekhar, 1989; Rao and Singbal, 1995). Though chlorine is the major halogen present in algae, it is not accumulated by algae, whereas iodine is accumulated to

Table 4.16. I:Cl ratios of different species of marine algae from Kerala coast.

	SM	RM	WM1	WM2	HS1	HS2
Ettikkulam						
Chlorophyceae						
Chaetomorpha antennina	0.22	0.15	0.20	0.24	0.10	0.10
Ulva lactuca	0.02	0.07	0.04	0.20	0.22	0.08
Enteromorpha intestinalis	0.06	*	*	*	*	*
Rhodophyceae						
Gracilaria corticata	0.19	0.28	0.25	0.14	0.19	0.33
Centroceras clavulatum	*	0.81	*	0.50	*	*
Grateloupia filicina	*	0.65	*	*	*	*
Narakkal						
Chlorophyceae						
Chaetomorpha antennina	0.05	0.03	*	*	*	*
Enteromorpha intestinalis	0.33	*	*	*	*	0.03
Rhodophyceae						
Centroceras clavulatum	0.18	0.39	0.61	0.84	0.79	*
Grateloupia filicina	0.37	0.65	0.71	*	0.34	*

SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid winter monsoon HS1: Mid-hot season HS2: Late hot season
* Algal species were absent / I:Cl ratios were not calculated

several orders of magnitude compared to their concentration in the ambient seawater (Rao and Indusekhar, 1989). A similar observation was made during the present study also. Taking the average chlorine and iodine concentrations of seawater as 1.94×10^4 mg kg^{-1} and 5×10^{-2} mg kg^{-1} respectively (Bruland, 1983), the concentration factors (CF) were found to be in the ranges 0.07 – 0.21 for chlorine and 3,600 – 18,000 for iodine (Table. 4.17). The largest concentrator of iodine was found to be the rhodophyte *Centroceras clavulatum* (CF: 18,000) belonging to the order Ceramiales. The members of this order have been reported to be good concentrators of iodine

Table 4.17 The concentration factors (CF) for chlorine and iodine in algae

	Chlorine		Iodine	
	Concentration (mg g^{-1})	CF	Concentration (mg g^{-1})	CF
Seawater *	19.4	—	5×10^{-5}	—
Marine algae				
Chaetomorpha antennina	4.07	0.21	0.42	8400
Ulva lactuca	2.68	0.14	0.21	4200
Enteromorpha intestinalis	3.20	0.16	0.18	3600
Gracilaria corticata	1.46	0.08	0.32	6400
Centroceras clavulatum	1.81	0.09	0.90	18000
Grateloupia filicina	1.43	0.07	0.77	15400

* Bruland (1983)

(Saenko *et al.*, 1978). The rhodophyte *Grateloupia filicina* of the order Cryptonemiales was also found to concentrate iodine (CF: 15,400). The lowest accumulation of iodine was shown by the chlorophyte *Enteromorpha intestinalis* (CF: 3600).

The determination of trace metal content of marine algae carried out during the present study showed that algae from Kerala coast contained considerable amounts of many of the essential trace metals needed for human and animal nutrition. They were also found to accumulate toxic heavy metals such as Pb, Cd etc. Most of the metals studied showed high levels of accumulation. Comparison of these results with previous studies from the study area is difficult due to the absence of published data. However, the metal concentrations recorded in this study showed variations when compared to those from other coastal regions.

In the present study, Cu contents of algae were found to vary in the range 0.60 – 14.59 μg g^{-1} with an average value of 6.58 μg g^{-1}. This was found to be well within the range 0.23 – 46.62 μg g^{-1} reported for the marine algae from different coastal regions of India (Rao, 1992). Pillai (1956) reported Cu contents varying from non-detectable level to 12 ug g^{-1} (mean = 3.25 μg g^{-1}) in marine algae from Mandapam. Zingde *et al.* (1976) obtained Cu contents varying in the range 3.2 – 27.5 μg g^{-1} (mean = 13.3 μg g^{-1}), whereas Agadi *et al.* (1978) reported Cu accumulation in the range 3.2 - 80.4 μg g^{-1} in marine algae from Goa coast. Marine algae from Maharashtra (Agadi *et al.*, 1984) were found to contain high levels of Cu varying from non-detectable level to 96.5 μg g^{-1} (mean = 14.4 μg g^{-1}). Algae from Saurashtra coast showed Cu contents varying in the range 5.08 – 32.4 μg g^{-1} with an average value of 12.2 μg g^{-1} (Rao and Indusekhar, 1986). Marine algae from Gulf of Mannar recorded high levels of Cu content in the range 18.5 – 45.0 μg g^{-1} (Ganesan *et al.*, 1991). Rao *et al.* (1995) reported Cu contents varying from 2.43 to 26.2 μg g^{-1} (mean = 14.2 μg g^{-1}) in marine algae from Visakhapatnam coast. From the above comparisons, it can be seen that marine algae from Kerala showed lower accumulation of Cu from seawater compared to the algae from most of the other coastal regions of India.

Cu contents recorded in the present study were also observed to show considerable variations from the Cu content of algae from other parts of the world. Shiber (1980) reported Cu contents in the range 3.8 – 110.0 μg g^{-1} in algae from Lebanon, while Burdon-Jones *et al.* (1982) obtained the values ranging from 1.4 to 11.1 μg g^{-1} in marine algae from Australia. Guimaraes *et al.* (1982) reported a range of 3.6 – 7.4 μg g^{-1}, whereas Lacerda *et al.* (1985) recorded a range of 1.5 – 21.6 μg g^{-1} for Cu contents in macroalgae from Brazil. Black Sea algae were found to accumulate Cu in the range 3.27 – 19.48 μg g^{-1} (Guven *et al.*, 1992). Villares *et al.* (2001)

reported Cu contents varying in the range 2.18 – 48.12 μg g^{-1} in algae from northwest coast of Spain. Farias *et al.* (2002) observed Cu contents varying from non-detectable level to 15.2 μg g^{-1} in Antarctic macroalgae. Marine macroalgae from Spain were reported to contain Cu below 5 μg g^{-1} (Ruperez, 2002).

The Cu contents recorded by different species of algae collected during the present study were found to be of similar magnitude to those reported by various authors for algae from supposedly clean areas (Hagerhall, 1973; Lacerda *et al.*, 1985; Ho, 1987& 1990; Muse *et al.*, 1999). All the species showed Cu contents below 30 μg g^{-1}, which is the maximum permissible limit of Cu in foods in India (PFA, 1955). FAO (1983) has prescribed a maximum Cu content of 10 μg g^{-1} for seafood for human consumption and in the present study only *Centroceras clavulatum* showed Cu contents greater than this value. This species recorded average Cu content of 10.20 μg g^{-1} with a maximum of 14.59 μg g^{-1}. This alga belongs to the order Ceramiales, members of which have been found to be good concentrators of metals (Saenko *et al.*, 1978). On the basis of mean values of Cu content (Table 4.7), algae selected for the present study may be arranged in the ascending order as follows: *Gracilaria corticata* (3.44 μg g^{-1}), *Ulva lactuca* (5.63 μg g^{-1}), *Grateloupia filicina* (5.74 μg g^{-1}), *Enteromorpha intestinalis* (6.11 μg g^{-1}), *Chaetomorpha antennina* (6.39 μg g^{-1}) and *Centroceras clavulatum* (10.20 μg g^{-1}).

During the present study, algae recorded Zn contents in the range 5.49 – 83.88 μg g^{-1} (mean = 26.96 μg g^{-1}). The mean concentration was found to be well within the range 1.28 – 61.5 μg g^{-1} reported for the mean Zn concentration of Indian marine algae (Rao, 1992). Algae from Mandapam coast were reported to show Zn contents varying from non-detectable level to 80 μg g^{-1} with a mean value of 34.6 μg g^{-1} (Pillai, 1956). Zingde *et al.* (1976) obtained Zn contents in the range 6.4 – 60.2 μg g^{-1} (mean = 27.9 μg g^{-1}), while Agadi *et al.* (1978) reported values varying from 2.8 to 203.9 μg g^{-1} in marine algae from Goa coast. Algae from Maharashtra exhibited high values of Zn content varying in the range 4.00 – 296 μg g^{-1} with mean value of 90.7 μg g^{-1} (Agadi *et al.*, 1984). Rao and Indusekhar (1986) reported Zn levels varying from 7.31 to 54.8 μg g^{-1} (mean = 23.7 μg g^{-1}) in marine algae from Saurashtra coast. Ganesan *et al.* (1991) observed high levels of Zn in algae from Gulf of Mannar (37.0 – 189.0 μg g^{-1}). Marine algae of Visakhapatnam coast (Rao *et al.*, 1995) also recorded high values of Zn content (range: 6.92 – 215 μg g^{-1}, mean: 128 μg g^{-1}). Thus, algae from Kerala coast were found to show a lower accumulation of Zn compared to algae from many other coastal regions of India.

Algae from several other countries were also reported to accumulate Zn at levels higher than that observed in the present study. Shiber (1980) obtained Zn contents in the range 2.6 – 206.3 μg g^{-1} in algae from Lebanon while Burdon-Jones *et al.* (1982) reported the values varying in the range 3.7 – 166 μg g^{-1} in algae from Australian coast. Although Guimaraes *et al.* (1982) reported low Zn contents of 18.0 – 25.9 μg g^{-1} in algae from Brazil, Lacerda *et al.* (1985) obtained high values in the range 4.7 – 145 μg g^{-1}. Black Sea algae were found to record Zn contents in the range 6 – 98 μg g^{-1} (Guven *et al.*, 1992). Villares *et al.* (2001) reported Zn contents ranging from 6.96 – 147 μg g^{-1} in algae from Spain. Antarctic macroalgae were observed to show low Zn contents varying from non-detectable level to 15 μg g^{-1} (Farias *et al.*, 2002)

The accumulation of Zn by various algal species collected in the present study were observed to be of similar magnitude to those reported by various authors for algae from supposedly clean areas (Hagerhall, 1973; Lacerda *et al.*, 1985; Ho, 1987 & 1990). All the species except *Grateloupia filicina* showed average Zn contents below 50 μg g^{-1}, which is the maximum permissible limit of Zn prescribed for foods for human consumption (PFA, 1955; FAO, 1983). *G. filicina* recorded average Zn content of 56.69 μg g^{-1} with a maximum of 83.88 μg g^{-1}. On the basis of mean Zn concentrations (Table 4.7), algae from Kerala may be arranged in the ascending order as follows: *Ulva lactuca* (12.84 μg g^{-1}), *Gracilaria corticata* (14.21 μg g^{-1}), *Enteromorpha intestinalis* (20.99 μg g^{-1}), *Chaetomorpha antennina* (21.40 μg g^{-1}), *Centroceras clavulatum* (31.98 μg g^{-1}) and *Grateloupia filicina* (56.69 μg g^{-1}).

In the present study, Mn content of algae varied in the range 14.02 – 202.30 μg g^{-1} with only three samples of *Gracilaria corticata* from Etti-kkulam showing Mn content greater than 150 μg g^{-1}. The mean value was 71.78 μg g^{-1}. This was well within the range 0.44 – 1286 μg g^{-1} reported for Indian marine algae (Rao, 1992). Algae from Mandapam showed Mn conte-nts varying from trace level to 575 μg g^{-1} with mean value of 132 μg g^{-1} (Pillai, 1956). Zingde *et al.* (1976) reported Mn accumulation in the range 37 - 586 μg g^{-1} (mean = 270 μg g^{-1}) in algae from Goa coast, whereas Agadi *et al.* (1978) reported values in the range 25.09 – 3420.56 μg g^{-1}. The macroalgae from Maharashtra recorded high levels of Mn varying in the range 35.7 – 1737 μg g^{-1} with mean value of 236 μg g^{-1} (Agadi *et al.*, 1984). Rao and Indusekhar (1986) obtained Mn concentrations ranging from 8.09 to 161 μg g^{-1} (mean = 57.2 μg g^{-1}) in algae from Saurashtra coast. Ganesan *et al.* (1991) reported Mn contents in the range 35.0 – 299.5 μg g^{-1} in algae from Gulf of Mannar. Rao *et al.* (1995) observed Mn levels varying from 25.1 to 378 μg g^{-1} (mean = 168 μg g^{-1}) in algae from Visakhapatnam. Algae from Tuticorin coast were found to accumulate Mn in the range 20 – 1077 μg g^{-1} (Ganesan and Kannan, 1995). Thus, it can be seen that as with Cu and Zn, Mn also shows lower level of accumulation in algae from Kerala coast in comparison with algae from most of the other Indian coasts.

Marine algae from Black Sea (Guven *et al.*, 1992) were found to show Mn contents lower than that observed in the present study (range: 14.43 – 149.98 μg g^{-1}). Villares *et al.* (2001) reported Mn contents in the range 4.59 – 512 μg g^{-1} in algae from Spain. Antarctic macroalgae were observed to contain very low levels of Mn varying in the range 0.3 – 6.5 μg g^{-1} (Farias *et al.*, 2002). The Mn level of algae observed in the present study were found to be higher than that recorded in algae from supposedly clean areas (Ho, 1987). However, in some cases, values were lower than that reported for polluted (Ho, 1990; Munda and Hudnik, 1991) as well as unpolluted areas (Wong *et al.*, 1979; Ho, 1990). On the basis of mean Mn contents (Table 4.7), algae of Kerala coast may be arranged in the ascending order as follows: *Ulva lactuca* (26.33 μg g^{-1}), *Chaetomorpha antennina* (41.57 μg g^{-1}), *Grateloupia filicina* (44.25 μg g^{-1}), *Enteromorpha intestinalis* (50.35 μg g^{-1}), *Centroceras clavulatum* (102.16 μg g^{-1}) and *Gracilaria corticata* (150.20 μg g^{-1}).

During the present investigation, Fe contents of algae varied in the range 180 – 18590 μg g^{-1} (mean = 4230 μg g^{-1}), but were most frequently in the range 1000 – 7500 μg g^{-1}. Only four samples of *Centroceras clavulatum* showed values higher than 7500 μg g^{-1}. The observed mean value was well within the range of mean values (3.7 – 6470 μg g^{-1}) reported for algae from different Indian coasts (Rao, 1992). Algae from Maharashtra were found to record Fe contents higher than that observed in the present study (Agadi *et al.*, 1984). The values ranged from 115 to 39496 μg g^{-1} with mean value of 4996 μg g^{-1}. Algae from Tuticorin coast (Ganesan and Kannan, 1995) recorded Fe contents comparable to that in the present study (range: 325 – 15922 μg g^{-1}). Algae from other Indian coast were found to show lower accumulation of Fe in comparison with algae from Kerala coast. Pillai (1956) reported very low Fe content of 35 – 504 μg g^{-1} (mean: 163 μg g^{-1}) in algae from Mandapam. Marine algae from Goa recorded Fe contents ranging from 128 to 1796 μg g^{-1} (Agadi *et al.*, 1978). Ganesan *et al.* (1991) obtained Fe contents in the range 135.5 – 1185 μg g^{-1} in macroalgae from Gulf of Mannar. Rao *et al.* (1995) reported Fe contents ranging from 630 to 2580 μg g^{-1} (mean: 1441 μg g^{-1}) in algae from Visakhapatnam. Algae from other parts of the world also showed lower Fe contents. Guven *et al.* (1992) reported Fe contents varying in the range 206 – 3506 μg g^{-1} in Black Sea algae. Marine algae from Spain were found to contain Fe in the range 38.6 – 5046 μg g^{-1} (Villares *et al.*, 2001). Antarctic macroalgae also recorded low levels of Fe ranging from 23.6 – 3095 μg g^{-1} (Farias *et al.*, 2002).

The amounts of Fe accumulated by marine algae in the present study were, in general, of similar magnitude to those reported for algae from polluted areas (Agadi *et al.*, 1984; Ho, 1987 & 1990; Guven *et al.*, 1993). Only the chlorophyte *Ulva lactuca* showed average Fe content less than 1000 μg g^{-1}. The rhodophyte *Centroceras clavulatum* belonging to the order Ceramiales showed highest average Fe content (9010 μg g^{-1}). This is characteristic of algae of the order Ceramiales (Saenko *et al.*, 1978). Based on the average Fe contents (Table 4.7), algae of Kerala coast may be arranged in the ascending order as follows: *Ulva lactuca* (740 µg g^{-1}), *Gracilaria corticata* (1420 µg g^{-1}), *Grateloupia filicina* (2830 µg g^{-1}), *Enteromorpha intestinalis* (4500 µg g^{-1}), *Chaetomorpha antennina* (4540 µg g^{-1}) and *Centroceras clavulatum* (9010 µg g^{-1}).

Cobalt contents of algae from Kerala coast were found to vary from non-detectable level to 10.08 µg g^{-1} with mean concentration of 5.59 µg g^{-1}. This was within the range of mean values of Co, 0.12 – 12.25 µg g^{-1} reported for Indian algae (Rao, 1992). Agadi *et al.* (1978) reported Co contents varying in the range 1.3 – 15.2 µg g^{-1} in algae from Goa coast. Algae from Maharashtra coast were found to record high values of Co content such as 31.4 µg g^{-1} (Agadi *et al.*, 1984). Algae from Australia showed Co contents varying from non-detectable level to 3.2 µg g^{-1} (Burdon-Jones *et al.*, 1982). Guimaraes *et al.* (1982) reported Co contents in the range 3.4 – 6.2 µg g^{-1} in algae from Brazil while Lacerda *et al.* (1985) reported values ranging from 1.6 to 13.5 µg g^{-1}. Black Sea algae recorded Co contents in the range 1.2 – 8.17 µg g^{-1} (Guven *et al.*, 1992). Antarctic macroalgae were found to record very high values of Co content in the range 2.4 – 357 µg g^{-1} (Farias *et al.*, 2002).

Cobalt contents recorded by algae in the present study were in accordance with those reported for unpolluted areas (Agadi *et al.*, 1978; Lacerda *et al.*, 1985). The average Co contents of various species did not vary much with the lowest being 4.61 µg g^{-1} (*Ulva lactuca*) and the highest 7.31 µg g^{-1} (*Enteromorpha intestinalis*). Based on the Co contents (Table 4.7), the algae selected for the present study may be arranged in the ascending order as follows: *Ulva lactuca* (4.61 µg g^{-1}), *Gracilaria corticata*

(4.64 μg g^{-1}), *Chaetomorpha antennina* (4.70 μg g^{-1}), *Centroceras clavulatum* (6.44 μg g^{-1}), *Grateloupia filicina* (6.81 μg g^{-1}) and *Enteromorpha intestinalis* (7.31 μg g^{-1}).

In the present study, Ni content of algae varied in the range 0.20 – 21.06 μg g^{-1} with mean value of 10.13 μg g^{-1}. The mean concentration was found to be within the range of mean values, 0.61 – 12.96 μg g^{-1}, reported for algae from Indian coast (Rao, 1992). Algae from Goa recorded Ni contents comparable to that obtained in the present study. The values ranged from 4.51 to 39.06 μg g^{-1} with mean value of 12.72 μg g^{-1} (Agadi *et al.*, 1978). Algae of Maharashtra coast (Agadi *et al.*, 1984) exhibited Ni contents varying from non-detectable level to 44.1 μg g^{-1} (mean = 13.4 μg g^{-1}). Rao and Indusekhar (1986) reported low values for Ni content ranging from 0.61 to 6.94 μg g^{-1} (mean = 2.53 μg g^{-1}) in algae from Saurashtra coast. Algae from Visakhapatnam also showed low accumulation of Ni varying from 0.04 to 3.26 μg g^{-1} (Rao *et al.*, 1995). Algae from northwest Spain were found to show Ni contents comparable to that obtained in the present study. The values varied from non-detectable level to 18.05 μg g^{-1} (Villares *et al.*, 2001). Antarctic macroalgae showed low levels of Ni varying in the range 1.8 – 8.8 μg g^{-1} (Farias *et al.*, 2002).

The average Ni contents recorded by some algal species during the present study were, in general, of similar magnitude to those reported for some polluted areas (Hagerhall, 1973; Ho, 1987; Catsiki and Papathanassiou, 1993; Haritonidis and Malea, 1995). However, in other cases, the recorded values were lower than that reported for supposedly clean areas (Ho, 1990). Based on the average Ni contents (Table 4.7), algae of Kerala coast may be arranged in the ascending order as: *Ulva lactuca* (6.16 μg g^{-1}), *Gracilaria* *corticata* (8.08 μg g^{-1}), *Chaetomorpha antennina* (8.62 μg g^{-1}), *Grateloupia filicina* (9.66 μg g^{-1}), *Centroceras clavulatum* (14.29 μg g^{-1}) and *Enteromorpha intestinalis* (14.68 μg g^{-1}).

Chromium content of algae selected for the present study varied from non-detectable level to 37.18 μg g^{-1} (mean = 13.86) with 50% of the samples (19 samples) showing values below 10 μg g^{-1}. Only 18% of the samples (7 samples) showed values above 20 μg g^{-1}. The mean Cr content obtained in the present study was considerably higher than that reported by Rao (1992) for algae from other Indian coasts (range: 0.13 – 4.2 μg g^{-1}). Algae from Saurashtra coast were found to show very low Cr contents in the range 0.13 – 3.25 μg g^{-1} (Rao and Indusekhar, 1987). However, algae collected from Visakhapatnam coast showed Cr contents comparable to that reported in the present study (Rao *et al.*, 1995). The values ranged from 2.43 to 28.3 μg g^{-1} (mean = 16.4 μg g^{-1}). Marine algae from the Sea of Japan showed Cr contents in the range 1.1 – 23.4 μg g^{-1} (Saenko *et al.*, 1976). Sivalingam (1978) reported high values such as 58.77 μg g^{-1} for Cr content of algae from Malaysia. Shiber (1980) recorded Cr contents varying from non-detectable level to 16.3 μg g^{-1} in algae collected from Lebanon. Algae from Australia showed low values of Cr varying in the range 1.4 – 10 μg g^{-1} (Burdon-Jones *et al.*, 1982). Guimaraes *et al.* (1982) reported Cr contents ranging from 2.2 to 7.9 μg g^{-1} in algae from Brazil while Lacerda *et al.* (1985) obtained higher values ranging from 1.0 to 25.7 μg g^{-1}. Black Sea algae exhibited Cr contents in the range 2.06 – 18.2 μg g^{-1} (Guven *et al.*, 1992). Antarctic macroalgae showed Cr contents in the range 1.6 – 12.1 μg g^{-1} (Farias *et al.*, 2002). Chromium contents recorded by algae in the present study were found to be in agreement with that shown by algae from polluted areas (Hagerhall, 1973; Haritonidis and Malea, 1995; Muse *et al.*, 1999). On the basis of mean Cr contents (Table 4.7), algae may be arranged in the ascending order as: *Ulva lactuca* (2.89 μg g^{-1}), *Gracilaria corticata* (4.83 μg g^{-1}), *Grateloupia filicina* (9.98 μg g^{-1}), *Enteromorpha intestinalis* (13.23 μg g^{-1}), *Centroceras clavulatum* (18.62 μg g^{-1}) and *Chaetomorpha antennina* (21.32 μg g^{-1}).

In the present study, Sr contents of algae varied in the range 2.19-103.90 μg g^{-1} (mean = 29.40 μg g^{-1}), but were most frequently in the range 10 – 50 μg g^{-1}. Only 13% of the samples showed values above 50 μg g^{-1} with a single sample (*Chaetomorpha antennina* from Narakkal in the summer monsoon season) showing Sr content greater than 100 μg g^{-1}. 16% of the samples showed Sr contents below 10 μg g^{-1}. The mean Sr content obtained in the present study was well within the wide range, 19 – 1458 μg g^{-1}, reported for mean Sr contents of algae from Indian coast (Rao, 1992). Black Sea algae were found to contain high levels of Sr varying in the range 76 – 2115 μg g^{-1} (Guven *et al.*, 1992). Antarctic algae also showed high levels of Sr accumulation in the range 6.1 – 500 μg g^{-1} (Farias *et al.*, 2002). In the present study, all the algal species except *Grateloupia filicina* showed average Sr contents below 50 μg g^{-1}. *G. filicina* recorded a value of 51.94 μg g^{-1}. In the increasing order of Sr content, algae may be arranged as: *Centroceras clavulatum* (14.48 μgg^{-1}), *Gracilaria corticata* (18.60 μg g^{-1}), *Enteromorpha intestinalis* (21.18 μg g^{-1}), *Ulva lactuca* (27.89 μg g^{-1}), *Chaetomorpha antennina* (41.62 μg g^{-1}) and *Grateloupia filicina* (51.94 μg g^{-1}) (Table 4.7).

Algae selected for the present study were observed to accumulate Pb in concentrations varying from non-detectable level to 18.92 μg g^{-1} with a mean value of 9.03 μg g^{-1}. The observed mean value for Pb was considerably higher than the mean values reported (range: 0.49 – 5.36 μg g^{-1}) for marine algae from some other Indian coasts (Rao, 1992). However, the present values were much lower than that reported from Goa coast (3.0 – 197.5 μg g^{-1}) which was supposed to be a clean area (Agadi *et al.*, 1978). Sivalingam (1978) reported average Pb content of 10 μg g^{-1} in algae from non-contaminated areas of Malaysia coast. Shiber (1980) reported Pb contents in the range <7.5 – 96.6 μg g^{-1} in algae from Lebanon. Burdon-Jones *et al.* (1982) obtained lower values for Pb accumulation (<0.3 – 10.2 μg g^{-1}) in algae from Australia. Guimaraes *et al.* (1982) reported Pb contents ranging from 9.7 to 21.9 μg g^{-1} in algae from Brazil, while Lacerda *et al.* (1985) obtained higher values varying in the range 3.6 – 42.8 μg g^{-1}. Rao and Indusekhar (1987) recorded very low Pb contents varying from 0.49 to 2.00 μg g^{-1} in algae from Saurashtra coast. Black Sea algae exhibited Pb contents in the range 5.34 – 24.56 μg g^{-1} (Guven *et al.*, 1992). Antarctic algae showed low levels of Pb accumulation in the range 1.6 – 12.1 μg g^{-1} (Farias *et al.*, 2002). All these coastal areas were supposed to be unpolluted.

From the above comparisons, it can be seen that Pb contents recorded by algae in the present study were of similar magnitude to those reported by various authors for uncontaminated areas. However, many authors attribute a Pb content of 10 μg g^{-1} to considerable pollution (Preston *et al.*, 1972; Lozano *et al.*, 2003). Lozano *et al.* (2003) observed that about 32% of the algal samples collected from Canary Islands accumulated Pb in concentrations above 10 μg g^{-1}. A similar result was obtained in the present study with 34% of the samples showing Pb contents above 10 μg g^{-1}. However, in the present study, maximum Pb content observed was 18.92 μg g^{-1}, while

Lozano *et al.* (2003) obtained a maximum value of 81.79 µg g^{-1} with eight samples showing values higher than 20 µg g^{-1}. The maximum permissible limit of Pb in macroalgal products in India is 10 µg g^{-1} (PFA, 1955) and in the present study, two of the algal species, *viz.*, *Gracilaria corticata* and *Grateloupia filicina* were found to record average Pb contents above 10 µg g^{-1} (12.93 and 12.87 µg g^{-1} respectively), while other species recorded values around 7 µg g^{-1}. Based on the Pb content (Table 4.7), algae may be arranged in the ascending order as: *Enteromorpha intestinalis* (6.95 µg g^{-1}), *Ulva lactuca* (7.05 µg g^{-1}), *Chaetomorpha antennina* (7.64 µg g^{-1}), *Centroceras clavulatum* (7.87 µg g^{-1}), *Grateloupia filicina* (12.87 µg g^{-1}) and *Gracilaria corticata* (12.93 µg g^{-1}).

In the present study, marine algae accumulated Cd in the range 0.30-6.69 µg g^{-1} with a mean value of 3.29 µg g^{-1}. This was comparable to the highest of mean values (3.12 µg g^{-1}) reported for Indian marine algae (Rao, 1992). Rao and Indusekhar (1987) recorded low levels of Cd accumulation (0.16 – 0.85) in marine algae from Saurashtra coast. Rao *et al.* (1995) obtained Cd contents varying from non-detectable level to 4.61 µg g^{-1} (mean = 1.80 µg g^{-1}) in algae from Visakhapatnam. Preston *et al.* (1972) reported Cd contents in the range 0.05 – 20.8 µg g^{-1} in algae from British Isles. Shiber (1980) obtained Cd contents varying in the range <0.4 – 5.9 µg g^{-1} in algae from Lebanon. Australian algae recorded Cd contents in the range 0.2 – 1.4 µg g^{-1} (Burdon-Jones *et al.*, 1982). Guimaraes *et al.* (1982) reported accumulation of Cd in the range 0.9 – 1.5 µg g^{-1} by Brazilian algae, while Lacerda *et al.* (1985) obtained values ranging from 0.4 to 3.6 µg g^{-1}. Black Sea algae exhibited Cd contents in the range 1.03 – 6.5 µg g^{-1} (Guven *et al.*, 1992). Antarctic macroalgae showed Cd contents varying in the range

<0.1 – 10.4 μg g^{-1} (Farias *et al.*, 2002). Algae from Canary Islands recorded Cd accumulation ranging from 0.19 to 5.13 μg g^{-1} (Lozano *et al.*, 2003). It was found that Cd contents of algae recorded in the present study were generally in accordance with those reported for algae from contaminated areas. Cd is one of the most toxic elements and its maximum permissible limit in foods in India is 1.5 μg g^{-1} (PFA, 1955). In the present study, only *Chaetomorpha antennina* from Narakkal and *Enteromorpha intestinalis* from Ettikkulam showed Cd contents lower than this limit. Based on the average Cd content (Table 4.7), algae of Kerala coast may be arranged in the ascending order as: *Enteromorpha intestinalis* (1.56 μg g^{-1}), *Centroceras clavulatum* (2.70 μg g^{-1}), *Grateloupia filicina* (3.10 μg g^{-1}), *Chaetomorpha antennina* (3.53 μg g^{-1}), *Ulva lactuca* (3.87 μg g^{-1}) and *Gracilaria corticata* (4.30 μg g^{-1}).

In the present study, algae were found to accumulate Ag in concentrations ranging from non-detectable level to 6.39 μg g^{-1} (mean = 1.80 μg g^{-1}). However, the values were most frequently less than 2 μg g^{-1}. Only 18% of the samples showed Ag contents higher than this value. Two of the algal species were found to record average Ag content above 2 μg g^{-1} (*Gracilaria corticata* and *Grateloupia filicina*). Comparison of Ag concentration of algae observed in the present study with previous studies on Indian algae was difficult due to the absence of published data. However, present values were found to be comparable with that reported for Black Sea algae, which varied in the range <0.10 – 5.04 μg g^{-1} (Guven *et al.*, 1992). Algae of Kerala coast may be arranged in the increasing order of Ag content as: *Chaetomorpha antennina* (1.08 μg g^{-1}), *Enteromorpha intestinalis* (1.50 μg g^{-1}), *Centroceras clavulatum* (1.53 μg g^{-1}), *Ulva lactuca* (1.97 μg g^{-1}).

Grateloupia filicina (2.25 μg g^{-1}) and *Gracilaria corticata* (2.79 μg g^{-1}) (Table 4.7).

The ongoing discussion reveals that the trace metal contents of marine algae from Kerala coast show considerable variation from those of marine algae collected from other coastal regions. These variations may be due to the differences in the algal species and the ambient environmental conditions. Different algal species have different uptake rates of metals depending upon the factors such as surface area: volume ratio of the thallus, type of the tissue, age of the plant etc. The environmental factors such as temperature, salinity, dissolved oxygen and particulate content of water are known to affect the availability of metals to algae. Since these factors vary with climate and among different geographic regions, they may give rise to considerable variability in the tissue metal concentrations (Brown and Depledge, 1998). The concentration of metals in the ambient medium, the position of algae on the shore and the phenological stages of algae also contribute to the variability in the metal accumulation by algae from different places.

Algae were observed to show significant variations in the levels of metals such as Fe, Zn, Cd, Mn and Ni between the locations selected for the present study. The other metals did not show any significant spatial variations. Zn, Fe and Ni showed higher accumulation in algae from Narakkal compared to those from Ettikkulam, while reverse was true for Cd. Chlorophyceae of Narakkal showed higher accumulation of Mn compared to that from Ettikkulam, while reverse was true for rhodophyceae. Thus, in general, metal contents were higher in algae from Narakkal. This may be attributed to possibly higher level of most of the metals in seawater at Narakkal due to its proximity to the Cochin barmouth through which the

Cochin backwaters open out into the Arabian Sea. This backwater is intensely polluted due to the inputs from several major and minor industries situated in the upstream region. Several rivers, irrigation channels and sewers open into this backwater making it polluted. The harbour activities and oil jetty operations add to the pollution. The organic and inorganic substances delivered to the coastal waters through barmouth may have an influence on the bioavailability and accumulation of metals by algae. Also, the sampling site at Narakkal was a sandy shore and hence algae might have been able to accumulate sediment borne metals, whereas Ettikkulam being a rocky shore algae were not in direct contact with bottom deposits and accumulation of metals was possible only during turbulence caused by wave action and water movement.

The lower level of Cd accumulation observed in the algae from Narakkal compared to those from Ettikkulam may be attributed to the environmental factors influencing the bioavailability and accumulation of Cd such as the concentrations of dissolved calcium, dissolved organic carbon (DOC) and hydrogen ions in the ambient water (Desy *et al.*, 2002). The calcium ions exert a competitive effect on the Cd assimilation since the active uptake of Cd usually takes place through the uptake systems involved in the Ca regulation. Thus, an increase in Ca concentration in ambient medium may result in a decline in the Cd accumulation. The natural DOC has been found to reduce the bioavailability of Cd by acting as a ligand for cadmium ions. An increase in hydrogen ion concentration also retards Cd ion uptake. The inputs through the Cochin barmouth may be resulting in increased levels of calcium ions, DOC and hydrogen ions in the vicinity of the sampling site at Narakkal. The reason for the variable trend observed in the case of some metals may be that their accumulation is more related to phenological stages of algae rather than the ambient medium concentration.

It can be seen from the foregoing discussion that different species of algae collected during the present study accumulate metals to different extent. Statistically significant interspecies variations were shown by the metals Fe, Cu, Zn, Sr, Mn and Cr. The rhodophyte *Centroceras clavulatum* showed highest accumulation of Fe and Cu and *Grateloupia filicina*, Zn and Sr. *Gracilaria corticata* accumulated highest level of Mn. The chlorophyte *Chaetomorpha antennina* showed highest accumulation of Cr. Although *Enteromorpha intestinalis* accumulated highest levels of Co and Ni and *Gracilaria corticata* Pb, Cd and Ag, these were not statistically different from the levels shown by other algal species. The least accumulation of most of the metals was shown by the chlorophyte *Ulva lactuca*, which was obtained only from Ettikkulam. There may be different reasons for the specieswise variations in the accumulation of the various metals. It has been found that the accumulation of metals by algae occurs by different mechanisms depending on the alga, metal ion species, ambient solution conditions etc. (Greene and Bedell, 1990). The trace nutrient heavy metals are accumulated intracellularly by active biological transport, whereas toxic heavy metal ions may be accumulated by at least one of the three ways: (1) intracellular chelation by biological polymers, (2) accretion or precipitation of the heavy metals on the cell wall surface or (3) by adsorptive surface binding to various cell wall chemical functional groups including amine, phosphate, thiol, sulphate, carboxylate, imidazole or other groups associated with various biopolymers found in the cell walls. Since wide variations occur in the cell wall structures depending upon the algal division, genera, species and variety, in addition to the individual environmental adaptations, there can be species-dependent variation in biopolymers, side chains and monomeric chemicals that would be available to coordinate or electrostatically bind various heavy metal ions. This may be the reason for the observed specieswise differences in metal accumulation. It could also originate from the specific environmental conditions surrounding each species. Different species of algae are susceptible to confounding environmental factors, which bring significant changes in metal content for a single species (Barreiro *et al.*, 2002). Even under similar ambient conditions, variations in surface area available for absorption, permeability of cells/ tissues, number and nature of binding sites, and metabolic rate can result in difference in the metal uptake between species and even between individuals of the same species (Brown and Depledge, 1998). Also, it has been found that different species of algae have different natural ability to evolve adaptive mechanisms of decontamination of metals.

Algae were found to show interclass variations in the accumulation of some of the trace metals. The variations were statistically significant for the metals Cr, Sr, Mn and Cd. Chlorophyceae showed greater accumulation of Cr and Sr, while rhodophyceae accumulated more Mn and Cd. The latter also showed higher accumulation of Cu, Zn and Pb. However, these were not significantly different from the levels shown by chlorophyceae. These results were partly in accordance with earlier reports. Guven *et al.* (1992) reported higher accumulation of Cu, Zn, Mn, Pb and Cd in rhodophyceae compared to chlorophyceae from the coast of Turkey. They also observed higher accumulation of Cr by chlorophyceae. Rao and Indusekhar (1987) and Rao (1992) reported higher accumulation of Cr by chlorophyceae than rhodophyceae of Indian coast. They also observed higher accumulation of Pb and Cd by rhodophyceae.

In the present study, the trace metal distribution pattern was in the following order:

Whole algae: Fe > Mn > Sr > Zn > Cr > Ni > Pb > Cu > Co > Cd > Ag

Ettikkulam:

Chlorophyceae: Fe > Mn > Sr > Cr > Zn > Ni = Pb > Cu > Co > Cd > Ag

Rhodophyceae: Fe > Mn > Zn > Sr > Pb > Ni > Cr > Cu > Co > Cd > Ag

Narakkal:

Chlorophyceae: Fe > Mn > Sr > Zn > Cr > Ni > Pb > Cu > Co > Ag > Cd

Rhodophyceae: Fe > Mn > Zn > Sr > Cr > Ni > Pb > Cu > Co > Cd > Ag

In general, the decreasing trend of Fe > Mn > Zn/Sr > Ni/ Pb > Cu > Co > Cd/Ag was observed in both the classes of marine algae from the two locations selected for the present study. A similar order of metal accumulation was reported from other coastal regions also (Lacerda *et al.*, 1985; Rao and Indusekhar, 1987; Ganesan *et al.*, 1991). The high Fe concentration encountered as compared to the other trace metals may probably be due to several factors such as the established need of iron for normal growth of marine plants, ability of most algal species to biomagnify iron from the surrounding environment and the contamination from industrial and other operations. The accumulation of higher levels of Mn over Zn is common in tropical algae (Rao, 1992) and this may be due to their metabolic requirements. Zingde *et al.* (1976) have suggested that marine algae were better representatives of Mn in seawater than other organisms. It has been reported that in the case of many metals, the uptake is generally in proportion to their concentration within the surrounding medium (Sanders and Riedel, 1998).

However, certain metals show concentration factors of several orders of magnitude even though their ambient concentration is very much low. This may be due to the fact that the elemental accumulation is channelized through cell wall and the inflow of an element is regulated by its ability to pass through membrane pores, its mobility, valency and also its binding affinity with binding sites on the cell surface of algae (Simkiss, 1998). Since the channels are largely impermeable to metal complexes, ionic species dominate the uptake and hence metal speciation plays an important role in the accumulation. The uptake of a number of heavy metals has been found to be affected by the availability of calcium since their most likely route for entry is the calcium ion channel. Since hydrogen ions can compete with many cations for attachment to various ligand sites in the channel, the water pH has been found to have a great effect upon the cellular uptake of a variety of metal ions.

Considering temporal variations in metal accumulation, no general pattern was observed during the present study. In the summer monsoon season, marine algae generally showed low accumulation of metals such as Cu, Fe, Pb and Ag, whereas the metals Zn, Mn and Cd were accumulated to moderate concentrations. Cr and Sr showed variable trend in accumulation with concentrations varying from lowest to highest among different species. Ni and Co were, in general, accumulated to high concentrations in this season compared to other seasons. In the retreating monsoon season, Zn, Mn, Ni and Pb generally showed low accumulation with Co and Sr showing fairly low values. Fe, Cu, Cr, Cd and Ag showed variable trend in accumulation with some species recording lowest levels, while others showing moderate to high values. In the early winter monsoon season, trace metal accumulation showed moderate to high values with the exception of

Ag, which showed non-detectable levels in all the species collected from both Ettikkulam and Narakkal. Cu, Zn, Cr and Pb were accumulated to higher levels in this season compared to other seasons, whereas Co and Cd showed moderate levels. Fe, Mn, Ni and Sr showed variable trend. In the mid winter monsoon season, most of the metals exhibited variable trends. These include Fe, Cu, Co, Ni, Cr, Pb and Ag. Zn showed fairly low accumulation, whereas Mn was accumulated to moderate concentrations. Ni and Cd showed fairly high levels of accumulation. In the hot season also, most of the metals showed variable trend in the accumulation levels. In the mid hot season, Fe, Mn, Co, Ni, Cr, Sr and Ag showed variable trend, while in the late hot season, Fe, Mn, Zn, Co, Cr, Sr, Cd and Ag showed variable trend. In the mid hot season, Zn and Pb were accumulated to moderate levels and Cu and Cd to high levels. In the late hot season, Cu generally showed moderate levels and Ni, low levels. Pb was accumulated to high levels.

There may be different reasons for the seasonal differences found, including the environmental factors such as variations in the metal concentrations of the ambient seawater, 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. It has been found that the trace metal concentrations of coastal waters are influenced, to a great extent, by fresh water inflow (Riley and Chester, 1971). The input of metals to coastal waters is expected to be high during the monsoon period due to the increased run-off and land drainage. However, metals such as Cu exhibit a decrease with the onset of monsoon. This may be due to the removal of dissolved Cu from water by flocculation with increased load of humic substances (Sholkovitz, 1978) or by adsorption on particles of hydrated ferric hydroxide and manganese oxide, the former being more abundant in coastal waters (Bourg, 1983). The concentration of certain other metals such as Zn in coastal waters has been found to increase during monsoon. This may be due to the release of metals adsorbed onto the suspended matter on contact with seawater. Thus, different metals show varying concentrations during the monsoon period. This may be one of the reasons for the observed variation in the accumulation of various metals during the summer monsoon season.

Comparatively low levels of accumulation in algae exhibited by most of the metals in the early retreating monsoon season may be due to a decreased land input in this season as a result of the cessation of summer monsoon rains. A decrease in the metal levels in coastal waters may also be resulted from their accumulation by phytoplankton during the summer monsoon season, which is the period of their peak production. The incorporation of trace elements by phytoplankton has been found to regulate their form and availability. Most simply, the uptake by phytoplankton reduces the amount of dissolved metals in seawater, thereby reducing the availability of dissolved species to other phytoplankton or macroalgae (Sanders and Riedel, 1998). Also, if new tissues are incorporated in algae faster than metal is accumulated, then growth will, in effect, dilute metal concentrations in algae.

The moderate to high concentrations of metals observed in algae during the early winter monsoon season may be due to different reasons. The fairly high rates of photosynthesis and respiration of algae in this season would favour the assimilation of metals from the surrounding medium. The metal concentration of ambient seawater may be considerably high in this season due to the increased land input during the late retreating monsoon season, which is the secondary rainy season. Also, the regeneration of metals by the biodegradation of organic materials with which the metals are associated may occur at an increased rate under the prevailing environmental conditions of the season. As metals are accumulated rapidly by phytoplankton they can also be released, particularly as cells die and decompose. It has been found that particle reactive elements such as Ag, associated with cell walls are released more slowly than the elements associated with cellular cytoplasm (Sanders and Riedel, 1998). Thus, Ag concentration may be considerably low in the ambient water. This explains the low accumulation of Ag shown by all the algal species during the early winter monsoon season. In the later period of winter monsoon season, metals such as Zn showed fairly low level of accumulation, probably due to the decreased input of these metals from land during this season. However, metals such as Cd showed fairly high levels of accumulation. This may be attributed to possibly high ambient levels resulting from its more quick regeneration under the favourable conditions of temperature that increases all the bacterial processes. The moderate to high levels of many metals observed in the mid hot season can be attributed to the active regeneration of metals by mineralization of organic materials and microbial biomass under the favourable environmental conditions. The highly variable trend shown by algae in the accumulation of metals in the late hot season may be due to the interactions between various environmental and metabolic factors affecting the accumulation of different metals.

The information obtained in the present study support the assumption on the selective ability of macroalgae to accumulate trace metals from seawater. The rhodophyceae members *Gracilaria corticata*, *Centroceras clavulatum* and *Grateloupia filicina* were found to be good concentrators of trace metals and these can be considered for use as bioindicators of trace metal levels in seawater. Although many authors have suggested that the chlorophytes *Ulva* and *Enteromorpha* are good bioindicators of metal contamination (Bryan *et al.*, 1985; Ho, 1990; Say *et al.*, 1990; Haritonidis and Målea, 1999; Villares *et al.*, 2001), their use for this purpose is restricted in the study area. In the present study, the species *Ulva lactuca* showed the least accumulation of most of the trace metals and hence may not act as a good indicator of trace metal levels. Although *Enteromorpha intestinalis* concentrated Co and Ni to the highest level at Ettikkulam and Ni, Pb and Ag at Narakkal, its use as bioindicator was restricted due to its unavailability during most period of the year. However, the chlorophyte *Chaetomorpha antennina* can be used as bioindicator for Cr, Sr and Cd levels at Ettikkulam. Its use at Narakkal was also restricted by its non-availability. Although the rhodophyte *Gracilaria corticata* showed low accumulation of most of the metals studied, it showed highest accumulation of toxic metals such as Pb, Cd and Ag. On the contrary, *Centroceras clavulatum* showed high accumulation of most of the metals with comparatively low accumulation of Sr, Pb, Cd and Ag. Thus, in the context of use of algae as biomonitors, *Gracilaria corticata* can be used for the monitoring of potentially toxic heavy metals while *Centroceras clavulatum* for the nutrient trace metals.

The different algal species investigated during the present study were found to have potential as sources of essential trace elements including iodine, which are needed for human nutrition. However, caution must be taken of toxic metals such as Cd and Pb. Most of the species studied were found to contain Cd in amounts greater than the maximum permitted level in foods. Two of the species, *viz.*, *Gracilaria corticata* and *Grateloupia* *filicina* recorded Pb contents exceeding the regulatory limit. These toxic metals are usually taken up by algae from seawater by adsorption onto charged polysaccharides in the cell wall and intercellular matrix (Greene and Bedell, 1990; Maeda and Sakaguchi, 1990; Lobban and Harrison, 1997). The binding of metals by polysaccharides is usually strong and this deactivates their toxicity (Maeda and Sakaguchi, 1990). Since these charged algal polysaccharides are weakly fermented by the human colonic bacteria (Ruperez, 2002), metals bound to them may be retained throughout the digestive tract and removed along with the polysaccharides, posing less threat to health. Nevertheless, considering the levels of trace metals – both nutrient and toxic – in marine algae from Kerala, their use as food supplement rather than as staple food item is recommended as a better means of introducing them into the diet. They can also serve as a source of minerals for animals and plants whose products are eventually consumed by humans.

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* *Not referred in original*

Chapter 5

AMINO ACIDS

5.1 Introduction

The world population is continually increasing and this has resulted in an ever-increasing demand for proteinaceous food for human consumption. In this context, the effective exploitation of marine resources is becoming increasingly important. Of the different kinds of marine organisms, fish is the most widely consumed item all over the world. Fisheries occupy an important part in the world protein supply systems accounting for about 10% of the total protein supplies (Rajammal, 1994). Marine algae, which have been found to contain substantial amount of proteins with potential for human and animal nutrition, might also help to alleviate the increasing deficit in the world's protein supply. Many of the algal proteins have been found to be superior in their nutrient quality to those of animal or vegetable origin because of the presence of all the eight essential amino acids needed for the maintenance of good health (Fowden, 1962; McKeith, 1998). These include lysine, valine, threonine, methionine, phenylalanine, leucine, isoleucine and tryptophan, which cannot be synthesised in adequate amounts in the body and must, therefore, be supplied by the protein in the diet. Algal proteins also contain semi-essential amino acids such as histidine, arginine, glycine, serine, tyrosine and cysteine; and the non-essential (dispensable) amino acids such as glutamic acid, aspartic acid, alanine, proline and hydroxyproline (Fowden, 1962; Dave and Parekh, 1994). These amino acids are needed by the mammalian body to meet the requirements such as the replacement of endogenous loss of body proteins due to wear and tear, the synthesis of new tissue proteins during growth, pregnancy and lactation and the synthesis of enzymes, blood proteins, hormones of protein nature etc. (Swaminathan, 1985). In addition to the amino acids bound in proteins and peptides, many of the marine algae contain high amounts of free amino acids. This free amino acid pool contains nearly all of the amino acids normally occurring in proteins (Fowden, 1962). Algae have also been found to contain iodoamino acids. Di-iodotyrosine is the major iodoamino acid in algae with minor contributions from mono-iodotyrosine and tri-iodo-thyronine (Fowden, 1962). They also contain rare amino acids like ornithine and γ-aminobutyric acid.

Several studies have been carried out on the protein quality and amino acid profiles of marine algae from different parts of the world. Some of the early studies include those of Fowden (1951, 1954), Channing and Young (1952, 1953), Coulson (1953, 1955), Smith and Young (1955) and Jones (1958). Pillai (1957) studied the amino acid content of some of the Indian marine algae. Extensive studies were carried out by Lewis and Gonzalves (1959 a, b & c; 1960; 1962 a, b & c) on the amino acid content of marine algae from Bombay (Mumbai) coast. A number of observations were made by Lewis (1962 a & b; 1963 a, b & c) on the protein, peptide and amino acid contents of marine algae from southeast coast of India. Marine algae from Bombay were also analysed by Lewis (1963 d) for their amino acid contents. Lewis (1967) carried out a review on the protein, peptide and amino acid contents of Indian marine algae. Dave and Lewis (1973) reported the free amino acid composition of marine green algae from Indian coast. Arasaki and Mino (1973) studied the amino acid profile of algal proteins. Dave and Lewis (1976) investigated free and combined amino acid composition of marine algae from Saurashtra coast. Munda and Gubensek (1976) carried out studies on the amino acid composition of different species of marine algae from Iceland. Munda (1977) examined the differences in the amino acid composition of estuarine and marine algae. Dave and Chauhan (1984) studied the seasonal variations in free and combined amino acid composition of marine algae from Saurashtra coast. Fujiwara-Arasaki *et al.* (1984) determined the amino acid composition of algal proteins. Rosell and Srivastava (1985) studied seasonal variations in amino acid contents of marine algae. Munda and Gubensek (1986) investigated the amino acid content of some marine algae from the northern Adriatic coast. Ochiai *et al.* (1987) made observations on the amino acid composition of algal proteins. Dave *et al.* (1988) determined the amino acid content of macroalgae from Okha coast. Dave *et al.* (1990) estimated amino acid content of three species of marine algae from Saurashtra coast. Seasonal variations in the amino acid contents of algae from Tuticorin coast were studied by Kumar (1993). Dave and Parekh (1994) estimated the amounts of free and combined amino acids in marine algae from northwest coast of India. Fleurence *et al.* (1995) determined the amino acid composition of algal proteins. Dave *et al.* (1996) studied the amino acid content of marine green alga *Caulerpa.* Dave and Parekh (1997) estimated amino acid contents of some marine algae from Saurashtra coast. Gomez *et al.* (1998) investigated the amino acid composition of marine algae from King George Island. Fleurence (1999) studied the biochemical and nutritional aspects of macroalgal proteins. Fleurence *et al.* (1999) studying the nutritional value of proteins reported the values for amino acid contents of marine algae. Tao and He (2000) reported amino acid contents of algae from Dalian coast. Wong and Cheung (2001) studied the amino acid profiles of marine algae from Hong Kong. Campanella *et al.* (2002) carried out studies on the free and total amino acid composition of algae.

Although there are a number of studies on the amino acid composition of the Indian marine algae, information on the amino acid profiles of algae from southwest coast of India, especially Kerala, is rather scanty. No detailed studies have been carried out so far on the amino acid content of marine algae from central and northern coasts of Kerala. The present study provides information on the total amino acid pool of selected species of marine algae from two locations (Ettikkulam and Narakkal) on the central and northern coasts of Kerala. In addition to the specieswise and classwise variations in the amino acid contents, temporal and spatial variations were also investigated during the current study.

5.2 Results

The total amino acid pool, which represents the amino acids occurring in free state as well as those bound in peptides and proteins was determined in the hydrolysates of different species of marine algae collected from Ettikkulam and Narakkal. The amino acids estimated in the present study (Table 5.1) include:

(i) Monoamino monocarboxylic acids – glycine, alanine, valine, leucine (together with isoleucine), serine, threonine and γ-aminobutyric acid

(ii) Monoamino dicarboxylic acids – aspartic acid and glutamic acid

(iii) Diamino monocarboxylic acids – arginine, lysine and ornithine

(iv) Sulphur containing amino acids – cysteine and methionine, and

(v) Aromatic and heterocyclic amino acids – phenylalanine, tyrosine, histidine, proline and hydroxyproline.

Tryptophan was not determined during the present study.

Table 5.1. The amino acids estimated during the present study.

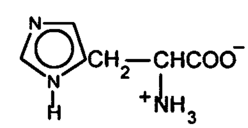
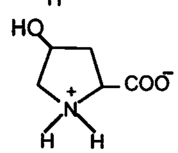
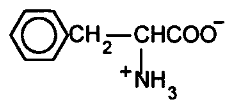
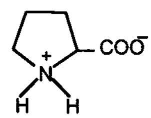
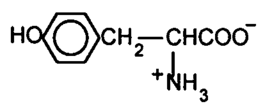
Name	Abbreviation	Formula
(+)-Alanine	Ala	$CH_3-CHCOO^-$ $^+NH_3$
γ – Aminobutyric acid	GABA	$H_2NCH_2CH_2CH_2COO$
(+)-Arginine	Arg	$H_2NCNHCH_2CH_2CH_2-CHCOO^-$ $^+NH_2$ NH_2
(+)-Aspartic acid	Asp	$HOOCCH_2-CHCOO^-$ $^+NH_3$
(-)-Cysteine	Cys	$HSCH_2-CHCOO^-$ $^+NH_3$
(+)-Glutamic acid	Glu	$HOOCCH_2CH_2-CHCOO^-$ $^+NH_3$
Glycine	Gly	CH_2COO^- $^+NH_3$
(-)-Histidine	His	
(-)-Hydroxyproline	Hyp	

Table 5.1. (*Continued*)

Name	Abbreviation	Formula
(+)-Isoleucine[e,*]	Ile	$CH_3CH_2CH(CH_3)-CH(^+NH_3)COO^-$
(-)-Leucine[e,*]	Leu	$(CH_3)_2CHCH_2-CH(^+NH_3)COO^-$
(+)-Lysine[e]	Lys	$^+H_3NCH_2CH_2CH_2CH_2-CH(NH_2)COO^-$
(-)-Methionine[e]	Met	$CH_3SCH_2CH_2-CH(^+NH_3)COO^-$
Ornithine	Orn	$^+H_3NCH_2CH_2CH_2CH(NH_2)COO^-$
(-)-Phenylalanine[e]	Phe	
(-)-Proline	Pro	
(-)-Serine	Ser	$HOCH_2-CH(^+NH_3)COO^-$
(-)-Threonine[e]	Thr	$CH_3CHOH-CH(^+NH_3)COO^-$
(-)-Tyrosine	Tyr	
(+)-Valine[e]	Val	$(CH_3)_2CH-CH(^+NH_3)COO^-$

[e] Essential amino acid (Morrison and Boyd, 1996)
* Estimated together

The methodologies used for the analysis of amino acid contents of algae are detailed in Chapter 2 and the results are presented in Table 5.2.

In the present study, no qualitative difference was found in the amino acid composition of different species of algae studied. However, quantitative differences were observed with regard to the individual amino acids. In general, aspartic acid, proline, phenylalanine, arginine, cysteine, leucine(s), glutamic acid and lysine were found to be the major constituents. Glycine, histidine and tyrosine were present in fairly good quantity. Hydroxyproline was a minor constituent in all the algal species studied. Although alanine, serine and threonine were found to be minor constituents in most of the algal samples, they were present in large amounts in some samples. Methionine, valine and ornithine were minor constituents in some algae, while they were present in trace amounts in others. γ-Aminobutyric acid occurred in fairly good quantity in some algae while in others, it was present in traces.

Specieswise variations in the amino acid composition were studied in marine algae collected from Ettikkulam in three different seasons, *viz.*, summer monsoon (August), winter monsoon (December) and hot season (May). The species studied include *Chaetomorpha antennina*, *Ulva lactuca*, *Enteromorpha intestinalis* and *Gracilaria corticata*. *Centroceras clavulatum* and *Grateloupia filicina* were not included because of their restricted availability in these seasons. At Narâkkal, interspecies variability in the amino acid pool was studied in marine algae collected in the summer monsoon season only, considering the maximum species diversity. The algal species studied include *Chaetomorpha antennina*, *Enteromorpha intestinalis*, *Centroceras clavulatum* and *Grateloupia filicina*.

Table 5.2. Amino acid content (mg g^{-1} dry wt.) of different species of marine algae from Kerala coast.

	Season	Asp	Glu	Ser	Gly	Thr	Ala	Tyr	Cys	Lys	Arg	His	Met	Val	Phe	Leu	Hyp	Pro	Orn	GABA	TAA[a]
Ettikkulam																					
Chlorophyceae																					
Ulva lactuca																					
	SM	3.22	2.07	2.19	2.70	4.34	9.34	4.89	6.13	7.26	8.83	7.33	*	*	17.03	6.22	3.03	24.08	3.3	9.48	121.44
	WM	5.64	6.26	3.69	5.01	9.60	15.37	6.99	7.55	7.63	15.24	9.58	*	*	22.08	20.63	2.21	18.98	3.99	6.68	167.13
	HS	26.83	13.86	10.82	9.40	11.35	14.23	8.77	16.08	6.79	11.98	6.58	*	*	12.22	10.14	3.74	18.26	8.37	10.98	200.40
Chaetomorpha antennina																					
	SM	22.97	16.52	10.80	8.54	5.71	5.52	6.44	17.28	11.59	7.34	5.78	*	*	16.01	9.81	3.34	17.83	*	8.84	174.32
	WM	19.74	7.99	7.94	6.61	3.22	4.44	5.87	10.53	13.16	5.56	2.45	*	*	8.03	5.18	3.21	12.67	*	5.31	121.91
	HS	19.62	11.32	10.81	6.04	3.53	3.89	4.78	10.98	10.61	7.22	6.94	*	*	10.82	3.54	1.97	14.27	*	5.39	131.73
Enteromorpha intestinalis																					
	SM	13.26	9.19	5.40	7.39	5.70	5.44	7.32	8.29	6.90	5.27	3.31	0.33	0.50	3.45	10.16	3.83	11.11	*	*	106.85
Rhodophyceae																					
Gracilaria corticata																					
	SM	18.98	14.74	8.97	6.84	7.01	5.99	6.20	11.69	9.53	9.35	3.15	0.93	0.89	5.79	18.00	1.56	12.61	*	*	142.23
	WM	16.15	5.22	6.64	5.80	3.61	10.33	6.65	10.20	8.97	13.60	8.85	4.02	3.36	12.47	8.06	2.37	20.02	*	*	146.32
	HS	2.32	0.96	1.11	2.12	0.77	4.15	6.47	11.11	6.93	6.76	3.53	2.48	1.37	6.60	4.75	3.07	20.99	*	*	85.49
Centroceras clavulatum																					
	RM	11.07	4.56	3.08	4.90	1.88	1.53	5.49	6.87	10.96	10.71	8.05	3.32	3.68	8.31	7.84	3.43	13.67	3.96	*	113.31
Narakkal																					
Chlorophyceae																					
Chaetomorpha antennina																					
	SM	5.18	3.99	2.53	4.02	4.14	4.85	6.50	8.56	10.22	6.41	6.09	*	*	16.58	5.01	3.42	19.39	*	5.20	112.09
Enteromorpha intestinalis																					
	SM	9.65	7.33	4.94	5.38	3.68	3.99	1.43	4.16	6.43	6.19	6.06	1.56	1.90	6.51	6.31	1.99	8.36	*	*	85.87
Rhodophyceae																					
Centroceras clavulatum																					
	SM	30.68	17.28	12.76	11.82	14.35	14.22	5.61	6.06	13.87	16.35	2.30	7.37	6.62	7.02	20.63	1.25	14.70	7.99	*	210.88
	RM	7.98	3.97	2.73	5.77	3.16	1.86	*	5.68	9.58	11.90	4.47	0.67	0.50	8.65	5.85	2.43	18.87	4.67	*	98.74
Grateloupia filicina																					
	SM	25.49	16.62	9.47	10.33	10.60	9.88	2.81	4.85	16.54	15.31	4.60	3.06	3.09	9.70	23.89	1.72	11.62	*	*	179.58

SM: Summer monsoon season RM: Retreating monsoon season WM: Winter monsoon season HS: Hot season

* below estimatable level

[a] Total amount of estimated amino acids

At Ettikkulam, all the species studied had the same amino acid composition, but showed considerable variations in the quantities of individual amino acids (Fig. 5.1) as well as in the total amount estimated (Fig. 5.2). In the summer monsoon season, the total amounts of estimated amino acids were 121.44, 174.32, 106.85 and 142.23 mg g^{-1} dry weight of algae for *Ulva lactuca, Chaetomorpha antennina, Enteromorpha intestinalis* and *Gracilaria corticata* respectively. In the chlorophyte *U. lactuca*, the most dominant amino acid was proline (24.08 mg g^{-1} dry weight) followed by phenylalanine (17.03 mg g^{-1}), while in *C. antennina* and *E. intestinalis*, aspartic acid recorded the highest value (22.97 and 13.26 mg g^{-1} respectively) followed by proline (17.83 and 11.11 mg g^{-1} respectively). In the rhodophyte *G. corticata* also the highest value was recorded by aspartic acid (18.98 mg g^{-1}). This was followed by leucine(s), glutamic acid, proline and cysteine. In *U. lactuca*, amino acids such as γ-aminobutyric acid, alanine, arginine, histidine, lysine, leucine(s) and cysteine were present in fairly good quantities while tyrosine, threonine, ornithine, aspartic acid, hydroxyproline, glycine, serine and glutamic acid were present in small amounts. Methionine and valine occurred in trace levels. In *C. antennina*, cysteine, glutamic acid and phenylalanine were present in large amounts. Lysine, serine, leucine(s), γ-aminobutyric acid and glycine were also well represented. Arginine, tyrosine, histidine, threonine and alanine were present in good quantities. Hydroxyproline occurred in small amounts while methionine, valine and ornithine were in traces. In *E. intestinalis*, leucine(s), glutamic acid, cysteine, glycine, tyrosine, lysine, threonine, alanine, serine and arginine were present in good quantities. Hydroxyproline, phenylalanine and histidine occurred in small quantities, whereas methionine and valine in very small amounts. γ-Aminobutyric acid and ornithine were present in trace

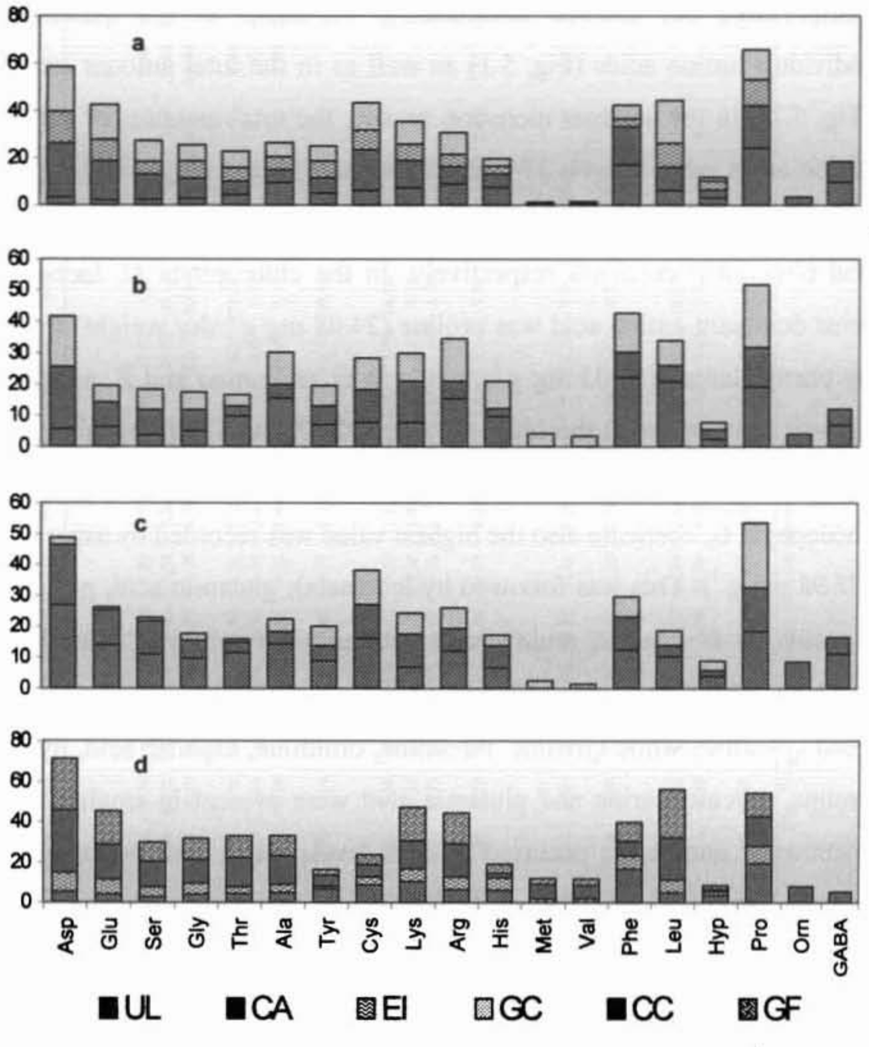


Fig. 5.1. Interspecies variations in the amino acid contents (mg g^{-1} dry wt.) of marine algae collected from Ettikkulam in the summer monsoon season (a), winter monsoon (b), and hot season (c) and that of algae collected from Narakkal in the summer monsoon season (d). (UL: *Ulva lactuca* CA: *Chaetomorpha antennina* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

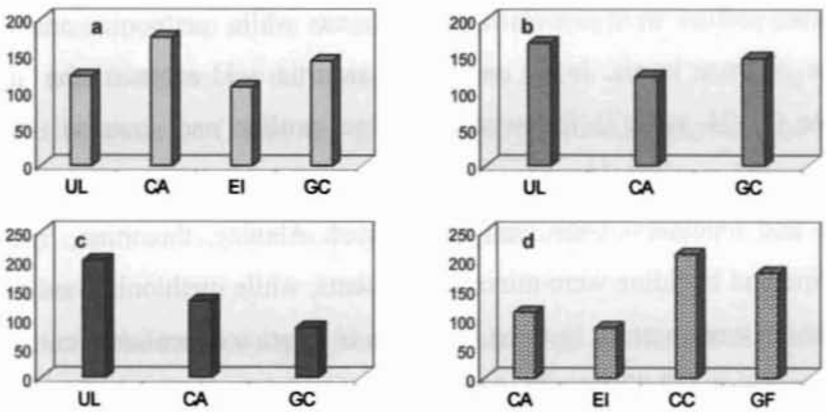


Fig. 5.2. Interspecies variations in the total amino acid contents (mg g^{-1} dry wt.) of marine algae collected from Ettikkulam in the summer monsoon season (a), winter monsoon (b), and hot season (c) and that of algae collected from Narakkal in the summer monsoon season (d). (UL: *Ulva lactuca* CA: *Chaetomorpha antennina* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

levels. In *G. corticata*, lysine, arginine, serine, threonine, glycine, tyrosine, alanine and phenylalanine were well represented. Histidine and hydroxyproline were present in small quantities and methionine and valine in very small amounts. In this species also, γ-Aminobutyric acid and ornithine occurred in trace levels.

In the winter monsoon season, the total amount of amino acids were found to be 167.13 mg g^{-1} for *U. lactuca*, 121.91 mg g^{-1} for *C. antennina* and 146.32 mg g^{-1} for *G. corticata*. *E. intestinalis* was absent in this season. In *U. lactuca*, the dominant amino acid was phenylalanine (22.08 mg g^{-1}) followed by leucine(s) (20.63 mg g^{-1}) and proline (18.98 mg g^{-1}). Alanine and arginine were also present in large amounts. Threonine, histidine, lysine, cysteine, tyrosine, γ-aminobutyric acid, glutamic acid, aspartic acid and glycine were present in fairly good quantities. Ornithine, serine and hydroxyproline were present in low amounts while methionine and valine were in trace levels. In *C. antennina*, aspartic acid recorded the highest value (19.74 mg g^{-1}) followed by lysine, proline and cysteine. Phenylalanine, glutamic acid, serine, glycine, tyrosine, arginine, γ-aminobutyric acid and leucine(s) were well represented. Alanine, threonine, hydroxyproline and histidine were minor constituents, while methionine, valine and ornithine were present in trace levels. In *G. corticata*, proline recorded the highest value (20.02 mg g^{-1}). Aspartic acid, arginine, phenylalanine, alanine and cysteine were also found to be major constituents. Lysine, histidine, leucine(s), tyrosine, serine, glycine and glutamic acid occurred in good amounts. Methionine, threonine, valine and hydroxyproline were present in low levels while γ-aminobutyric acid and ornithine occurred in trace levels.

In the hot season, the values for the total amount of estimated amino acids were 200.40 mg g^{-1} for *U. lactuca*, 131.73 mg g^{-1} for *C. antennina* and 85.49 mg g^{-1} for *G. corticata*. In this season, aspartic acid was found to be the dominant amino acid in *U. lactuca* and *C. antennina* (26.83 and 19.62 mg g^{-1} respectively) followed by proline (18.26 and 14.27 mg g^{-1} respectively). Cysteine, alanine, glutamic acid, phenylalanine, arginine, threonine, γ-aminobutyric acid, serine and leucine(s) in *U. lactuca* and glutamic acid, cysteine, phenylalanine, serine and lysine in *C. antennina* were found to be major constituents. In *U. lactuca*, glycine, tyrosine, ornithine, lysine and histidine were present in fairly high amounts and in *C. antennina*, arginine, histidine, glycine and γ-aminobutyric acid were well represented. Low level of hydroxyproline was observed in *U. lactuca* while tyrosine, alanine, leucine(s), threonine and hydroxyproline were present in small amounts in *C. antennina*. Methionine and valine occurred in trace levels in both the species. Ornithine was also present in traces in *C. antennina*. In *G. corticata*, most of the amino acids recorded low levels in the hot season. Proline was found to be the most dominant amino acid (20.99 mg g^{-1}) followed by cysteine (11.11 mg g^{-1}). Lysine, arginine, phenylalanine and tyrosine were also present in good amounts, while all others were found to be minor constituents. γ-Aminobutyric acid and ornithine were present only in trace levels.

As in the case of algae from Ettikkulam, no qualitative differences were observed in the amino acid composition of different species of algae collected from Narakkal also. However, considerable variations were found in the concentrations of individual amino acids (Fig. 5.1). The total amount of estimated amino acids also showed notable variations (Fig. 5.2). The values were 112.09 mg g^{-1} for *Chaetomorpha antennina*, 85.87 mg g^{-1} for *Enteromorpha intestinalis*, 210.88 mg g^{-1} for *Centroceras clavulatum* and 179.58 mg g^{-1} for *Grateloupia filicina*. In the chlorophyte *C. antennina*, proline recorded the highest value (19.39 mg g^{-1}) followed by phenylalanine (16.58 mg g^{-1}) and lysine (10.22 mg g^{-1}). Cysteine, tyrosine, arginine, histidine, γ-aminobutyric acid, aspartic acid and leucine(s) were well represented. Alanine, threonine, glycine, glutamic acid, hydroxyproline and serine were present in small quantities and methionine, valine and ornithine in trace levels. In *E. intestinalis*, no amino acid was present in large amounts. Aspartic acid recorded the highest level (9.65 mg g^{-1}) followed by proline (8.36 mg g^{-1}). Glutamic acid, phenylalanine, lysine, leucine(s), arginine, histidine and glycine occurred in fairly good quantities while others were found to be minor constituents with γ-aminobutyric acid and ornithine present only in trace levels. In the rhodophytes *Centroceras clavulatum* and *Grateloupia filicina*, aspartic acid was the most dominant amino acid (30.68 and 25.49 mg g^{-1} respectively) followed by leucine(s) (20.63 and 23.89 mg g^{-1} respectively). In *C. clavulatum*, glutamic acid, arginine, proline, threonine, alanine, lysine, serine and glycine were also present in large amounts. Ornithine, methionine, phenylalanine, valine, cysteine and tyrosine occurred in good amounts. Histidine and hydroxyproline were present in small amounts and γ-aminobutyric acid in trace level. In *G. filicina*, glutamic acid, lysine, arginine and proline were present in large amounts. Threonine, glycine, alanine, phenylalanine and serine occurred in fairly high levels. Cysteine, histidine, valine, methionine, tyrosine and hydroxyproline were found to be minor constituents. γ-Aminobutyric acid and ornithine occurred in trace amounts.

From the above results, it can be seen that among the marine algae collected from Ettikkulam in the summer monsoon season, *Ulva lactuca* recorded the highest levels of alanine, histidine, phenylalanine, proline, γ-aminobutyric acid and ornithine; *Chaetomorpha antennina*, aspartic acid, glutamic acid, serine, glycine, cysteine and lysine; *Enteromorpha intestinalis*, tyrosine and hydroxyproline; and *Gracilaria corticata*, threonine, arginine, methionine, valine and leucine(s). In the winter monsoon season, *U. lactuca* showed highest levels of threonine, alanine, tyrosine, arginine, histidine, phenylalanine, leucine(s), γ-aminobutyric acid and ornithine; *C. antennina*, aspartic acid, glutamic acid, serine, glycine, cysteine, lysine and hydroxyproline; and *G. corticata*, methionine, valine and proline (*E. intestinalis* was absent in this season). In the hot season, the highest levels of most of the amino acids studied were recorded by *Ulva lactuca* (fourteen out of nineteen amino acids estimated). *C. antennina* showed highest levels of lysine and histidine while *G. corticata* recorded highest values of proline, methionine and valine. Among the algae collected from Narakkal in the summer monsoon season, *C. antennina* recorded highest levels of tyrosine, cysteine, histidine, phenylalanine, proline, hydroxyproline and γ-amino-butyric acid; *Centroceras clavulatum*, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, arginine, methionine, valine and ornithine; and *Grateloupia filicina*, lysine and leucine(s).

Classwise variations in the amino acid composition were also studied. At Narakkal, this study was carried out only in the summer monsoon season. Here, considerable quantitative variations were shown by most of the amino acids between rhodophyceae and chlorophyceae (Fig. 5.3). Twelve of the nineteen amino acids studied recorded higher values in rhodophyceae and in most cases values were more than twice that of chlorophyceae. Cysteine, tyrosine and proline showed comparable values in the two classes of algae, while histidine, phenylalanine, hydroxyproline and γ-aminobutyric acid showed higher values in chlorophyceae. Considering the total amino acid content, it was found that the value for rhodophyceae of Narakkal was almost twice as high as that for chlorophyceae (Fig. 5.4). At Ettikkulam, the only rhodophyte studied was *Gracilaria corticata* and for the study of classwise variations in the amino acid composition, the mean values for chlorophyceae members were compared with the values obtained for the single rhodophyte *G. corticata*. The study was carried out in three different seasons, *viz.*, summer monsoon, winter monsoon and hot season. In the summer monsoon season, total amino acid content was slightly higher in rhodophyceae than in chlorophyceae (Fig. 5.4). The rhodophyceae member was found to record higher values of aspartic acid, glutamic acid, serine, threonine, arginine and leucine(s) while chlorophyceae showed higher levels of histidine, phenylalanine, hydroxyproline, proline, γ-aminobutyric acid and ornithine (Fig. 5.3). Tyrosine, glycine, alanine, cysteine, lysine, valine

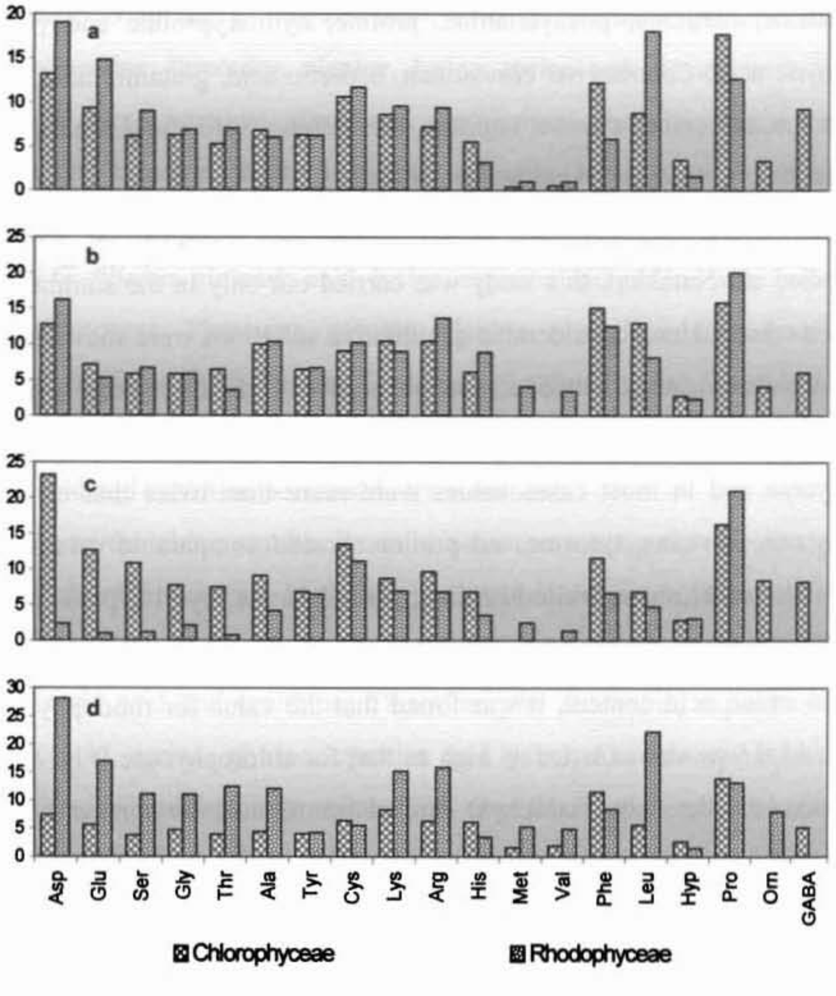


Fig. 5.3. Interclass variations in the amino acid contents (mg g^{-1} dry wt.) of marine algae collected from Ettikkulam in the summer monsoon season (a), winter monsoon (b), and hot season (c) and that of algae collected from Narakkal in the summer monsoon season (d).

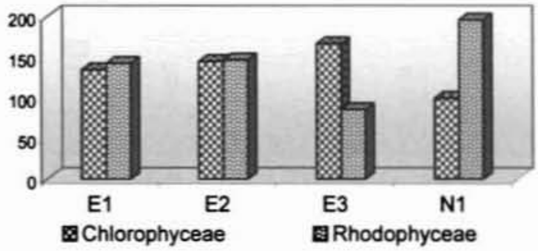


Fig. 5.4. Interclass variations in the amino acid contents (mg g^{-1} dry wt.) of marine algae from Kerala coast (E1: Ettikkulam – summer monsoon E2: Ettikkulam – winter monsoon E3: Ettikkulam – hot season N1: Narakkal – summer monsoon)

and methionine recorded comparable values in the two classes of algae. In the winter monsoon season, total amino acid contents were comparable in the two classes of algae (Fig. 5.4). The rhodophyceae member recorded higher values of aspartic acid, cysteine, arginine, histidine, methionine, valine and proline, whereas chlorophyceae showed higher levels of glutamic acid, threonine, lysine phenylalanine, leucine(s), γ-aminobutyric acid and ornithine (Fig. 5.3). Glycine, tyrosine, alanine, serine and hydroxyproline recorded comparable values in rhodophyceae and chlorophyceae. In the hot season, total amino acid content of chlorophyceae was almost twice as high as that of rhodophyceae (Fig. 5.4). Considering the individual amino acids, it was found that fourteen of the nineteen amino acids studied recorded higher values in chlorophyceae (Fig. 5.3). Methionine, valine and proline showed higher values in rhodophyceae while tyrosine and hydroxyproline showed comparable values in the two classes.

During the present study, temporal variations in the amino acid composition were determined for marine algae collected from Ettikkulam. The variations were studied for *Chaetomorpha antennina*, *Ulva lactuca* and

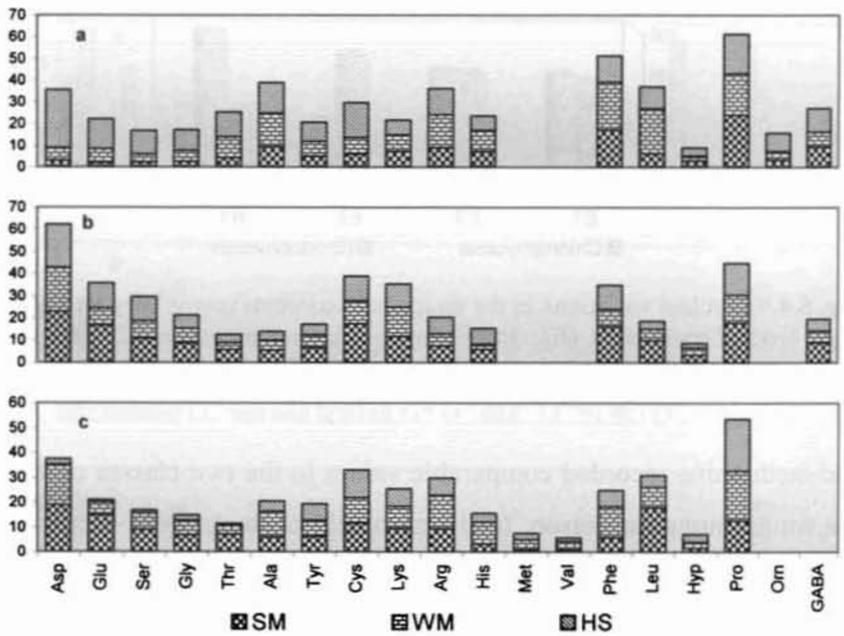


Fig. 5.5. Temporal variations in the amino acid contents (mg g^{-1} dry wt.) of marine algae from Ettikkulam a) *Ulva lactuca* b) *Chaetomorpha antennina* c) *Gracilaria corticata* (SM: summer monsoon WM: winter monsoon HS: hot season)

Gracilaria corticata in three different seasons, *viz.*, summer monsoon, winter monsoon and hot season. *Enteromorpha intestinalis* was not included in this study since it was present only in the summer monsoon season. No qualitative differences were observed in the amino acid composition of algae in different seasons, but variations were found in the amount of individual amino acids (Fig. 5.5) as well as in the total amount estimated (Fig. 5.6). *U. lactuca* recorded the values 121.44, 167.13 and 200.40 mg g^{-1} for the total amount of amino acids in the summer monsoon, winter monsoon and hot season respectively. It was found that this species recorded the highest levels of most of the amino acids in the hot season (Fig. 5.5). Proline

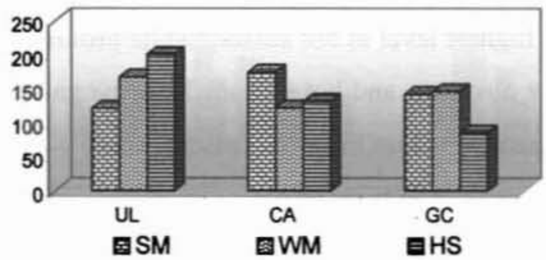


Fig. 5.6. Temporal variations in the total amino acid contents (mg g^{-1}) of marine algae from Ettikkulam (UL: *Ulva lactuca* CA: *Chaetomorpha antennina* GC: *Gracilaria corticata* SM: summer monsoon WM: winter monsoon HS: hot season)

was highest in the summer monsoon season while alanine, lysine, arginine, histidine, phenylalanine and leucine(s) recorded highest levels in the winter monsoon season. Methionine and valine were in trace levels in all the three seasons. In *C. antennina*, total amounts of amino acids were 174.32, 121.91 and 131.73 mg g^{-1} in summer monsoon, winter monsoon and hot season respectively. This species recorded the highest levels of most of the amino acids in the summer monsoon season (twelve out of nineteen amino acids estimated) (Fig. 5.5). Lysine was highest in the winter monsoon season and histidine in the hot season. Serine recorded highest value in both summer monsoon and hot season while hydroxyproline showed high values in the summer monsoon and winter monsoon seasons. Methionine, valine and ornithine were in trace levels in all the three seasons. In *G. corticata*, the total amount of amino acids recorded comparable values in the summer monsoon and winter monsoon seasons (142.23 and 146.32 mg g^{-1} respectively), whereas in the hot season, the value was considerably lower (85.49 mg g^{-1}). Aspartic acid, glutamic acid, serine, glycine, threonine, lysine and leucine(s) recorded highest levels in the summer monsoon season, while alanine, arginine, histidine, methionine, valine and phenylalanine showed maximum values in the winter monsoon season (Fig. 5.5). Hydroxyproline exhibited the highest level in hot season, while proline recorded high levels in both winter monsoon and hot season. Tyrosine and cysteine were found to show comparable values in all the seasons, while γ-aminobutyric acid and ornithine were present in trace levels.

In the present study, spatial variations in the amino acid composition were determined for *Chaetomorpha antennina* and *Enteromorpha intestinalis* collected in the summer monsoon season (August) and *Centroceras clavulatum* in the retreating monsoon season (October). In all the studied cases, total amount of amino acids showed higher values at Ettikkulam than at Narakkal (Fig. 5.7). In the case of *C. antennina*, most of the amino acids recorded higher values at Ettikkulam and only proline showed higher value at Narakkal (Fig. 5.8). However, the difference was not much pronounced in the latter case. Alanine, tyrosine, histidine, phenylalanine and hydroxyproline recorded comparable values at the two locations, while methionine, valine and ornithine were present in trace levels. In *E. intestinalis* also, most

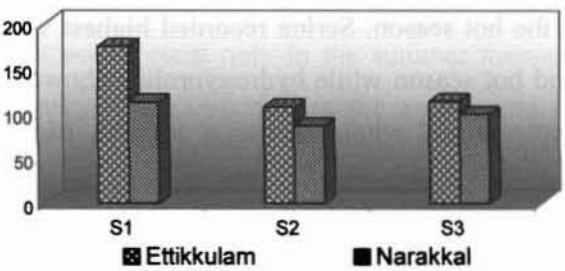


Fig. 5.7. Spatial variations in the total amino acid content (mg g^{-1}) of marine algae from Kerala coast (S1: *Chaetomorpha antennina* in summer monsoon S2: *Enteromorpha intestinalis* in summer monsoon S3: *Centroceras clavulatum* in retreating monsoon)

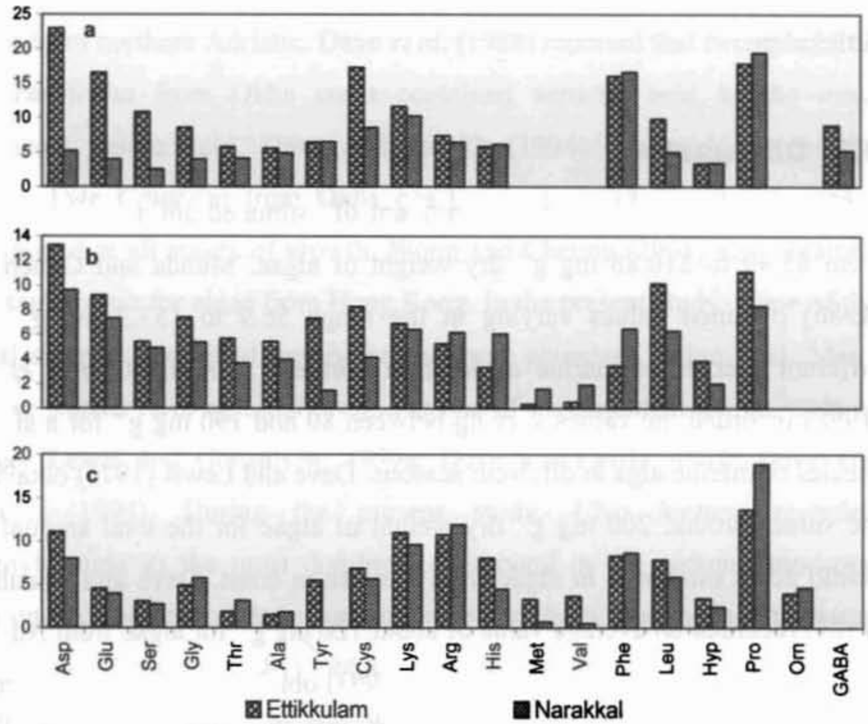


Fig. 5.8. Spatial variations in the amino acid contents (mg g^{-1}) of marine algae from Kerala coast a) *Chaetomorpha antennina* in the summer monsoon b) *Enteromorpha intestinalis* in the summer monsoon and c) *Centroceras clavulatum* in the retreating monsoon)

of the amino acids recorded higher values at Ettikkulam. However, in this case, four of the amino acids, *viz*., histidine, methionine, valine and phenylalanine showed higher values at Narakkal. Serine, lysine and arginine recorded comparable values at the two locations, while ornithine and γ-aminobutyric acid were present only in trace levels. In the case of *C. clavulatum*, threonine, arginine and proline showed higher values at Narakkal, whereas glutamic acid, serine, glycine, alanine, ornithine and phenylalanine recorded comparable values at the two locations. γ-Aminobutyric acid was present in trace levels. All the other estimated amino acids showed higher levels at Ettikkulam.

5.3 Discussion

In the present study, total amount of estimated amino acids varied from 85.49 to 210.88 mg g^{-1} dry weight of algae. Munda and Gubensek (1986) obtained values varying in the range 56.9 to 153.3 mg g^{-1} for different species of marine algae from northern Adriatic. Gomez *et al.* (1998) recorded the values varying between 80 and 190 mg g^{-1} for a single species of marine alga in different seasons. Dave and Lewis (1976) obtained the values around 200 mg g^{-1} dry weight of algae for the total amount of amino acids estimated in algae from Saurashtra coast. Dave and Chauhan (1984) recorded an average value of about 120 mg g^{-1} for algae from Adatra reef along the Saurashtra coast. Kumar (1993) obtained values ranging from 4.0 to 36.6 mg g^{-1} fresh weight of algae for the total amino acid content of marine algae from Tuticorin coast. The estimated levels of individual amino acids were found to vary in the range 0.33 – 30.68 mg g^{-1} dry weight of algae during the present study (Some amino acids were present in trace levels in some algal samples). This was comparable with the range, 0.1 - 25.0 mg g^{-1}, obtained by Munda and Gubensek (1986) for the total amino acid pool of algae from West Istrian coast.

During the present study, the most dominant amino acid in most of the algae was found to be aspartic acid. This was in accordance with earlier reports. Dave and Lewis (1976) obtained aspartic acid as the dominant compound in the protein hydrolysates of algae from Saurashtra coast. Dave and Chauhan (1984) also observed aspartic acid to be the dominant amino acid in algae from Adatra reef of Saurashtra coast. Munda and Gubensek

(1986) showed that aspartic acid was one of the dominant amino acids in algae from northern Adriatic. Dave *et al.* (1988) reported that two species of *Enteromorpha* from Okha coast contained aspartic acid as the most dominant amino acid. Dave and Parekh (1994) observed that in the chlorophyte *Caulerpa* from Okha coast, aspartic acid was the dominant compound at all stages of growth. Wong and Cheung (2001) also obtained the same result for algae from Hong Kong. In the present study, some of the algal samples contained proline as the most abundant amino acid. Many earlier studies have reported proline as one of the dominant amino acids of algae (Lewis and Gonzalves, 1959a; Dave and Lewis, 1976; Dave and Parekh, 1994). During the present study, *Ulva lactuca* recorded phenylalanine as the most dominant compound in the summer monsoon season and the second dominant compound in the winter monsoon season. Dave and Chauhan (1984) obtained phenylalanine as one of the most abundant amino acids in the winter monsoon season in algae from Saurashtra coast. Dave and Lewis (1976) also recorded phenylalanine as one of the abundant amino acids in algae from Saurashtra coast.

In the present study, amino acids such as arginine, cysteine, leucine(s), glutamic acid and lysine were also found to be major constituents. Glycine, histidine and tyrosine were found to present in fairly good quantity. Similar results were reported earlier (Dave and Chauhan, 1984; Dave *et al.*, 1988). In the current investigation, some of the algal samples were observed to contain alanine, serine and threonine in large amounts, while in others they present as minor constituents. This was in agreement with previous studies (Dave and Lewis, 1976; Dave and Chauhan, 1984; Munda and Gubensek, 1986; Dave *et al.*, 1988; Dave and Parekh, 1994). The presence of methionine and ornithine in very small quantities was in accordance with earlier reports (Dave and Lewis, 1976; Dave and Chauhan, 1984; Dave *et al.*, 1988; Dave and Parekh, 1994). In the present study, valine was found in trace levels in some algae and γ-aminobutyric acid in fairly good quantities. This was contrary to previous reports (Dave and Lewis, 1976; Dave *et al.*, 1988; Dave and Parekh, 1994).

In this study, variations were observed in the amino acid composition of algae with respect to the place and period of collection. The dominant amino acids were found to change with season and habitat. There may be different reasons for these variations. The different phenological stages of algae and the various factors affecting the nitrogen metabolism influence the amino acid composition of algae. It has been found that the amino acid composition of algae is influenced by the factors such as age, plant part and the growing conditions like light, temperature, salinity, nutrient availability, period of emersion etc (Fowden, 1962).

A comparison of the total amino acid content of algae with their protein content (Fig. 5.9) showed that in most cases, amino acid contents were higher than protein contents indicating the presence of free amino acid pool. Previous studies have shown that algae contain fairly high levels of free amino acids (Dave and Lewis, 1976; Dave and Chauhan, 1984; Dave *et al.*, 1988; Dave and Parekh, 1994). It has been found that free amino acid pool in algae contains almost all the amino acids present in the protein (Fowden, 1962). In a few cases, amino acid contents were less than protein contents and similar results were reported earlier (Kumar, 1993; Gomez *et al.*, 1998). This may be due to the partial destruction of some amino acids during the preparation of acid hydrolysates of algal meal. Also, in the present study, all the constituent amino acids were not determined (e.g., tryptophan).

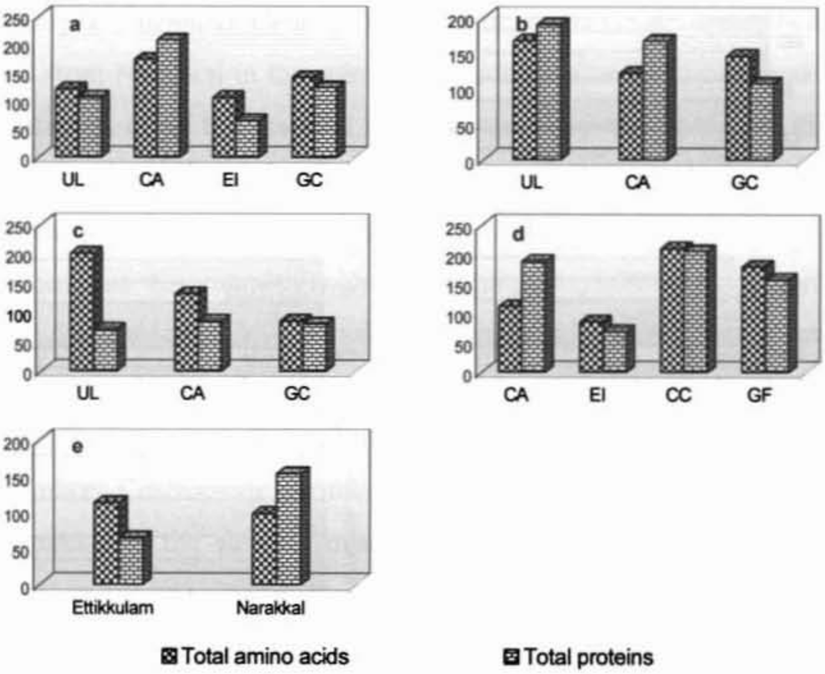


Fig. 5.9. Comparison of total amino acid contents (mg g^{-1} dry wt.) of different species of marine algae with their total protein contents (mg g^{-1} dry wt.) a) Ettikkulam – summer monsoon b) Ettikkulam – winter monsoon c) Ettikkulam – hot season d) Narakkal – summer monsoon and e) *Centroceras clavulatum* – retreating monsoon (UL: *Ulva lactuca* CA: *Chaetomorpha antennina* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

Considering the total amount of essential amino acids (excluding tryptophan), it was found that 20.2 – 37.2% of the total amino acid pool of the studied algae was constituted by essential amino acids (Fig. 5.10). Wong and Cheung (2001) included cysteine and tyrosine in the essential amino acid pool and showed that essential amino acids accounted for 36.2 – 40.2% of the total amino acid content. A similar calculation in the present study

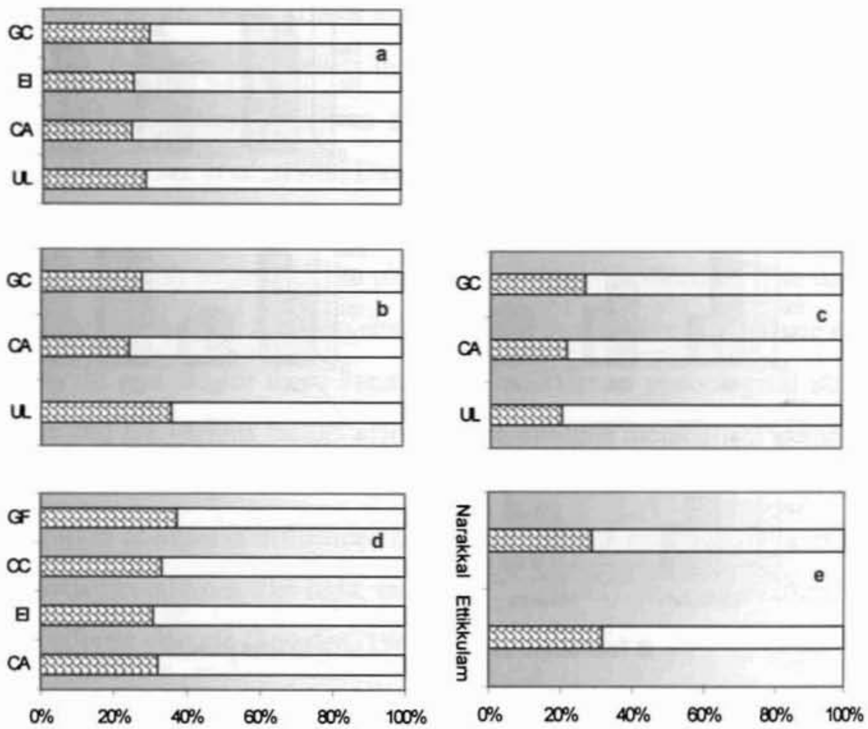


Fig. 5.10. Essential amino acid content (expresses as percent of total amino acid content) of different species of marine algae from Kerala coast a) Ettikkulam - summer monsoon b) Ettikkulam – winter monsoon c) Ettikkulam – hot season d) Narakkal – summer monsoon and e) *Centroceras clavulatum* – retreating monsoon (UL: *Ulva lactuca* CA: *Chaetomorpha antennina* EI: *Enteromorpha intestinalis* GC: *Gracilaria corticata* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*).

showed that essential amino acids constituted 32.2 – 47.4% of the total amino acid content. Comparable values were reported earlier (Fujiwara-Arasaki *et al.*, 1984; Ochiai *et al.*, 1987; Fleurence *et al.*, 1995). Among the essential amino acids, phenylalanine and leucine(s) were found to be dominant compounds during the present study. Lysine and threonine were present in fairly high levels. However, methionine and valine were found to be limiting amino acids in many cases, especially in *Ulva lactuca* and *Chaetomorpha antennina*. *Centroceras clavulatum* and *Grateloupia filicina* collected from Narakkal in the summer monsoon season were observed to be rich sources of all the essential amino acids. They also contained high levels of other amino acids. Total amino acid contents were relatively high in these species. *Ulva lactuca* was rich in phenylalanine and leucine(s) in all the seasons and *Chaetomorpha antennina* in phenylalanine and lysine. However, these species contained valine and methionine in very low or trace levels.

In conclusion, with respect to the amino acid content, the rhodophyceae members *Centroceras clavulatum* and *Grateloupia filicina* collected from Narakkal in the summer monsoon season were found to be more nutritive than the other algal species studied. The chlorophyceae members were found to be limiting in methionine and valine. Thus, the rhodophytes of Narakkal especially, those available in the summer monsoon season can be exploited for use as an additional source of essential amino acids.

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Chapter 6

AGAR

6.1 Introduction

Gelling and viscous substances have been extracted from marine macroalgae, especially red and brown algae, for centuries and usually used in food preparations. These algal extracts are commonly known as phycocolloids or hydrocolloids because they can form colloidal systems in water. These include agar and carrageenan extracted from red algae and alginates from brown algae. They serve four basic functions in food applications – thickening, gelling, emulsification and stabilization. The first phycocolloid to achieve commercial status for purposes other than food use was agar, which was used as a microbiological culture medium (Chapman, 1970). The modern uses of agar include biotechnological (electrophoresis, chromatography etc), medical (as laxative, anticoagulant etc.) and dental (impression materials) applications. It finds several applications in plant tissue culture, biochemistry, molecular biology and drug industry (Mouradi-Givernaud *et al.*, 1992). The excellent gelling properties of agar at moderate temperatures make it much useful in bakery products, confectionery making, and in puddings, creams and jellied products. It is also used in canned meats and fish, and as a clarifying agent in wines and beers (Levring *et al.*, 1969). Table 6.1 summarizes the applications of agar in prepared foods.

Agar production, from its first reported production in 17^{th} century in Japan, has now grown into a multi-million dollar industry. About 1.8×10^4 tonne yr^{-1} (wet weight) of algae are consumed for the production of about 11,000 tonne yr^{-1} of agar (Lobban and Harrison, 1997). Asian countries lead the world in agar production with over 50% of the agar produced, and Japan is the largest producer of agar with over 400 agar-extracting firms (Guist, 1990). Agar industries in India produce about 60 tonnes of agar annually. Since, this is quite insufficient to meet the demand, India imports about 10-12 tonnes of agar annually (Zaidi *et al.*, 1999).

Agar occurs in certain red algae, especially in the members of the Gelidiaceae and Gracilariaceae, as a major constituent of cell walls and is

Table 6.1. Food applications of agar.

System	Product	Function
Water-based	Icings	Production and storage stability
	Frozen foods	Moisture retention, texture, body, mouth feel
	Fruit juices	Clarification
	Candies	Texture, mouth feel
	Dessert gels	Texture, body
	Canned meat, fish, poultry	Production and storage stability
Milk-based	Cheeses	Texture, production and storage stability
	Yogurt	Texture, production and storage stability

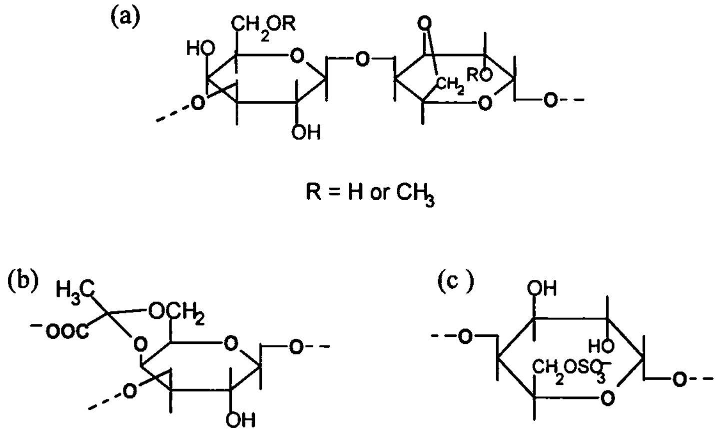

Fig. 6.1. (a) The disaccharide repeating structure of agar showing the positions of commonly encountered methyl ether substituents; (b) 1,3-linked β-D-galactopyranosyl unit with a pyruvate acetal substituent; (c) 1,4-linked α-L-galactopyranosyl-6-sulphate unit.

located in the intercellular matrix (McCandless, 1981). It helps the algae to cope with the externally induced stress in their environment. In addition to adding rigidity to the cell walls, it provides a certain amount of elasticity necessary in the aquatic environment. It also provides protection for the cells against desiccation (McCandless, 1981; Lobban and Harrison, 1997). Agar is usually considered to be a complex mixture of closely related polysaccharides (galactans) possessing varying degrees of chemical substitution to a basic structure composed of alternating 1,3 linked β-D-galactopyranose and 1,4 linked 3,6-anhydro-α-L- galactopyranose residues (Fig. 6.1). The family of galactans may vary among three extreme forms: a neutral agarose, which may contain methoxyl groups on C-6 of D-galactose or C-2 of L-galactose residues, a slightly acidic pyruvated agarose in which D-galactose residues are partially replaced with 4,6-O-(1-carboxy ethylidene) D-galactose, and a sulphated agarose in which D-galactose may be sulphated on C-4, C-6 or C-2 and L-galactose on C-6 (Duckworth and Yaphe, 1971a,b; Falshaw *et al.*, 1999). The 6-sulphated L-galactose residue is usually considered as the precursor of 3,6-anhydro-α-L- galactose (Araki, 1966) and the conversion can be effected by alkali treatment. The gelling properties of agars depend on the degree and type of substitution. Sulphated residues strongly decrease the gel strength, while 3,6-anhydro-α-L- galactose residues improve the gel strength (Yaphe and Duckworth, 1972; Whyte *et al.*, 1981). Methyl-ether groups modify the elasticity and gelling temperature (Guiseley, 1970; Lahaye *et al.*, 1986). The relative proportions of the three major fractions of agar polysaccharides may be different in different algae and may also vary under different growth conditions giving rise to variable gelling properties (Friedlander *et al.*, 1981).

Several studies have been carried out on the agar characteristics of various red algae especially *Gelidium* and *Gracilaria* species occurring in different parts of the world. DeLoach *et al.* (1946) studied agar characteristics of the rhodophyte *Gracilaria confervoides* from North Carolina. Funaki and Kojima (1951) also carried out studies on the agar from this species. Jones (1959) examined the effects of environmental factors on agar from *G. verrucosa.* Minghou *et al.* (1965) studied the characteristics of agar from the same species. Araki (1966) carried out structural studies of agar. Tsuchiya and Hong (1967) made studies on agar from *Gelidium* and *Gracilaria* species. Duckworth and Yaphe (1970) carried out thin layer chromatographic analysis of agar. The relationship between methoxyl content and gelling temperature of agarose was determined by Guiseley (1970). Some structural studies on agar were carried out by Duckworth and Yaphe

(1971a,b). Duckworth *et al.* (1971) investigated agar polysaccharides of *Gracilaria* sp. Young *et al.* (1971) made structural studies of agar. Izumi (1971, 1972) studied the chemical heterogeneity of agar from *Gelidium* and *Gracilaria* species. Kim and Yaphe (1972) examined the parameters affecting the gel formation of algae. The relationship between structure and biological properties of agars was studied by Yaphe and Duckworth (1972). Matsuhashi and Hayashi (1972) carried out studies on the agar extracted from *G. foliifera* of Florida. Chou (1973) and Young (1974) investigated *Gracilaria* agars. John and Asare (1975) studied variations and properties of agar from Ghanaian agarophytes. Seasonal aspects of agar characteristics of Hawaiian *Gracilaria* species were studied by Hoyle (1978). DeBoer (1978) studied the effects of nitrogen enrichment on growth rate and phycocolloid content of *G. foliifera.* Kim and Henriquez (1978) recorded the yield and gel strength of agar from cystocarpic and tetrasporic plants of *G. verrucosa.* Agars from different generations of *G. verrucosa* were compared by Usov *et al.* (1979). Whyte and Englar (1979) investigated the variations in agar quality with seasons and reproductive conditions of *Gracilaria* sp. from Haines Island. Nicolaisen *et al.* (1980) carried out spectral analysis of agarose. Wang and Yang (1980) studied seasonal variation of agar quality of *Gracilaria* species from Taiwan. Asare (1980) determined the seasonal changes in sulphate and 3,6-anhydrogalactose contents of agar from *G. tikvahiae*. Zanlungo (1980) examined the composition of agar from Chilean *Gelidium* sp. Whyte and Englar (1980) investigated the chemical composition and quality of agar in the morphotypes of *Gracilaria* from British Columbia. Whyte *et al.* (1981) studied variations in the quality and quantity of agar from the reproductive and vegetative stages of *Gracilaria* sp. Bird *et al.* (1981) carried out studies on the chemical quality and production of agars extracted from *G. tikvahiae* grown in different nitrogen enrichment conditions. Durairatnam and Santos (1981) examined the agars from *G. verrucosa* and *G. sjoestedtii* from northeast Brazil. Friedlander *et al.* (1981) investigated the composition of agar from *Gracilaria* and *Pterocladia* species. Whyte and Englar (1981a) studied agar characteristics of an inter-tidal population of *Gracilaria* sp. Yang *et al.* (1981) reported seasonal variations in agar of algae from Taiwan area. Whyte and Englar (1981b) examined the agar component of *Gelidium* sp. Structural study of agars from New Zealand red algae of the family Gelidiaceae was carried out by Miller and Furneaux (1982). Shengyao and Zhanxiang (1982) investigated the effects of alkali treatment on the yield and gel strength of *Gracilaria* agar. Patwary and Meer (1983) studied the properties of agar extracted from *G. tikvahiae.* Usov *et al.* (1983) carried out chemical and spectral studies on the gel forming polysaccharides from Japan Sea red algae. Santos and Doty (1983) examined the agarose from *G. cylindrica.* Yang and Wang (1983) studied the effects of environmental factors on the agar characteristics of *Gracilaria* from Taiwan. The yield and quality of agar from species of *Gracilaria* collected from Taiwan and Micronesia were determined by Nelson *et al.* (1983). Craigie and Wen (1984) investigated the effects of temperature and tissue age on the gel strength and composition of agar from *G. tikvahiae.* Yao *et al.* (1984) studied the yield and properties of agar extracted from different life stages of *G. verrucosa.* Yaphe (1984) studied the properties of *Gracilaria* agar. The growth rates, phycocolloid yield and quality of several red algae including *Gracilaria* species from Israel were studied by Friedlander and Zelikovitch (1984). Craigie *et al.* (1984) determined variations in the composition of agars from Chinese *Gracilaria* spp. Minghou *et al.* (1985) carried out structural analysis of agar from *Gracilaria* collected from China. Lahaye *et al.* (1986) determined the heterogeneity of agar polymers in the cell walls of *Gracilaria* spp. The seasonal growth and agar contents of *Gelidium* spp. from South Africa were studied by Carter and Anderson (1986). Cote and Hanisak (1986) studied the production and properties of agars from *Gracilaria tikvahiae* and other red algae. Miller and Furneaux (1987) carried out the chemical characterization of galactans from *G. secundata* from New Zealand. Durairatnam (1987) reported the yield, gel strength and quality of agar of *G. edulis* from Brazil. Christiaen *et al.* (1987) investigated the structure, yield and biochemical quality of agar of *G. verrucosa* from northern France. Onraet and Roberston (1987) studied seasonal variation in yield and properties of agar from sporophytic and gametophytic phases of algae of family Gelidiaceae. Lahaye and Yaphe (1988) examined the effects of seasons on the chemical structure and gel strength of *G. pseudoverrucosa* agar. Lahaye *et al.* (1988) determined the chemical structure of agars from *G. crassissima* and *G. tikvahiae*. The effects of salinity and temperature on agar production from *G. verrucosa* were investigated by Daugherty and Bird (1988). Hurtado-Ponce and Umezaki (1988) studied physical properties of agar gel from *Gracilaria* of Philippines. Lahaye and Yaphe (1989) studied the chemical structure of agar from *G. compressa, G. cervicornis, G. damaecornis* and *G. domingensis*. Christeller and Laing (1989) examined the effects of environment on the agar yield and gel characteristics of *G. sordida*. Furneaux *et al.* (1990) carried out studies on agar from New Zealand members of Gracilariaceae. Ekman and Pedersen (1990) investigated the influence of environmental factors and growth rate on agar composition of *G. sordida* and *G. verrucosa*. Lemus *et al.* (1991) determined the agar yield, quality and standing crop biomass of agarophytes from Venezuela. The seasonal variations in agar yield and quality from North

Carolina agarophytes were studied by Bird and Hinson (1992). Murano *et al.* (1992) carried out chemical characterization of agar polymers from *G. dura*. Price and Beilig (1992) reported the agar yield from *G. edulis* collected from eastern Australia. Agars from *Gelidiella acerosa* were studied by Usov and Ivanova (1992). Luhan (1992) determined the agar yield and gel strength of *Gracilaria heteroclada* from central Philippines. Mouradi-Givernaud *et al.* (1992) studied the biochemical composition and seasonal variation of agar from *Gelidium* sp. of France. Durairatnam and Sena (1993) carried out studies on the yield and gel strength of *Gracilaria cornea*. Nelson *et al.* (1994) examined the agar chemistry of *Gelidium* sp. from northern New Zealand. Takano *et al.* (1995) studied the agar characteristics of *Gracilaria eucheumoides*. Oliveiro *et al.* (1996) reported temporal and spatial variations in agar from Brazilian algae of the order Gelidiales. Seasonal changes in agar characteristics of agarophytes from Spain were studied by Freile-Pelegrin *et al.* (1996). Pondevida and Hurtado-Ponce (1996) studied seasonal variations in the agars of *Gracilariopsis* and *Gracilaria* from coastal areas of Philippines. Rebello *et al.* (1996) determined the growth rates and agar quality of *G. gracilis* from Namibia. Hurtado-Ponce and Pondevida (1997) examined the interactive effects of environmental factors on the growth, agar yield and quality of *Gracilariopsis* sp. Freile-Pelegrin and Robledo (1997) studied the effects of season on the agar content and chemical characteristics of *Gracilaria cornea* from Mexico. Falshaw *et al.* (1998) carried out studies on the agars from nine species of red algae of family Gracilariaceae. Falshaw *et al.* (1999) reported the yield, gel strength and chemical structure of agars from three Fijian *Gracilaria* species. Arano *et al.* (2000) examined the growth, agar yield and quality of agarophytes including *Gracilaria* from Philippines. Freile-Pelegrin (2000) studied the agar yield and quality characteristics of *G. cornea* from Mexico.

Many studies have also been carried out on Indian agarophytes. Some of the early studies include those of Bose *et al.* (1943), Chakraborty (1945), Joseph and Mahadevan (1948), Karunakar *et al.* (1948), Thivy (1952, 1960), Pillai (1955), Kappanna and Rao (1963), Rao *et al.* (1965), Srinivasan and Santhanaraja (1967), Doshi and Rao (1967a,b), Doshi *et al.* (1968) and Rao (1970). Thomas *et al.* (1975) studied the periodicity in growth and agar production of *Gelidiella acerosa.* Thomas and Krishnamurthy (1976) examined the agar from cultured *Gracilaria edulis*. Thomas (1977) reported seasonal variations in yield and physical properties of agar from *G. verrucosa*. Rao *et al.* (1977) listed the contents and properties of phycocolloids from six species of red algae including *G. corticata, G. foliifera* and *G. fergusonii*. Chennubhotla *et al.* (1977) made a comparative study on the yield and physical properties of agar from *G. edulis*, *G. verrucosa* and *Gelidiella acerosa*. Rao (1978) reported the yield and quality of agar extracted from *Gracilaria corticata* collected from Visakhapatnam area. Oza (1978) studied the seasonal variations in the yield and physical properties of agar from *G. corticata* occurring in Veraval coast. Chennubhotla *et al.* (1979) investigated seasonal variations in the yield and gel forming properties of agar from some of the commonly occurring agarophytes around Mandapam. Kaliaperumal and Rao (1981) carried out studies on the phycocolloids of algae from Visakhapatnam coast. Doshi *et al.* (1984) reviewed studies on extraction of agar from different agarophytes and Kaliaperumal *et al.* (1987) reviewed studies on agar characteristics of Indian agarophytes. Kaliaperumal *et al.* (1989) examined the agar component of algae from Lakshadweep. The yield and physical properties of agar of *G. edulis* were studied by Mal and Subbaramaiah (1989). Kaliaperumal *et al.* (1992) determined the yield and physical properties of agars of *G. corticata* var. *cylindrica* and *G. arcuata* from Gulf of Mannar. Doshi *et al.* (1992) studied the utilization and management of Indian agarophytes. Mathew *et al.* (1993) studied the preparation of high gel strength agar from *Gelidiella acerosa* collected from Tamil Nadu coast. Nambisan (1998) carried out studies on the improvement of marine algae for enhanced production of agar. Koya (2000) studied seasonal variations in yield and gel strength of *Gracilaria edulis* from Lakshadweep. Mazumder *et al.* (2002) carried out chemical analysis of agar isolated from *G. corticata* collected from Gujarat coast.

The rhodophyceae members belonging to the genera *Gelidium* and *Gracilaria* have been used traditionally for the production of agar (Levring *et al.*, 1969). Species of *Gelidiella*, *Gelidiopsis* and *Pterocladia* are also used for this purpose. *Gelidium* is the most preferred commercial agarophyte because high quality bacteriological grade agar is obtained almost exclusively from this genus. However, the decreasing wild stocks due to their over-exploitation has led to the fast-growing *Gracilaria* becoming the principal source of agar worldwide (Freile-Pelegrin and Robledo, 1997). Approximately 60% of the world's present production of agar is derived from *Gracilaria* species and this is mainly used for food applications. *Gracilaria*, with some 150 species described in the subtidal and intertidal zones of temporate, tropical and Antarctic regions are harvested in over 20 countries of which Chile provides the largest quantity. In India, the availability of *Gracilaria* for agar production amounts to 2,500 – 3,000 tonne yr^{-1} (Zaidi *et al.*, 1999). The commonly occurring *Gracilaria* species in India include *G. corticata*, *G. edulis*, *G. crassa*, *G. verrucosa* and *G. foliifera*. Of these, *G. corticata* is the predominant species with availability of over 850 tonne yr^{-1} (Zaidi *et al.*, 1999).

Agar industries in India use agarophytes occurring mainly in coastal regions of Tamil Nadu and Gujarat. In Kerala, the agarophytes are harvested mainly from Vizhinjam on the southern coast. Since the starting of many agar industries in 80s, the increased need for raw materials has caused vast depletion of natural agarophytes along these coastal areas and presently agar-producing algae are short in supply. Although attempts are being made to cultivate agarophytes to meet the demand of industry, a sustainable exploitation of natural resources of agarophytes occurring on the other Indian coasts is also necessary. However, the lack of studies on the seasonality and quality of agar obtained from many of these agarophytes is an obstacle to commercial harvesting and improvement of wild stocks. The purpose of the present study was to investigate the agar characteristics of the agarophyte *Gracilaria corticata* occurring along the Ettikkulam coast of northern Kerala in order to determine whether it has potential as raw material for commercial agar production. The gelling properties as well as chemical characteristics were studied. Structural study was carried out using Fourier Transform infrared (FTIR) spectroscopic technique to characterize the agar and to correlate its gel forming ability with its structure.

6.2 Results

The yield and physicochemical parameters of agar isolated from *Gracilaria corticata* collected from Ettikkulam in different seasons were determined. The methodologies used for the analyses are detailed in Chapter 2 and the results are given in Table 6.2. The agar yield was found to vary from 20.6 to 26.7% on dry weight basis with a mean value of 24%. The

Table 6.2. Yield and physicochemical parameters of agar extracted from *Gracilaria corticata*

Parameter	Minimum	Mean ± SD	Maximum
Agar yield (%)	20.6	24.0 ± 2.4	26.7
Melting temperature (^{0}C)	85.1	86.2 ± 0.9	87.8
Gelling temperature (^{0}C)	44.0	44.8 ± 0.9	46.4
3,6-anhydrogalactose (%)	26.9	30.2 ± 3.1	35.3
Sulphate (%)	3.4	4.0 ± 0.6	5.0

yield was lowest in the hottest period (late hot season) and highest in the mid hot season (Fig. 6.2). The yield in the summer monsoon season was close to the highest value, while that in the retreating monsoon season was close to the lowest value. The agar yield in the winter monsoon season was almost same as the mean value and appeared to remain almost the same throughout the season.

3,6-Anhydrogalactose (3,6-AG) content varied from 26.9% dry weight of agar to 35.3% with minimum in the mid hot season and maximum in the retreating monsoon season (Fig. 6.2). The sulphate content of agar was found to vary in the range 3.4 – 5.0% dry weight of agar. The lowest value was recorded in the early winter monsoon season and highest in the mid winter monsoon season (Fig. 6.2). The sulphate contents showed lower values during the period from late hot season to early winter monsoon season and higher values from mid winter monsoon to mid hot season. Gelling and melting temperatures of agar recorded very narrow ranges (44.0 – 46.4 ^{0}C and 85.1 – 87.8 ^{0}C respectively). Correlation analysis (Fig. 6.3) showed a significant positive correlation of melting temperature with 3,6-AG content

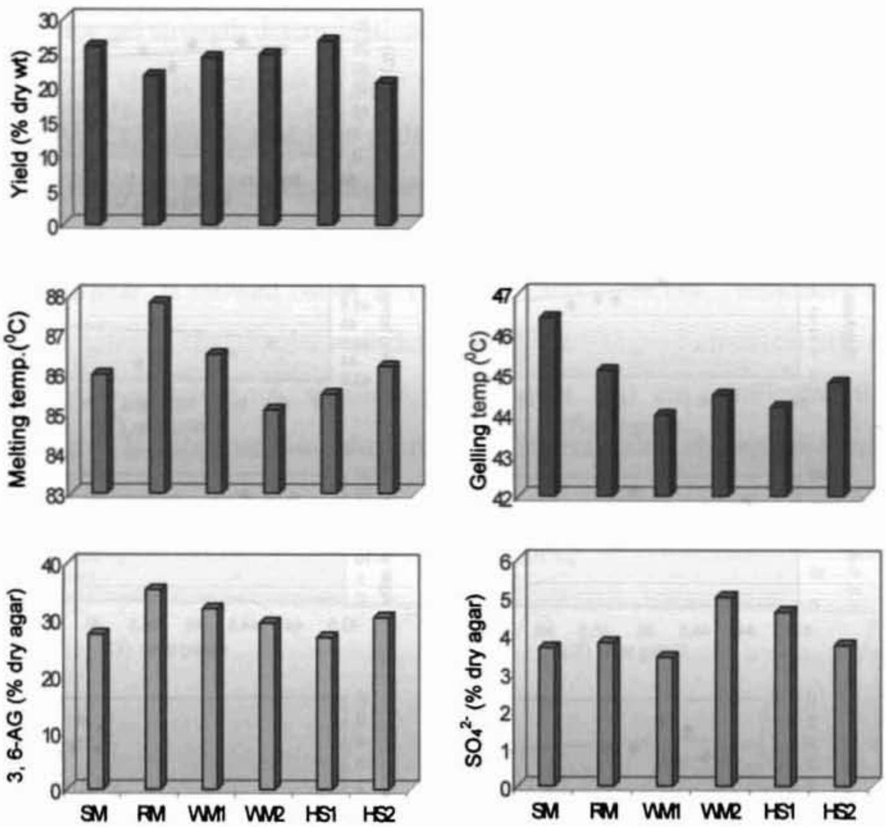

Fig. 6.2. Temporal variations in the yield and physicochemical parameters of afar isolated from *Gracilaria corticata* (SM: Summer monsoon RM: Retreating monsoon WM1: Early winter monsoon WM2: Mid-winter monsoon HS1: Mid-hot season HS2: Late hot season)

($r = 0.851$, $P<0.05$). No significant correlations were observed among other parameters ($P>0.05$).

The gel strength analysis was carried out only with the agar sample isolated from the algae collected in the early winter monsoon season and the value was found to be 76 g cm^{-2}. The quantities of *Gracilaria corticata*

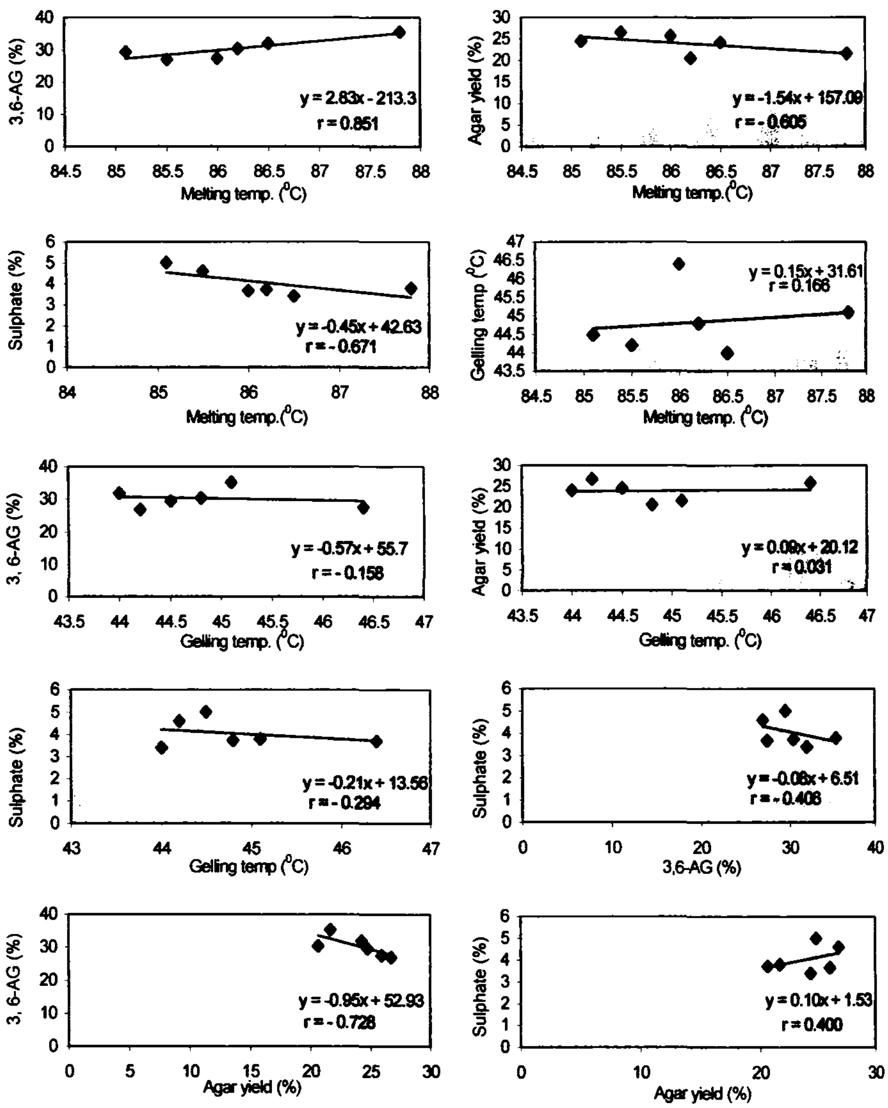

Fig. 6.3. The relationships among various physicochemical parameters of agar isolated from *Gracilaria corticata* (r > 0.755 is significant at 95% level)

collected in other seasons were not sufficient to provide the required amount of agar for gel strength determination.

The characterization of agar sample was carried out by FTIR spectroscopy. The spectrum of KBr pellet of agar was recorded on Bruker IFS 66V FTIR Spectrometer scanning between 400 and 4000 cm^{-1}. The result is given in Fig. 6.4. The spectrum showed the main characteristic absorption bands of agar. It showed bands at 1264 cm^{-1} and 1374 cm^{-1}, indicative of sulphate ester. It contained a well-defined peak at 932 cm^{-1} characteristic of 3,6-anhydro-galactose. A shoulder observed at 850 cm^{-1} indicated the presence of D-galactose-4-sulphate residue. Several other absorption bands were also present.

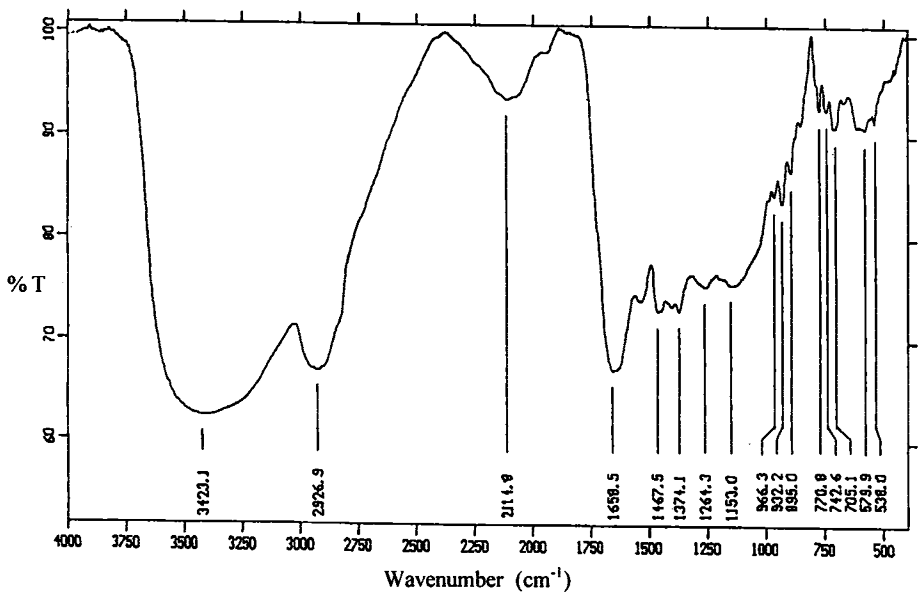

Fig. 6.4. The FTIR spectrum of agar isolated from *Gracilaria corticata*

6.3 Discussion

During the present study, agar isolated from *Gracilaria corticata* recorded an average yield of 24% dry weight of algae. This was higher than the agar yield reported by Oza (1978) for the same species from Veraval coast of Gujarat (14.5 – 22.5%). However, the present yield was found to be considerably lower than that reported for the same species from many other Indian coasts (Rao *et al.*, 1977; Rao, 1978; Chennubhotla *et al.*, 1979; Zaidi *et al.*, 1999). Rao *et al.* (1977) reported a yield of 44%. Rao (1978) recorded agar yield of 45% in *G. corticata* from Visakhapatnam while Chennubhotla *et al.* (1979) reported a value of 43% for the same species from Mandapam region. Kaliaperumal *et al.* (1992) reported agar contents varying from 33.1 to 48.6% in *G. corticata* var. *cylindrica* from Gulf of Mannar. The present value was found to be comparable to that reported for several other species of the genus *Gracilaria*. Chennubhotla *et al.* (1977) reported agar yield of 23% from *G. verrucosa*, while Rao *et al.* (1977) recorded a yield of 25% in *G. foliifera*. Kim and Henriquez (1978) obtained agar yields of 17 and 22% from Chilean *G. verrucosa*. Bird and Hinson (1992) reported agar contents of 20.9 – 22.2% in *G. tikvahiae* and 24.2 – 25.7% in *G. blodgettii*. Falshaw *et al.* (1999) recorded agar contents of 21% in *G. arcuata* and 23% in *G. maramae* from Fiji. Arano *et al.* (2000) reported agar yield of 23.5% in *G. firma* from Philippines. Agar yields lower than that reported for *G. corticata* in the present study were recorded in *Gracilaria* sp. (Arano *et al.*, 2000) and *G. heteroclada* (Luhan, 1992) from Philippines. Whyte and Englar (1980) also recorded lower values in *G. chorda* (15.7%), *G. verrucosa* (13.5%), *G. pseudoverrucosa* (11.3%) and *G. pseudoverrucosa variant* (16.8%) from British Columbia.

Several authors have reported seasonal variations in the agar content of *Gracilaria* species (Humm, 1951; Pillai, 1955; Kim and Humm, 1965; Rao, 1969; John and Asare, 1975; Penniman, 1977; Thomas, 1977; Oza, 1978; Whyte *et al.*, 1981; Christiaen *et al.*, 1987; Luhan, 1992; Kaliaperumal *et al.*, 1992; Freile-Pelegrin and Robledo, 1997). In the present study, agar yields of *G. corticata* varied from 20.62% in the late hot season (May) to 26.71% in the mid hot season (April). Relatively high values were recorded in the summer monsoon and winter monsoon seasons, and low value in the retreating monsoon season. Oza (1978) obtained somewhat similar results for *G. corticata* from Gujarat coast. He recorded high yields in the period June – July and November – April, and low yield in May and August – October. Kaliaperumal *et al.* (1992) recorded the maximum yield of agar in *G. corticata* from Gulf of Mannar in the hot month of March. Luhan (1992) also reported the highest agar yield in the hot period of April in *G. heteroclada* from Philippines.

Seasonality observed in the agar yield may be due to various reasons. The variations in the environmental conditions, which influence growth and reproduction of algae, may affect their agar content. It is possible that the various environmental stimuli such as temperature, salinity, rainfall and nutrients, which vary from season to season, affect the physiological responses of algae including the agar deposition in the cell walls (Luhan, 1992). The agar yield has also been found to be a function of different life phases of algae. Whyte *et al.* (1981) observed that the seasonal fluctuation in the agar yield was dependent, among other parameters, on the reproductive condition of the plant, with gametophytic, tetrasporic and vegetative plants giving different yields of agar. Oza (1978) observed the coincidence of low agar yield with shedding of branches after liberation of tetraspores.

The gel properties of agars have been found to depend on the number and position of sulphate groups as well as on the amount of 3,6-anhydrogalactose fraction of the agar (Duckworth and Yaphe, 1971a). These components are considered as gel regulating factors (Asare, 1980). It has been found that high contents of 3,6-AG and low levels of sulphation usually result in strong gels (Nelson *et al.*, 1983). The suphated residues when present in the polysaccharide chains comprising the gel, causes kinks in the helical structure responsible for the gel formation and this results in weaker and less rigid gels (Rees, 1972). Firmer gels are provided by the idealized agarose molecules consisting of alternating β-D-galactopyranose and 3,6-anhydro-α-L- galactopyranose residues (Fig. 6.1a), the latter forming 47% of the neutral polymer by weight (Whyte and Englar, 1980). A lower level of 3,6-AG indicates a low relative proportion of neutral agarose in the agar.

In the present study, 3,6-AG contents of agar varied in the range 26.91 – 35.32% and sulphate contents in the range 3.41 – 5.01%. This is in agreement with earlier reports. Nelson *et al.* (1983) reported 3,6-AG contents varying in the range 26.5 – 44.0% and sulphate levels in the range 2.5 – 5.3% for different species of *Gracilaria* from Micronesia and Taiwan. Whyte and Englar (1980) reported 3,6-AG contents in the range 28.2 - 34.4% and sulphate contents in the range 3.74 – 6.13% in different morphotypes of *Gracilaria* from British Columbia. *Gracilaria* from Chile recorded sulphate contents of 3.9 and 4.8% (Cote and Hanisak, 1986). Asare (1980) recorded higher values of 3,6-AG (34 – 43%) and wider range of sulphate contents (1.6 – 8%) in *G. tikvahiae* from Rhode Island. However, Bird and Hinson (1992) recorded lower values of 3,6-AG (20.3 – 27.3%) and sulphate (2.5 – 3.5%) in the same species from North Carolina. *G. cornea* from Mexico showed 3,6-AG contents in the range 31.6 – 32.5% and sulphate contents in the range 4.80 – 5.47% (Freile-Pelegrin and Robledo, 1997). Arano *et al.* (2000) recorded maximum 3,6-AG contents of 25.2% and 28% in *Gracilaria* sp. and *G. firma* respectively collected from Philippines.

During the present study, agar samples showed relatively high sulphate contents (>4.5%) and low 3,6-AG contents (<30%) in the mid winter monsoon and mid hot seasons. This may indicate weaker but more viscous gels at these times of the year. In the late hot season, retreating monsoon and early winter monsoon seasons, agar contained relatively high level of 3,6-AG and low level of sulphates. Possibly these times of the year represent the best period to obtain relatively strong gels.

The physical properties of agar such as gelling and melting temperatures showed little seasonal variations during the present study. Similar observations were made earlier (Thomas, 1977; Bird and Hinson, 1992). In the present study, gelling temperature varied from 44.0 to 46.4 ^{0}C and melting temperature from 85.1 to 87.8 ^{0}C. Gelling temperatures were comparable with earlier reports for agars from *G. corticata* (Oza, 1978; Chennubhotla *et al.*, 1979; Kaliaperumal *et al.*, 1992), *G. verrucosa* (Thomas, 1977), *G. edulis* (Chennubhotla *et al.*, 1977, 1979) and *G. tikvahiae* (Bird and Hinson, 1992). The present values were considerably higher than those reported by Rao *et al.* (1977) for *G. corticata* (33 ^{0}C), *G. fergusonii* (22 ^{0}C) and *G. foliifera* (41 ^{0}C). Agars from *G. chorda, G. verrucosa, G. pseudoverrucosa* (Whyte and Englar, 1980), *G. lemanaeformis* (Cote and Hanisak, 1986), *G. heteroclada* (Luhan, 1992), *G. cornea* (Freile-Pelegrin and Robledo, 1997), *G. firma* (Arano *et al.*, 2000) recorded lower gelling temperatures than that recorded by *G. corticata* in the present study. Melting temperatures recorded in the present study were higher than those reported earlier for the same species by many authors (Rao *et al*, 1977; Oza, 1978; Chennubhotla *et al.*, 1979). The present values were lower than those reported by Kaliaperumal *et al.* (1992) for *G. corticata* and *G. arcuata* from Gulf of Mannar. Agars from *G. foliifera* (Rao *et al.*, 1977; Chennubhotla *et al.*, 1979), *G. fergusonii* (Rao *et al.*, 1977), *G. edulis* (Chennubhotla *et al.*, 1977, 1979), *G. tikvahiae* (Bird and Hinson, 1992), *G. lemanaeformis* (Cote and Hanisak, 1986) and *G. cornea* (Freile-Pelegrin and Robledo, 1997) also recorded lower melting temperatures than the present values.

The gelling and melting temperatures exhibited by *G. corticata* during the present study were found to be greater than the values (35 ^{0}C and 83 ^{0}C respectively) reported by Cote and Hanisak (1986) for bacteriological grade Difco Bacto-Agar. It has been reported that the higher gelling temperature is indicative of higher methoxyl content in the polysaccharide chain constituting the agar (Guiseley, 1970). Similarly, higher melting temperature is an indicator of greater molecular weight and size of the agar polymer (Selby and Wynne, 1973). The longer polymers may be more capable of interacting with each other and forming a greater three-dimensional lattice between water and the gel helices. It has been reported that increasing amounts of 3,6-AG and decreasing amounts of sulphate in agar lead to higher melting temperature (Cote and Hanisak, 1986). This is evident in the significant positive correlation observed in the present study between melting temperature and 3,6-AG content ($r = 0.851$, $P<0.05$). Although a negative correlation was observed between melting temperature and sulphate content, it was significant only at 90% level ($r = -0.671$, $P<0.10$).

Gel strength of agar is considered as an important quality parameter distinguishing between the strong brittle gels used in bacteriological and biomedical applications and soft elastic gels used in the food industry (Yaphe and Duckworth, 1972). In the present study, gel strength of agar was determined only for the sample obtained in the early winter monsoon season and the value was 76 g cm^{-2}. This sample recorded the lowest sulphate content (3.41%) during the present study. It also contained fairly high level of 3,6-AG (32%). Thus, this sample possibly represents one of the strongest gels obtained during the present study. The present gel strength was considerably higher than the values reported for agars from some of the Indian *Gracilaria corticata.* Rao *et al.* (1977) reported gel strength of 19 g cm^{-2} and Chennubhotla *et al.* (1979) reported a value of 22 g cm^{-2}. Kaliaperumal *et al.* (1992) recorded gel strengths varying in the range 12 – 67 g cm^{-2} in *G. corticata* var. *cylindrica* from Gulf of Mannar. Oza (1978) recorded gel strengths varying from 17 to 27 g cm^{-2} in *G. corticata* from Veraval coast. Other *Gracilaria* species such as *G. foliifera* (Rao *et al.*, 1977; Chennubhotla *et al.*, 1979), *G. fergusonii* (Rao *et al.*, 1977), *G. edulis* (Chennubhotla *et al.*, 1977) also recorded lower gel strengths than that recorded in the present study. *G. corticata* from Visakhapatnam recorded a higher gel strength of 134 g cm^{-2} (Rao, 1978). Several other *Gracilaria* species also recorded much higher gel strengths than the present value. Luhan (1992) reported values in the range 43 – 494 g cm^{-2} for agar from *G. heteroclada* of Philippines. Bird and Hinson (1992) obtained the range of values 73 – 153 g cm^{-2} for *G. tikvahiae* and much higher values in the range 257 – 593 g cm^{-2} for *G. blodgettii* from North Carolina.

The gel strength recorded for the agar of *G. corticata* in the present study was found to be much lower than that of bacteriological grade Difco Bacto-Agar (270 g cm^{-2}; Cote and Hanisak, 1986). Thus, the agar isolated from *G. corticata* in the present study is not suitable for bacteriological purposes. This is in agreement with previous reports on *Gracilaria* agar (Friedlander *et al.*, 1981; Lobban and Harrison, 1997). The yield and gel strength of agar of *G. corticata* collected from Ettikkulam coast of Kerala during the present study were found to be in accordance with those specified for Grade-II *Gracilaria* species (25% and 70 g cm^{-2} respectively; BIS, 1989). This may be suited for use in food industry, probably after the enhancement of agar quality by techniques such as fractionation (Duckworth and Yaphe, 1971; Friedlander *et al.*, 1981), alkali treatment (Duckworth *et al.*, 1971a; Nelson *et al.*, 1983; Craigie *et al.*, 1984) etc.

The gel-forming properties of agar have been found to depend on its chemical structure. These properties are known to be affected considerably by small modifications in the polymer structure (Rees, 1969). Therefore, structure information is required for possible chemical modification of agar to improve its gel-forming properties. The use of infrared spectroscopy for the structural analysis of agars is well established (Christiaen and Bodard, 1983; Whyte *et al.*, 1985; Rochas *et al.*, 1986). The FTIR spectrum of the present phycocolloid sample showed the main characteristic absorption bands of agar (Christiaen and Bodard, 1983), thus confirming its identity as agar. The spectrum showed a broad band with peak at 1264 cm^{-1}. This band was due to the S–O stretching vibrations and hence, common to all sulphated polysaccharides (Zablackis and Perez, 1990; Duarte *et al.*, 2002). The band at 1374 cm^{-1} was also due to sulphate (Christiaen and Bodard, 1983; Rochas *et al.*, 1986). The broad band at 1153 cm^{-1} may also be attributed to the presence of sulphate residues (Christiaen and Bodard, 1983). The presence of considerable amount of 3,6-anhydrogalactose in the agar polymer was evident in the well-defined peak at 932 cm^{-1} (Christiaen and Bodard, 1983). The shoulder at 850 cm^{-1} showed the presence of D-galactose-4-sulphate residues in the polymer (Rochas *et al.*, 1986). The band at 705 cm^{-1} may also be due to sulphate on C-4 of D-galactose. The absence of absorption bands in the region 800–840 cm^{-1} indicated that the residues such as galactose-2-sulphate, galactose-6-sulphate and 3,6-anhydrogalactose-2-sulphate were absent in the agar polysaccharide and if present, only in very small amounts (Rochas *et al.*, 1986). The band at 2926 cm^{-1} was due to the stretching of C–H groups present in the sugar units (Rochas *et al.*, 1986). The band at 1658 cm^{-1} may be attributed to water present in the agar (Zundel, 1969).

A possible absence of significant amount of L-galactose-6-sulphate residue in the polymer indicates that the well-known alkali treatment may not be effective in improving the gel-forming properties of agar isolated in the present study because this technique is based on the conversion of this residue into 3,6-anhydro-α-L- galactose (Rees, 1961). Duckworth *et al.* (1971) and Usov *et al.* (1983) had not observed any considerable improvement in the gelling properties of *Gracilaria* agar following the alkali treatment. However, the gel-forming properties of the present agar may be improved by fractionation of the mixture of polysaccharides in the agar to get a fraction rich in agarose component having high gel strength (Duckworth and Yaphe, 1971a; Friedlander *et al.*, 1981).

In conclusion, based on the specifications of BIS (1989) for the yield and gel strength of agar isolated, *G. corticata* collected from Ettikkulam coast of Kerala may be categorized as Grade-II. The rheological properties of the agar isolated from this species were found to be far from those required for bacteriological purposes, however, it may be suitable for food industry, probably after enhancement of agar quality. The quality improvement may be carried out by fractionation technique. Considering the gel regulating factors as well as the yield, the best time of the year to obtain a firmer and less viscous gel seems to be the early winter monsoon season. The late hot season and retreating monsoon season may also provide firm and rigid gel, but yield may be low. In other seasons, the gel may be less firm and more viscous.

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Chapter 7

CONCLUSION

Marine macroalgae, which are the most conspicuous inhabitants of rocky shores, constitute an important marine living resource. They are used as human food, live stock feed and agricultural fertilizer in many countries. They also serve as sources of commercially and pharmaceutically important compounds. They are the sole source of commercial phycocolloids such as agars, carrageenans, alginates etc. The various uses of marine algae depend mainly on the richness of biochemical constituents such as proteins, lipids, carbohydrates, amino acids, fatty acids, vitamins, macrominerals, trace elements, bioactive compounds etc. The present study investigates the biochemical composition of some selected species of marine macroalgae from two locations (Ettikkulam and Narakkal) along Kerala coast. The study provides information on the potential of these algae as sources of food for human and animal consumption. In addition, it reveals the interspecies and interclass variability of various organic and inorganic constituents of algae on a spatial and temporal scale. The study gives information on the potential use of the different species of algae studied as bioindicators of heavy metal pollution. It also evaluates the quality and quantity of agar isolated from the agarophyte *Gracilaria*. The salient features of this study are summarized in the following paragraphs.

The different algal species studied during the present investigation were found to contain substantial amounts of proteins (average, 7 – 19%) and carbohydrates (average, 12 – 34%). In most cases, carbohydrates constituted the largest proportions of the dry weight of algae compared to proteins. There were a few exceptions with proteins showing the highest proportion. In the case of the chlorophyte *Chaetomorpha antennina*, fibres represented the largest component. In all the marine algae studied, lipids exhibited relatively smallest proportion (average, 0.4 – 2.2%). The average energy contents varied from 1.5 to 2.1 kcal g^{-1}.

In the present study, marine algae did not show any significant interspecies or interclass variations in the levels of proteins and lipids. Algae from Ettikkulam showed significant specieswise and classwise variations in the levels of carbohydrates including low-molecular-weight carbohydrates (LMWC) and polysaccharides. Rhodophyceae recorded higher values compared to chlorophyceae. Calorific values and fibre contents of algae from this location also exhibited significant variations among different species and classes. Marine algae from Narakkal showed statistically significant interspecies variations in fibre contents only. They did not exhibit any significant interclass variations in the major biochemical constituents.

The biochemical compositions of marine algae from Kerala coast exhibited considerable variations from those of marine algae from other parts of Indian coast. However, variations were not much pronounced between the locations selected for the present study. Significant spatial variations were observed only for the protein and carbohydrate (LMWC, polysaccharides and total carbohydrates) contents of rhodophyceae members. Rhodophyceae from Narakkal showed higher protein contents than those from Ettikkulam, while reverse was true for their carbohydrate contents.

All the algal species studied were found to exhibit considerable temporal variations in their biochemical compositions. Protein and carbohydrate levels were high in the summer monsoon and early winter monsoon seasons. Lipid levels were higher in the period from mid winter monsoon to mid hot season compared to other seasons. Biochemical constituents recorded comparatively low levels in the late hot season. Crude fibre contents of algae did not show any notable seasonal variations. Calorific values were high in the summer monsoon and early winter monsoon seasons and low in late hot seasons. Thus, with respect to the biochemical composition and calorific value, the best seasons for harvest of algae for food purposes appears to be summer monsoon and early winter monsoon seasons while the least suitable period is the late hot season. Considering the levels of major biochemical constituents and energy contents, *Centroceras clavulatum, Grateloupia filicina, Gracilaria corticata* and *Ulva lactuca* were found to have potential as food sources. Among the different species of marine algae studied, *Chaetomorpha antennina* with very high levels of crude fibre and low calorific value appeared to be least suitable for food purposes.

Study on the amino acid composition of marine algae from Kerala showed the presence of both essential and non-essential amino acids. The total amount of amino acids (both free and combined) varied in the range 85 – 211 mg g^{-1} on dry weight basis. Of these, about 20 – 37% was constituted by essential amino acids. The most dominant amino acid in most of the algae studied was aspartic acid. Arginine, cysteine and glutamic acid were also found to be major constituents. Glycine, histidine and tyrosine were found to present in fairly good quantities. Among the essential amino acids, leucine(s) and phenylalanine were found to be dominant compounds. Lysine and threonine were present in fairly high levels. However, methionine and valine were found to be limiting amino acids in many cases. The amino acid compositions showed considerable temporal and spatial variations. The rhodophyceae members *Centroceras clavulatum* and *Grateloupia filicina* collected from Narakkal in the summer monsoon season were found to be more nutritive with respect to the amino acid contents than the other algal species studied. The chlorophyceae members were found to be limiting in methionine and valine. Thus, the rhodophytes of Narakkal especially, those available in the summer monsoon season can be exploited for use as an additional source of essential amino acids.

Marine algae from Kerala coast were found to contain considerable amounts of many of the essential trace elements needed for human and animal nutrition. The total mineral constituents of algae, represented by their ash contents, were found to show mean values varying in the range 7.8 – 21.5%. Only the rhodophyte *Gracilaria corticata* recorded ash contents below 10%, whereas all other species recorded average values above 12%. *Centroceras clavulatum* from Ettikkulam (21.5%) and *Enteromorpha intestinalis* from Narakkal (21%) recorded the highest values. Although classwise variations in ash contents were not significant at both the locations, specieswise variations were observed to be significant at Ettikkulam. The ash content of rhodophyceae showed significant differences between the two locations, whereas that of chlorophyceae was statistically indistinguishable. Algae were found to show temporal variations in their ash content. Low values were observed in the summer monsoon and retreating monsoon seasons, whereas comparatively high values in the period from mid winter monsoon to hot season.

Among the various inorganic constituents of algae, chlorine and iodine were found to show mean values varying in the ranges 1.18 – 6.08

mg g^{-1} and 0.16 – 1.47 mg g^{-1} respectively. Iodine contents of algae from Ettikkulam showed highly significant interspecies variations, while their chlorine contents were not significantly different. A reverse case was observed at Narakkal. Here, iodine contents of different species were statistically indistinguishable, whereas chlorine contents showed significant specieswise variation. Both iodine and chlorine exhibited significant interclass variations at Narakkal, while differences were significant only for chlorine at Ettikkulam. Although significant spatial variations were not observed in the halogen contents, temporal variations were notable. All the algal species recorded lowest iodine contents in the summer monsoon season and most of them showed highest values in the mid hot season. Intermediate values were obtained in other seasons. Such a general trend was not observed in the chlorine content of algae. The period of minimum and maximum chlorine contents were found to differ from one species to another and also between locations. It was found that chlorine contents of all the algal species studied were higher than their iodine contents in all the seasons. However, chlorine was not concentrated by any of these species, whereas iodine was accumulated to several orders of magnitude compared to its concentration in the ambient seawater. The largest concentrator of iodine was found to be the rhodophyte *Centroceras clavulatum* followed by *Grateloupia filicina* and the lowest accumulation of iodine was shown by the chlorophyte *Enteromorpha intestinalis*. The former two species of algae can be considered for use as dietary sources of iodine.

Marine macroalgae from Kerala were observed to contain notable amounts of many of the essential trace metals. They were also found to accumulate toxic heavy metals such as Pb and Cd. Most of the metals studied showed high levels of accumulation. The levels of Cu, Zn and Co in different species of algae were found to be of similar magnitude to those reported for algae from supposedly clean areas, while Fe, Cr and Cd contents were generally in accordance with those reported for algae from contaminated areas. Mn, Ni and Pb contents of algae were in agreement with those reported for algae from some polluted areas, but in other cases, values were lower than those from clean areas. Different species of algae were found to accumulate metals to different extent. Significant interspecies variations were observed in the accumulation of metals such as Fe, Cu, Zn, Sr, Mn and Cr. The rhodophyte *Centroceras clavulatum* showed highest accumulation of Fe and Cu, while *Grateloupia filicina* showed high accumulation of Zn and Sr. *Gracilaria corticata* accumulated highest level of Mn. The chlorophyte *Chaetomorpha antennina* showed highest accumulation of Cr. The least accumulation of most of the metals was shown by the chlorophyte *Ulva lactuca.* Interclass variations were significant only for the metals Cr, Sr, Mn and Cd. Chlorophyceae showed greater accumulation of Cr and Sr, while rhodophyceae accumulated more Mn and Cd. The general pattern of trace metal distribution in the two classes of marine algae from both Ettikkulam and Narakkal were found to be Fe > Mn > Zn/Sr > Ni/Pb > Cu > Co > Cd/Ag. The accumulation level of Cr was in between that of Mn and Cu. Algae were observed to show significant spatial variations in the levels of metals such as Fe, Zn, Cd, Mn and Ni. Other metals did not show any significant spatial variations. In general, metal contents were higher in algae from Narakkal, which may be due to the proximity of this site to the Cochin barmouth through which the intensely polluted Cochin backwaters open out into the Arabian Sea. The temporal variations in metal accumulation did not follow any general pattern and different metals were accumulated to different extents in various seasons.

Information obtained on the trace metal composition of marine algae from Kerala coast suggests a possible use of some of the species as bioindicators of trace metal levels in seawater. The rhodophyceae members *Gracilaria corticata*, *Centroceras clavulatum* and *Grateloupia filicina*, which were found to be good concentrators of trace metals can be considered for this purpose. *G. corticata* can be used for the monitoring of potentially toxic heavy metals, while *C. clavulatum* for the nutrient trace metals.

The qualitative and quantitative evaluation of agar isolated from *Gracilaria corticata* collected from Ettikkulam coast of Kerala showed that yield and gel strength of this agar was in accordance with those specified for agar from Grade-II *Gracilaria*. The average yield was 24% on dry weight basis and the gel strength was 76 g cm^{-2}. The yield was high in summer and winter monsoon seasons and low in the retreating monsoon season. Gelling and melting temperatures showed average values of 44.8 and 86.2 ^0C respectively. Mean values of 3,6-anhydrogalactose and sulphate contents of agar were 30% and 4% respectively. Spectral studies revealed the presence of D-galactose-4-sulphate residues and possible absence of galactose-2-sulphate, galactose-6-sulphate and 3,6-anhydrogalactose-2-sulphate residues in the agar isolated in the present study. The physicochemical properties of this agar appeared to be far from those required for bacteriological purposes. However, this agar may be suitable for food industry, probably after enhancement of its quality, which may be carried out by techniques such as fractionation. Considering the gel regulating factors as well as the yield, the best time of the year to obtain a firmer and less viscous gel seems to be the early winter monsoon season. The late hot season and retreating monsoon season may also provide firm and rigid gel, but yield may be low.

In short, some of the algal species investigated during the present study, especially *Centroceras clavulatum* and *Grateloupia filicina* have potential as sources of proteins, carbohydrates, lipids, fibres, essential amino acids, iodine and trace metals, which are needed for human and animal nutrition. However, caution must be taken of toxic metals such as Cd and Pb. Since these heavy metals are usually detoxified in algae by adsorption onto charged polysaccharides in the cell wall and intercellular matrix, which are weakly fermented in the digestive tract, they may pose less threat to health. Nevertheless, considering the levels of trace metals – both nutrient and toxic – in marine algae from Kerala, their use as food supplement rather than as staple food item is recommended as a better means of introducing them into the diet. They can also serve as a source of minerals for plants whose products are eventually consumed by humans and animals. The rhodophyte *Gracilaria corticata* occurring at Ettikkulam can be used as a source of food-grade agar and the species such as *Gracilaria corticata, Centroceras clavulatum* and *Grateloupia filicina* can be considered for use as biomonitors of heavy metal pollution in coastal waters.

APPENDIX I

Results of multiple comparisons (Tukey's HSD method) for specieswise differences in the organic and inorganic constituents of marine algae from Ettikkulam

Dependent Variable	Species (I)	Species (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total carbohydrates	CA	UL	-9.7942(*)	2.0857	0.001	-15.7229	-3.8655
		GC	-22.0785(*)	2.0857	0.000	-28.0072	-16.1498
		CC	-13.8459(*)	2.5544	0.000	-21.1070	-6.5847
	UL	CA	9.7942(*)	2.0857	0.001	3.8655	15.7229
		GC	-12.2843(*)	2.0857	0.000	-18.2130	-6.3556
		CC	-4.0517	2.5544	0.412	-11.3128	3.2095
	GC	CA	22.0785(*)	2.0857	0.000	16.1498	28.0072
		UL	12.2843(*)	2.0857	0.000	6.3556	18.2130
		CC	8.2326(*)	2.5544	0.023	0.9715	15.4938
	CC	CA	13.8459(*)	2.5544	0.000	6.5847	21.1070
		UL	4.0517	2.5544	0.412	-3.2095	11.3128
		GC	-8.2326(*)	2.5544	0.023	-15.4938	-0.9715
LMWC	CA	UL	2.433E-02	0.6078	1.000	-1.7034	1.7521
		GC	-3.3357(*)	0.6078	0.000	-5.0634	-1.6079
		CC	0.5066	0.7444	0.903	-1.6095	2.6226
	UL	CA	-2.4333E-02	0.6078	1.000	-1.7521	1.7034
		GC	-3.3600(*)	0.6078	0.000	-5.0877	-1.6323
		CC	0.4822	0.7444	0.915	-1.6338	2.5983
	GC	CA	3.3357(*)	0.6078	0.000	1.6079	5.0634

		UL	3.3600(*)	0.6078	0.000	1.6323	5.0877
		CC	3.8422(*)	0.7444	0.000	1.7262	5.9583
	CC	CA	-0.5066	0.7444	0.903	-2.6226	1.6095
		UL	-0.4822	0.7444	0.915	-2.5983	1.6338
		GC	-3.8422(*)	0.7444	0.000	-5.9583	-1.7262
Polysaccharides	CA	UL	-9.8217(*)	2.0032	0.001	-15.5161	-4.1272
		GC	-18.7433(*)	2.0032	0.000	-24.4378	-13.0489
		CC	-14.3533(*)	2.4535	0.000	-21.3276	-7.3791
	UL	CA	9.8217(*)	2.0032	0.001	4.1272	15.5161
		GC	-8.9217(*)	2.0032	0.002	-14.6161	-3.2272
		CC	-4.5317	2.4535	0.287	-11.5059	2.4426
	GC	CA	18.7433(*)	2.0032	0.000	13.0489	24.4378
		UL	8.9217(*)	2.0032	0.002	3.2272	14.6161
		CC	4.3900	2.4535	0.312	-2.5842	11.3642
	CC	CA	14.3533(*)	2.4535	0.000	7.3791	21.3276
		UL	4.5317	2.4535	0.287	-2.4426	11.5059
		UL	-4.3900	2.4535	0.312	-11.3642	2.5842
Fibres	CA	CC	28.4650(*)	0.8115	0.000	26.1583	30.7717
		GC	24.9867(*)	0.8115	0.000	22.6800	27.2934
		CC	23.4433(*)	0.9938	0.000	20.6182	26.2684
	UL	CA	-28.4650(*)	0.8115	0.000	-30.7717	-26.1583
		GC	-3.4783(*)	0.8115	0.003	-5.7850	-1.1716
		CC	-5.0217(*)	0.9938	0.001	-7.8468	-2.1966
	GC	CA	-24.9867(*)	0.8115	0.000	-27.2934	-22.6800
		UL	3.4783(*)	0.8115	0.003	1.1716	5.7850
		CC	-1.5433	0.9938	0.430	-4.3684	1.2818
	CC	CA	-23.4433(*)	0.9938	0.000	-26.2684	-20.6182

		UL	5.0217(*)	0.9938	0.001	2.1966	7.8468
		GC	1.5433	0.9938	0.430	-1.2818	4.3684
Calorific value	CA	UL	-0.3517	0.1530	0.138	-0.7867	8.336E-02
		GC	-.6500(*)	0.1530	0.003	-1.0850	-0.2150
		CC	-0.1700	0.1874	0.801	-0.7028	0.3628
	UL	CA	0.3517	0.1530	0.138	-8.3365E-02	0.7867
		GC	-0.2983	0.1530	0.245	-0.7334	0.1367
		CC	0.1817	0.1874	0.768	-0.3511	0.7145
	GC	CA	0.6500(*)	0.1530	0.003	0.2150	1.0850
		UL	0.2983	0.1530	0.245	-0.1367	0.7334
		CC	0.4800	0.1874	0.086	-5.2802E-02	1.0128
	CC	CA	0.1700	0.1874	0.801	-0.3628	0.7028
		UL	-0.1817	0.1874	0.768	-0.7145	0.3511
		GC	-0.4800	0.1874	0.086	-1.0128	5.280E-02
Ash	CA	UL	-1.5850	2.0278	0.862	-7.3491	4.1791
		GC	5.6667	2.0278	0.055	-9.7460E-02	11.4308
		CC	-8.0450(*)	2.4835	0.023	-15.1046	-0.9854
	UL	CA	1.5850	2.0278	0.862	-4.1791	7.3491
		GC	7.2517(*)	2.0278	0.011	1.4875	13.0158
		CC	-6.4600	2.4835	0.079	-13.5196	0.5996
	GC	CA	-5.6667	2.0278	0.055	-11.4308	9.746E-02
		UL	-7.2517(*)	2.0278	0.011	-13.0158	-1.4875

		CC	-13.7117(*)	2.4835	0.000	-20.7713	-6.6521
	CC	CA	8.0450(*)	2.4835	0.023	0.9854	15.1046
		UL	6.4600	2.4835	0.079	-0.5996	13.5196
		GC	13.7117(*)	2.4835	0.000	6.6521	20.7713
I	CA	UL	0.2691(*)	7.548E-02	0.012	5.309E-02	0.4850
		GC	0.1578	7.548E-02	0.198	-5.8179E-02	0.3737
		CC	-0.9939(*)	0.1067	0.000	-1.2994	-0.6885
	UL	CA	-0.2691(*)	7.548E-02	0.012	-0.4850	-5.3087E-02
		GC	-0.1113	7.548E-02	0.475	-0.3272	0.1047
		CC	-1.2630(*)	0.1067	0.000	-1.5684	-0.9576
	GC	CA	-0.1578	7.548E-02	0.198	-0.3737	5.818E-02
		UL	0.1113	7.548E-02	0.475	-0.1047	0.3272
		CC	-1.1517(*)	0.1067	0.000	-1.4571	-0.8463
	CC	CA	0.9939(*)	0.1067	0.000	0.6885	1.2994
		UL	1.2630(*)	0.1067	0.000	0.9576	1.5684
		GC	1.1517(*)	0.1067	0.000	0.8463	1.4571
Cu	CA	UL	0.6733	1.0599	0.919	-2.3395	3.6861
		GC	2.8667	1.0599	0.065	-0.1461	5.8795
		CC	-4.8167(*)	1.2981	0.009	-8.5066	-1.1268
	UL	CA	-0.6733	1.0599	0.919	-3.6861	2.3395
		GC	2.1933	1.0599	0.203	-0.8195	5.2061
		CC	-5.4900(*)	1.2981	0.003	-9.1799	-1.8001

	GC	CA	-2.8667	1.0599	0.065	-5.8795	0.1461
		UL	-2.1933	1.0599	0.203	-5.2061	0.8195
		CC	-7.6833(*)	1.2981	0.000	-11.3732	-3.9934
	CC	CA	4.8167(*)	1.2981	0.009	1.1268	8.5066
		UL	5.4900(*)	1.2981	0.003	1.8001	9.1799
		GC	7.6833(*)	1.2981	0.000	3.9934	11.3732
Zn	CA	UL	5.2750	2.9545	0.314	-3.1235	13.6735
		GC	3.9000	2.9545	0.563	-4.4985	12.2985
		CC	-8.8867	3.6185	0.104	-19.1727	1.3994
	UL	CA	-5.2750	2.9545	0.314	-13.6735	3.1235
		GC	-1.3750	2.9545	0.966	-9.7735	7.0235
		CC	-14.1617(*)	3.6185	0.006	-24.4477	-3.8756
	GC	CA	-3.9000	2.9545	0.563	-12.2985	4.4985
		UL	1.3750	2.9545	0.966	-7.0235	9.7735
		CC	-12.7867(*)	3.6185	0.012	-23.0727	-2.5006
	CC	CA	8.8867	3.6185	0.104	-1.3994	19.1727
		UL	14.1617(*)	3.6185	0.006	3.8756	24.4477
		GC	12.7867(*)	3.6185	0.012	2.5006	23.0727
Mn	CA	UL	16.2667	18.3164	0.811	-35.7994	68.3328
		GC	-107.6033(*)	18.3164	0.000	-159.6694	-55.5372
		CC	-72.1850(*)	22.4329	0.024	-135.9527	-8.4173
	UL	CA	-16.2667	18.3164	0.811	-68.3328	35.7994
		GC	-123.8700(*)	18.3164	0.000	-175.9361	-71.8039
		CC	-88.4517(*)	22.4329	0.005	-152.2194	-24.6840

	GC	CA	107.6033(*)	18.3164	0.000	55.5372	159.6694
		UL	123.8700(*)	18.3164	0.000	71.8039	175.9361
		CC	35.4183	22.4329	0.416	-28.3494	99.1860
	CC	CA	72.1850(*)	22.4329	0.024	8.4173	135.9527
		UL	88.4517(*)	22.4329	0.005	24.6840	152.2194
		GC	-35.4183	22.4329	0.416	-99.1860	28.3494
Cr	CA	UL	15.3100(*)	5.3590	0.037	-0.4194	31.0394
		GC	16.3580(*)	5.0525	0.029	1.5282	31.1878
		CC	8.8867	5.8341	0.452	-8.2374	26.0107
	UL	CA	-15.3100(*)	5.3590	0.037	-31.0394	0.4194
		GC	1.0480	5.3590	0.997	-14.6814	16.7774
		CC	-16.4233(*)	6.1015	0.043	-24.3321	11.4854
	GC	CA	-16.3580(*)	5.0525	0.029	-31.1878	-1.5282
		UL	-1.0480	5.3590	0.997	-16.7774	14.6814
		CC	-17.4713(*)	5.8341	0.049	-24.5954	9.6527
	CC	CA	-8.8867	5.8341	0.452	-26.0107	8.2374
		UL	16.4233	6.1015	0.043	-11.4854	24.3321
		GC	17.4713	5.8341	0.049	-9.6527	24.5954
Sr	CA	UL	5.7983	5.5688	0.728	-10.0315	21.6281
		GC	15.0817	5.5688	0.065	-0.7481	30.9115
		CC	26.7117(*)	6.8203	0.006	7.3242	46.0991
	UL	CA	-5.7983	5.5688	0.728	-21.6281	10.0315
		GC	9.2833	5.5688	0.370	-6.5465	25.1131
		CC	20.9133(*)	6.8203	0.032	1.5259	40.3008
	GC	CA	-15.0817	5.5688	0.065	-30.9115	0.7481
		UL	-9.2833	5.5688	0.370	-25.1131	6.5465
		CC	11.6300	6.8203	0.351	-7.7575	31.0175

	CC	CA	-26.7117(*)	6.8203	0.006	-46.0991	-7.3242
		UL	-20.9133(*)	6.8203	0.032	-40.3008	-1.5259
		GC	-11.6300	6.8203	0.351	-31.0175	7.7575
Fe	CA	UL	4.1656(*)	1.4545	0.048	3.096E-02	8.3002
		GC	3.4861	1.4545	0.116	-0.6485	7.6207
		CC	-6.8232(*)	1.7814	0.007	-11.8871	-1.7594
	UL	CA	-4.1656(*)	1.4545	0.048	-8.3002	-3.0957E-02
		GC	-0.6795	1.4545	0.965	-4.8141	3.4551
		CC	-10.9888(*)	1.7814	0.000	-16.0526	-5.9250
	GC	CA	-3.4861	1.4545	0.116	-7.6207	0.6485
		UL	0.6795	1.4545	0.965	-3.4551	4.8141
		CC	-10.3093(*)	1.7814	0.000	-15.3731	-5.2455
	CC	CA	6.8232(*)	1.7814	0.007	1.7594	11.8871
		UL	10.9888(*)	1.7814	0.000	5.9250	16.0526
		GC	10.3093(*)	1.7814	0.000	5.2455	15.3731

CA: *Chaetomorpha antennina* UL: *Ulva lactuca* GC: *Gracilaria corticata* CC: *Centroceras clavulatum*
* The mean difference is significant at the .05 level.

APPENDIX II

Results of multiple comparisons (Tukey's HSD method) for specieswise differences in the organic and inorganic constituents of marine algae from Narakkal

Dependent Variable	Species (I)	Species (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Fibres	CA	EI	27.8800(*)	2.4486	0.000	19.7745	35.9855
		CC	22.6590(*)	2.0487	0.000	15.8774	29.4406
		GF	23.7500(*)	2.4486	0.000	15.6445	31.8555
	EI	CA	-27.8800(*)	2.4486	0.000	-35.9855	-19.7745
		CC	-5.2210	2.0487	0.135	-1EI26	1.5606
		GF	-4.1300	2.4486	0.396	-12.2355	3.9755
	CC	CA	-22.6590(*)	2.0487	0.000	-29.4406	-15.8774
		EI	5.2210	2.0487	0.135	-1.5606	1EI26
		GF	1.0910	2.0487	0.948	-5.6906	7.8726
	GF	CA	-23.7500(*)	2.4486	0.000	-31.8555	-15.6445
		EI	4.1300	2.4486	0.396	-3.9755	12.2355
		CC	-1.0910	2.0487	0.948	-7.8726	5.6906
Cl	CA	EI	2.5874	1.7296	0.472	-2.6180	7.7927
		CC	4.9017(*)	1.4122	0.023	0.6515	9.1519
		GF	4.6340(*)	1.4471	0.036	0.2789	8.9892
	EI	CA	-2.5874	1.7296	0.472	-7.7927	2.6180
		CC	2.3143	1.4122	0.398	-1.9359	6.5645
		GF	2.0467	1.4471	0.517	-2.3085	6.4018
	CC	CA	-4.9017(*)	1.4122	0.023	-9.1519	-0.6515
		EI	-2.3143	1.4122	0.398	-6.5645	1.9359

		GF	-0.2677	1.0473	0.994	-3.4197	2.8844
	GF	CA	-4.6340(*)	1.4471	0.036	-8.9892	-0.2789
		EI	-2.0467	1.4471	0.517	-6.4018	2.3085
		CC	0.2677	1.0473	0.994	-2.8844	3.4197
Cu	CA	EI	-0.4300	2.5713	0.998	-8.1686	7.3086
		CC	-3.1100	2.0995	0.480	-9.4285	3.2085
		GF	1.5480	2.1513	0.887	-4.9266	8.0226
	EI	CA	0.4300	2.5713	0.998	-7.3086	8.1686
		CC	-2.6800	2.0995	0.595	-8.9985	3.6385
		GF	1.9780	2.1513	0.795	-4.4966	8.4526
	CC	CA	3.1100	2.0995	0.480	-3.2085	9.4285
		EI	2.6800	2.0995	0.595	-3.6385	8.9985
		GF	4.6580	1.5570	0.052	-2.7962E-02	9.3440
	GF	CA	-1.5480	2.1513	0.887	-8.0226	4.9266
		EI	-1.9780	2.1513	0.795	-8.4526	4.4966
		CC	-4.6580	1.5570	0.052	-9.3440	2.796E-02
Zn	CA	EI	7.2600	14.7045	0.959	-36.9944	51.5144
		CC	-3.2217	1EI62	0.993	-39.3552	32.9119
		GF	-29.9720	12.3027	0.127	-66.9979	7.0539
	EI	CA	-7.2600	14.7045	0.959	-51.5144	36.9944
		CC	-10.4817	1EI62	0.819	-46.6152	25.6519
		GF	-37.2320(*)	12.3027	0.049	-74.2579	-0.2061
	CC	CA	3.2217	1EI62	0.993	-32.9119	39.3552
		EI	10.4817	1EI62	0.819	-25.6519	46.6152
		GF	-26.7503	8.9040	0.050	-53.5477	4.703E-02
	GF	CA	29.9720	12.3027	0.127	-7.0539	66.9979

		EI	37.2320(*)	12.3027	0.049	0.2061	74.2579
		CC	26.7503	8.9040	0.050	-4.7027E-02	53.5477
Mn	CA	EI	-23.6700	19.1319	0.618	-81.2490	33.9090
		CC	-57.3683(*)	15.6211	0.017	-104.3814	-10.3553
		GF	-2.7180	16.0069	0.998	-50.8921	45.4561
	EI	CA	23.6700	19.1319	0.618	-33.9090	81.2490
		CC	-33.6983	15.6211	0.195	-80.7114	13.3147
		GF	20.9520	16.0069	0.576	-27.2221	69.1261
	CC	CA	57.3683(*)	15.6211	0.017	10.3553	104.3814
		EI	33.6983	15.6211	0.195	-13.3147	80.7114
		GF	54.6503(*)	11.5849	0.003	19.7845	89.5162
	GF	CA	2.7180	16.0069	0.998	-45.4561	50.8921
		EI	-20.9520	16.0069	0.576	-69.1261	27.2221
		CC	-54.6503(*)	11.5849	0.003	-89.5162	-19.7845
Fe	CA	EI	-2.5230	2.5587	0.760	-10.2237	5.1777
		CC	-4.2046	2.0892	0.241	-10.4922	2.0830
		GF	1.2916	2.1408	0.929	-5.1512	7.7345
	EI	CA	2.5230	2.5587	0.760	-5.1777	10.2237
		CC	-1.6816	2.0892	0.851	-7.9692	4.6060
		GF	3.8146	2.1408	0.331	-2.6282	10.2575
	CC	CA	4.2046	2.0892	0.241	-2.0830	10.4922
		EI	1.6816	2.0892	0.851	-4.6060	7.9692
		GF	5.4962(*)	1.5494	0.020	0.8332	10.1592
	GF	CA	-1.2916	2.1408	0.929	-7.7345	5.1512
		EI	-3.8146	2.1408	0.331	-10.2575	2.6282
		CC	-5.4962(*)	1.5494	0.020	-10.1592	-0.8332

CA: *Chaetomorpha antennina* EI: *Enteromorpha intestinalis* CC: *Centroceras clavulatum* GF: *Grateloupia filicina*
* The mean difference is significant at 0.05 level.