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4) Thesis

Kaliaperumal, N. 1979. *Ecological studies on some red algae of Visakhapatnam coast.* Ph.D thesis, Andhra University, Visakhapatnam. 134 p.

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Studies on value added products from Indian marine algae – A review

N. KALIAPERUMAL[1] and V.S.K. CHENNUBHOTLA[2]

Central Marine Fisheries Research Institute, Kochi - 682018, Kerala, India

Present address: [1]N-163, Maya Indraprastha, Kanakapura Main Road, Bangalore - 560078, Karnataka, India

[2]Plot No. 597, Vaidehi Nagar, Vsanthalipurarm, Hyderabad - 500070, Andra Pradesh, India

ABSTRACT

Studies made during 1948-2016 on the value added products from Indian seaweeds such as phycocolloids (agar, agarose, agaroid, carrageenan, alginate and mannitol), human food, animal feed, fertilizer, medicinal compounds, nutraceutical and cosmeceutical products and biogas are reviewed in this paper with emphasis on utilisation of these products. The future R&D and extension activities to be carried out on value added seaweed products are recommended.

Introduction

Seaweed are wonder plants of the sea (Krishnamurthy, 2005a). They belong to the four classes namely Chlorophyceae, Phaeophyceae, Rhodophyceae and Cyanophyceae. They are distributed in the intertidal, shallow and deep waters of the sea up to 150 meters. They also occur in estuaries and backwaters and grow on rocks, dead corals, pebbles, solid substrates and on other plants as epiphytes. Some seaweeds grow on other plants as ephiphytes. Some others grow on the sandy substratum also. They are harvested from the coastal waters in numerous countries due to their value as food, feed for animals and source of various minerals, trace elements and phycocolloids. The diversity and abundance of seaweeds depend on many environmental, chemical and biological factors. Red seaweeds are found mostly in subtropical and tropical waters, while brown seaweeds are more common in temperate waters. This primitive macro-flora is well adapted to polar, temperate and tropical regions. It was estimated that about 90% of the species of marine plants are algae and about 50% of global photosynthesis is contributed by them. Suitable substratum along 8000 km coastline of India is available along Tamil Nadu from Rameswaram to Kanyakumari covering 21 islands of Gulf of Mannar, along Gujarat coast at Okha, Dwaraka, Porbandar, Veraval and Gopnath around Goa, Daman and Diu Island, Lakshadweep and Andaman Nicobar Islands. Fairly rich seaweed beds are present in the vicinity of Mumbai, Ratnagiri, Karwar, Varkala, Vizhinjam, Vishakapatnam and coastal lakes like Pulicat and Chilka.

According to FAO fisheries statistics 2016, the estimated marine algal production in the world is more than 27.3 million tons in 2014. The annual standing crop of marine algae of India has been estimated as 3,01,646 tonnes. The number of species reported from Indian waters till the year 2015 are 283 genera and 1019 species. Rhodophyceae is dominating with 442 species followed by 212 species of Chlorophyceae, 211 species of Phaeophyceae and 148 species of blue-green algae (Rao and Gupta, 2015). The abundance and diversity of algae have made them prime material for the human use as a source of food, feed, medicine and fertilizer. The earliest reference about the uses of algae dates back to BC 3000 in China. Asian counties have traditionally employed macroalgae as a natural source of food and medicine especially in Japan, Korea, China, Vietnam, Indonesia and Taiwan. Worldwide, there are 250 macro algal species which have been listed as commercially valuable, among which 150 are consumed as human food. It is estimated that around 8.6 million metric tonnes/year are utilised for human consumption, phycocolloids, feed supplements, agrichemicals, nutraceuticals and pharmaceuticals. They have been employed as dressing, ointments and in gynecology also. In general, marine algae have huge economic potential as they have high amounts of mineral and bioactive compounds. A diverse group of bioactive compounds such as vitamins, minerals, amino acids, sugars, lipids and other biologically active compounds are extracted from the algae and these

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compounds are used for varieties of cosmetic products (Kaliaperumal *et al.,* 1995, Kaliaperumal, 2000a, 2003, 2011, 2016; Umamaheswara Rao, 2011; Chennubhotla *et al.,* 2013, 2015; Baby Ushakiran *et al.,* 2014; Chennubhotla, 2016).

Considerable works have been done by several workers during the last fifty years on the chemical aspects of marine algae such as minerals, trace elements, iodine, bromine, vitamins, protein, carbohydrates, lipids, agar, align, mannitol, bioactive compounds etc. But these is no detailed and consolidated information on the value added products from Indian marine algae. Hence, the research and development activities carried out during 1948-2016 on various value added seaweed products and their utilization are reviewed and given in detail in this paper.

Phycolloids

Agar

Agar is an important phycocolloid extracated from certain red algae such as *Gelidiella*, *Gelidium* and *Gracialria*. The important and commonly occurring agarophytes of India are *Gelidiella acerosa*, *Gracilaria edulis*, *G. crassa*, *G. verrucosa*, *G. corticata* and *G. foliifera* (Chennubhotla *et al.,* 1991; Umamaheswara Rao, 1969). Studies were made on the yield and quality of agar from 17 species of red algae (Kaliaperumal and Chennubhotla, 2017 in-press). Based on the cottage and large scale industry method developed for agar production (Joseph and Mahadevan, 1948; Thivy, 1960; Visweswara Rao *et al.,* 1965; Kaliaperumal and Uthirasivam, 2001; Ramalingam *et al.,* 2002; Thirupathi and Subba Rao, 2004; Ramalingam, 2006), agar industry is established in India. At present, about 15 agar industries located in different states of the country are manufacturing bacteriological grade agar from *Gelidiella acerosa* and food grade agar from species of *Gracilaria* (Rajasekaran and Revathi, 2006) exploited from the natural seaweed beds mostly in Tamil Nadu. About 5 tons of bacteriological grade agar and 50 tons of food grade agar are produced annually from 250 tons of raw materials. Some quantity of agar is exported to few foreign countries and rest of the agar is marketed within the country (Kaladharan and Kaliaperumal, 1999; Kaliaperumal ad Kalimuthu, 2004; Kaliaperumal *et al.,* 2004).

Agar is used for various applications in food industries as thickening agents in the preparation of fruit salad, fruit jellies, yogurt, bakery products, as a preservative in canned foods and meat industry and in liquor industry to increase the viscosity. Higher concentration of agar is also used in fabricating molds for sculpture, archeological and dental impression to decrease the concentration of blood glucose and exerts an anti-aggregation effect on red blood cells and it is reported to affect absorption of ultraviolet rays (Subba Rao *et al.,* 2016).

Agarose

Agarose is also a polysaccharide like agar and obtained by fraction of agar the other fraction is agaropection. It is produced from agar obtained from species of *Gelidiella*, *Gelidium* and *Gracilaria*. It can be directly obtained from *Gracilaria dura* (Siddantha *et al.,* 1987). Some of the application of agarose are immune-diffusion and diffusion techniques, conventional electrophoresis, reverse electrophoresis, immune electrophoresis or electro focusing chromotographyic technique in gel chromatography, affinity chromatography or chromoto-focusing, bioengineering applications, microbiology and tissue culture. Agarose is not manufactured by the seaweed industries in India and is imported from foreign counties.

Agaroids

The gel-like extracts produced from certain red seaweeds are commonly known as agaroids. The organic sulphate content is very much higher in these compounds and the chemical nature and properties of agaroids vary from agar. Pure solution of agaroids are viscous and do not form gel when cooled. However, various inorganic and organic solutes alter the properties and increase the gelling power of agaroids. Investigations were made on the agaroid content in 19 species of red algae growing in different parts of Indian coast (Chennubhotla *et al.,* 1991; Kaliaperumal and Chennubhotla, 2017 in press).

Carrageenan

Carrageenans are complex sulphated polysaccharides. They are commercially important hydrocolloids derived from various red seaweeds. Its production was expanded during early 1970's. It is the third most important hydrocolloids in the world after starch and gelatin and occurs as cell-wall matrix materials in various species of red seaweeds. Based on the number and position of sulphate group, carageenans are classified into four types viz. kappa, iota, beta and lambda carrageenans. In India, the yield and quality of carrageenan was studied from 5 speices of *Hypnea* i.e. *Hypnea musciformis*, *H. muttukadensis, H. pannosa*, *H. valentia* and *Hypnea* sp. collected from wild and *Kappaphycus alvarezii* from the cultivation (Chennubhotla *et al.,* 1991; Kaliaperumal and Chennubhotla, 2017 in press). Only about 6 seaweed industries are manufacturing carrageenan from *K. alvarezii*. About 250 tons of kappa carrageenan is produced from 1000 tons of raw material and the entire finished product is marketed within the country. More than 250 applications were identified for carrageenan in different fields such as food products and processing, pharmaceutical industry, cosmetics, coating paints and inks etc. From human health perspective, it has been reported that carrageenans have antitumor and antiviral properties. The

various other uses of carrageenan are given in detail Subba Rao *et al.,* (2006).

Alginate

Algin or alginic acid is the main polysaccharide occurring in the cell-wall of brown algae. It consists of D-Mannuronic acid and 2-Guluronic acid in various proportions. The sodium, potiassium and magnesium salts of alginic acid are soulable in water and they give viscous solution without gel formation. Calcium alginate and other salts of copper, cobalt, mercury etc are insoluble in water. Species of *Sargassum*, *Turbinaria*, *Cystoseira*, *Hormophysa*, *Padina*, *Spatoglossum*, *Stoechospermum and Colpomenia* are some of the algin yielding seaweeds of Indian waters. The algin yielding seaweeds of India is given by Umamaheswara Rao (1969). Totally 30 species of brown algae belonging to 12 genera were studied for their alginic acid content. Based on the process developed for extraction of Alginic acid, Sodium alginate and Calcium alginate (Pillai, 1957, Sadasivan Pillai, 1961; Visweswara Rao and Mody, 1964; Desai, 1967; Chennubhotla *et al.,* 1976; Thirupathi and Subba Rao, 2004) alginate industries were started in our country. Now there are about 6 industries located in different places of India and they are producting 350 tons of alginates by using 2500–3000 tons of raw materials of *Sargassum* spp. and *Turbinaria* spp. collected mostly from Tamil Nadu coast. (Kaladharan and Kaliaperumal 1999; Rajesekaran and Revathi, 2006).

Algin is also equally and extensively used in the preparation of various pharmaceutical, food and rubber products (natural and synthetic latex creaming and thickening, finished articles, automobile carpeting, electrical insulations, foam cushions and rubber coating on tyres), textile products (size compound for cotton and rayon, textile print pastes and plastic laundry starch), adhesives (for all boards, paper bags, shipping containers, gummed tapes), paper products, food packages, pharmaceutical and detergents, packages, milk containers, butter cartons, frozen food packages, insulation boards, food wrappers, greaseproof paper and acoustical tiles and miscellaneous products such as paints, ceramic glazes, porcelain wares, leather finishers, autopolishes, welding-rod coatings, boiler compounds, battery plate separators, wall-board-joint cement, beet-sugar processing and wax emulsion (Chennubhotla *et al.,* 1987).

Mannitol

Mannitol is a sugar alcohol present in the cell sap of many brown seaweeds. Studies were made on the mannitol content of 30 species of brown algae by various workers (Kailaperumal *et al.,* 1987). In India, mannitol is not commercially produced from seaweeds. In pharmacy, mannitol is applied for the production of tablets. It is also used for making diabetic food, chewing gum etc. Mannitol is employed as dusting powder in the paint and varnish industry, leather and paper industry, pyrotechniques and in making explosives. In organic synthesis and in plastic production mannitol is used as plasticizers for the production of resins (Kaladharan *et al.,* 1998).

Food products

The fresh, dried and processed seaweeds are used for human consumption. The algal carbohydrates are not easily digestable and the food value of seaweed depends on the minerals, trace elements, proteins and vitamins present in them. Many seaweeds such as species of *Caulerpa*, *Codium Hydroclathrus*, *Sargassum*, *Porphyra*, *Gracilaria*, *Acanthophora* and *Laurencia* are used as food in Japan, Indonesia, China, Phillippines and other countries of Indo-pacific regions (Subba Rao, 1965). They are eaten as salad, curry, soup or vegetables. These are large industries in Japan using edible seaweeds like *Porphyra*. Thin algal sheets are prepared by washing and drying *Porphyra* plants and this forms an important food item in Japan. Some of the edible seaweeds occurring in different localities along the Indian coasts are species of *Ulva*, *Enteromorpha*, *Chaetomorpha*, *Caulerpa*, *Codium*, *Dictyota*, *Padina*, *Colpomenia*, *Hydroclathrus*, *Rosenvingea*, *Chnoospora*, *Sargassum*, *Turbinaria*, *Porphyra*, *Halymenia*, *Grateloupia*, *Gracilaria*, *Hypnea*, *Rhodymenia*, *Centroceras*, *Acanthophora and Laurencia* (Chennubhotla *et al.,* 1987). The seaweed *Gracilaria edulis* is being used since decades for making gruel in the coastal areas of Tamil Nadu.

The nutritive value of seaweeds is given by some workers (Chennubhotla, 1977; Kaliaperumal, 2000b; Krishnamurthy, 2005b; Thahira and Tajunnisha Begum, 2006). The methods of preparing various food products such as jam, jelly, chocolate, halwa, tea, toffy, squash, pickle, ice cream, marmalade, blancmange, masala, wafer and recipes namely parridge, salad, thoron, tomato soup, tomato spread, vegetable soup, nutrient health mix, pakoda, cutlet and biriyani from different species of seaweeds viz. *Ulva*, *Enteromorpha*, *Caulerpa*, *Codium*, *Sargassum*, *Hydroclathrus*, *Porphyra*, *Gracilaria*, *Gelidiella*, *Acanthophora*, *Laurencia* and *Padina* are given in detail by many workers (Thivy, 1958; Chennubhotla *et al.,* 1981, 1987; Kaladharan *et al.,* 1998; Vijaya Parthasarathy, 2006; Thahira Banu and Sangeetha, 2007; Biju Mathew *et al.,* 2008; Sobha *et al.,* 2008; Thahira Banu and Umamaheswari, 2011; Thahira Banu, 2016).

Animal feed

Seaweeds are cheap source of minerals and trace elements. Hence the meal prepared from seaweeds can be given as supplements to the daily rations of cattle, poultry and other farm animals. Seaweed meal can be obtained by grinding the cleaned and washed seaweeds such as *Ulva*, *Enteromorpha*, *Sargassum*, *Padina*, *Dictyota*, *Gracilaria* and

Hypnea. A simple method for the preparation of seaweed meal from *Gracilaria edulis* is given by Thivy (1960). The preparation of seaweed meal for the feeding of farm animals is given by Dave *et al.* (1979). Experiments were conducted with the seaweeds *Chaetomorpha linoides*, *Caulerpa taxifolia*, *Ulva lactuca*, *Enteromorpha intestinalis*, *Padina* sp, *Dictyota* sp., feed *Sargassum wightii*, *Gracilaria edulis* and *Kappaphycus alvarezii* to use as animal food for chicks, sheep, cattle, pig, prawn, fish, earthworm, silkworm and holothurian by several workers (Deva *et al.,* 1977; Chaturvedi *et al.,* 1979; Jagannathan and Venkatakrishnan, 1979; Krishnamurthy *et al.,* 1982; Sobha *et al.,* 1999; Kaladharan, 2004, 2006; Sivakumar and Sundararaman, 2006; Sreenathkumar *et al.,* 2008; Subhakaran, 2010; Lakshmi and Maharajaebeneser, 2010; Felix and Pradeepa, 2012; Sobha and Chitra, 2014). In general, promising results were obtained in all these studies. In India only about 30% of seaweed biomass is utilised for polysaccharide and fertilizer production. The remaining 70% of marine algal biomass is unutilised. Hence, these unutilised and underutilised seaweed resources can be used as fodder or feed for animals either as raw or in processed forms. Species of *Chaetomorpha*, *Enteromorpha*, *Ulva*, *Sargassum*, *Chnoospora*, *Acanthophora*, *Gracilaria* and *Hypnea* can be tried as fodder for cattle. These seaweeds grow luxuriantly in the intertidal and subtidal waters along the peninsular coastline and the Andaman–Nicobar and Lakshawadeep archipelago.

Fertilizer

The use of seaweeds as fertilizer is a common practice in coastal areas throughout the world. In India, it is used for coconut plantation especially in coastal areas of Tamil Nadu and Kerala. The high amount of water soluble potash, other minerals and trace elements present in seaweeds are readily absorbed by plants and they control deficiently diseases. The carbohydrates and other organic matter present in seaweeds alter the nature of the soil and improve its moisture retaining capacity. Hence, large quantity of seaweeds and also seagrasses such as C*ymodocea*, *Syringodium*, *Enhalus*, *Diplanthara* and *Halophila* can be used as manure either directly or in different forms (Chennubhotla *et al.,* 1987; Verkleij, 1992; Zodape, 2001; Rengasamy, 2006; Venkataraman Kumar, 2011, 2015; Mohanty *et al.,* 2013; Sujatha and Anand, 2016; Subba Rao *et al.,* 2016). In the field trial experiments conducted by several workers on the effect of Seaweed Liquid Fertilizer in 45 land crops consisting of cereals, millets, pulses, oil seeds, vegetable & fruit crops and ornamental plant (Table-1), good results were obtained and totally 40 seaweeds were used in these studies (Table-2).

A method for composting the seaweeds with cow dung was described by Thivy (1958 and 1960). The method of preparation and properties of liquid seaweed fertilizer from *Sargassum* was given by Sreenivasa Rao *et al.* (1979a). Five methods of extracting Seaweed Liquid Fertilizer (SLF) were given by Venkataraman Kumar (2015). The production of SLF on cottage industry scale and its cost benefits are given by Senthi and Adhikary (2011). Now some seaweed industries are manufacturing biofertilizers like Aqua Sap from *Kappaphycus alvarezii* (Aquagri Processing Private Limited, New Delhi) Organic 6 from *Sargassum* spp. (SNAP Natural & Alginate Products Pvt. Ltd., Ranipet, Tamil Nadu). PHYCOLINN from *Sargassum*, *Turbinaria* and *Kappaphycus* (Linn Plantae Limited, Madurai, Tamil Nadu) and Chitokin-33 from *Sargassum* spp. (Karthick Marine, Ramanathapuram, Tamil Nadu) and they are applied by the farmers to increase the yield and production of many cash crops.

Table-1. Land crops manured with seaweed fertilizer

Names of the land crops	
Cereals	*Sesamum indicum*
Oriza sativa L. Ambai-16	*Jatropha curcas*
O. sativa var. *ponmani*	**Vegetables & Fruits**
Pennisetum typhoides	*Abelmoschus esculentus*
Sorghum biocolor	*Amaranthus viridis*
S. vulgare	*Brassica juncesa*
Triticum aestivum	*B. nigraCamellia sinensis*
Zea mays	*Capsicum annum*
Millet	Carrot
Eleusine coracana	Cucumber
Pulses	Drum stick
Cejanus cajan	Gouards
Cicer avietinum	Keser
Cyamopsis tetragonolobia	Mango
Dolichos biflorus	*Lycopersicon esculentus*
Mucuna prurens	*L. lycopersicum*
Phaseolus aureus	Lime
P. vugaris	*Memoridica charanta*
Vigna catajung	Papaya
V. faba	*Rephanus sativus*
V. mungo	*Solanum melongena*
V. radiata	Topioca
V. unguiculata	*Zizyphus mauritine*
Winged bean	**Ornamental plant**
Oil Seeds	*Tagetes erecta*
Arachis hypogea	

Medicinal products

Seaweeds were considered of medicinal value in the Orient as early as 3000 BC. The Chinese and Japanese used them in the treatment of goitre and other glandular diseases. The Romans used the seaweeds for healing the wounds, burns and rashes. The British used *Porphyra* to prevent scurvy (Vitamin-C deficiency disease) during long voyages. Various red algae particularly *Corallina officinalis* and *Alsidium helminthocorton* were employed as vermifuge. Some other red algae such as *Chondrus*, *Gracilaria*, *Gelidium* and *Pterocladia* have been used to treat various stomach and intestinal

Table-2. Seaweeds used as fertilizer for different land crops

Names of the seaweeds	
Green Seaweeds	*S. tenerriumum*
Caulerpa corynophora	*S. wightii*
C. racemose	*Sargassum* sp.
C. scalpelliformis	*Stoechospermum marginatum*
Chaetomorpha antennina	*Turbinaria conoides*
C. linum	*T. decurrens*
Enteromorpha intestinalis	*Zonaria* sp.
Ulva fasicata	**Red Seaweeds**
U. lactuca	*Acanthophora spicifera*
U. reticulate	*Amphiroa fragilissima*
Brown Seaweeds	*Asparogopsis taxiformis*
Dictyota dichotoma	*Gracilaria corticata*
Hydroclathrus clathratus	*G. crassa*
Padina gymnospora	*G. edulis*
P. pavonica	*G. foliifera*
P. tetrastromatica	*Gracilaria textorii*
Sargassum johnstonii	*G. verrucosa*
S. linearifolium	*Grateloupia filicina*
S. muticum	*Hypnea musciformis*
S. myriocystum	*Kappaphycus alvarezii*
S. plagiophyllum	*Spyridia hypnoides*
S. polycystum	

disorders and also helped to relieve from constipation and other discomforts. *Laminaria cloustoni* has been used as a pain-killer and also to distend the uterus. Carrageenan may be useful in ulcer therapy and the alginates are found to prolong the rate of activity in certain drugs. Species of *Sargassum* were used for cooling and blood cleaning effect. *Hypnea musciformis* was employed as vermifuge or worm expelling agent and *Centroceras clavulatum* as cathartic agent. The iodine rich seaweeds such as *Asparagopsis taxiformis* and *Sarconema* can be used for controlling goitre disease caused by the enlargement of thyroid gland (Umamaheswara Rao, 1970; Chennubhotla *et al.,* 1987; Anantharaman *et al.,* 2006). The biomedical properties and application of seaweeds for degenerative diseases are given by Siddhanta *et al.* (1992), Venkata Rao and Bheemasankara Rao (2004), Murugan (2016), Maria Victorial Rani (2016), Pragasam Viswanathan (2016) and Ravi and Baskaran (2016). Many researchers investigated the antimicrobial, antibacterial, antifungal, antiviral, antibiotic, antioxidant and anticancer activities of many seaweeds. Subramanian *et al.* (1999) reported heparin from the red alga *Grateloupia filicina*. The Central Marine Fisheries Research Institute, Kochi, Kerala released the following 3 medicinal products (web:cmfri.org.in).

Cadalmin Green Algal extracts (GAe) for arthritis and joint pain

Cadalmin Antibiotic extracts (ADe) for Type-II diabetes

Cadalmin Antihypercholestrolemic extract (Ace) for obesity and high B.P.

Nutraceuticals

Nutraceuticals which are commonly referred to as functional foods, are substances that can be considered as food or part of a food which provide beneficial health effects of medical importance. The nutraceutical concept was introduced by Stephen DeFelice, the founder and chairman of the Foundation for Innovation in Medicine in 1989. Nutraceuticals antioxidants are dietary supplements that can exert positive pharmacological effects on specific human diseases by alleviating the negative effects of reactive oxygen species. As per the World Health Organisation (WHO), in developing countries mortality due to nutrition related factors is about 40%, which denotes the need for neutraceutical products to balance the nutritional intake of an individual. Most of the seaweeds contain high level of unsaturated fatty acids, which supports that antioxidants activity of marine algae derived from the lipophilic extracts. Astaxanthin is vibrantly red pigmented. Therefore, it can be used as a source of pigments. Further, antaxanthin has several key biological functions such as a precursor of vitamin-A and is associated with cell reproduction, embryo development, as well as protection against oxidative damage. Astaxanthin has been gaining widespread popularity as a human dietary supplement owing to its superior antioxidant properties compared to those of β-carotene, α-carotene, lutein, lycopene, canthaxanthin and Vitamin-E. Several astaxanthin-based products are now commercially available and are being promoted as anticancer, anti-inflammatory and immune stimulating agents. The fucoxanthin supplement led to the reduced activity of the hepatic lipogenic enzymes and the enhanced activity of a-oxidation. Fucoxanthin from brown seaweeds is a potentially useful compound for regulating inflammation in diabetic mellitus as well as anti-obesity (Ninawe, 2016; Padmakumar, 2016; Ravi and Baskaran, 2016).

Cosmeceuticals

The concept of Cosmeceuticals was created by Raymond Reed in 1961. Cosmeceuticals are products intended to be applied to the human body for cleansing, beautifying, promoting attractiveness or altering the appearance without affecting body structure or functions. Cosmeceuticals contain active ingredients or nutrients believed to promote healthy skin, hair and nails at cellular levels and their key ingredients often include vitamins, minerals, botanical extracts and antioxidants. As per the World Health Organization (WHO), in developing countries mortality due to nutrition related factors is about 40% which denotes the need for nutraceutical products to balance the nutritional intake of an individual (Padmakumar, 2016). The beneficial activities of marine algae for cosmeceuticals are given by Hanan *et al.* (2016). They prepared as dermal cream using *Gracilaria verrucosa* extract and the cell viability test was done. As the cream has not affected cell viability, it has become clear that

G.verrucosa can be substituted for other nutritious fruits and vegetables of various application in cosmetic industry.

Biofuel

The growing worldwide energy demands and its associated fossil fuels limitation have led to the decade's quest of alternative energy from renewable sources. Hence, the need to find an alternative source to fossil fuel-derived energy is important today as the oil reserves are dwindling rapidly. New sources of biofuel feedstock such as marine biomass, cellulosic biomass and other 'non-food' biomass are commonly referred to as next generation biofuel feed stocks. In general, biofuel promises higher productivity, a lower greenhouse gas profile and improved sustainability performance when compared with first generation feed stocks. Seaweeds that are producing the highest percentage of biomass in lesser time are exploited today for the production of biofuels. Seaweeds lack lignin and have a low cellulose content which makes them more suitable for complete biological degradation than land plants. They are used for generating methane and made into biogas energy via anaerobic digestion. They can also be used to produce ethanol by fermentation and there are efforts being done to convert macroalgae into butanol. The advantages of seaweed as biofuel feed stock are as follows: They grow fast and they are capable of producing more biomass per square metre than terrestrial plants. A seaweed farm can produce 50 tons of crop per acre (Ninawe, 2016). Sreenivasa Rao *et al.* (1979b) have conducted experiments on production of fuel gas for deomestive use utilising *Sargassum* as raw material. The methods of biogas production form marine macroalgae are given in detail by Anantharaman *et al.* (2016). The production of biodiesel from seaweeds and the studies made on biodiesel production from the green alga *Chaetomorpha linum* and red algae *Gracilariopsis longissima* are given by Anantharaman (2016). Studies were made on agar factory discharge for fuel (Kunda and Kaladharan, 2003) and *Kappaphycus alvarezii* as a source of bioethanol (Khambhaty *et al.,* 2012).

Conclusion

It is quite evident from the foregoing account that a good amount of research works was carried out by many researchers on the value added seaweed products from Indian marine algae for the last six decades especially on phycolloids and fertilizer. The technologies for processing of seaweeds with improved yield and quality of phycocolloids namely agar, alginate and carrageenan should be developed to meet the specifications and requirements of Indian user industries and also to export these products to earn good foreign exchange to our country and to discontinue the import of these value added seaweed products. The use of seaweeds as human food in various forms should be popularised. The standardization, production and marketing of edible seaweed products such as jelly, jam, pickle, wafer etc. should be made on priority. The animal feeds should be manufactured from seaweeds on commercial scale and used as daily rations for the poultry, cattle fishes and other farm animals. More and more quantities of fertilizers should be produced from seaweeds for using them as biofertilizer in the place of chemical fertilizers to increase the yield and production of various land crops and cash crops. Extensive studies should be undertaken on the bioactive and biochemical compounds from marine algae. Similarly, intensive studies should be carried out on the nutraceutical and cosmeceutical aspects of seaweeds. The R&D activities on the application of marine algae as biogas, biodiesel and biofuel should be intensified. Training and demonstration should be given in processing and production of various value added products and recipes from seaweeds to the fisher folk and rural population. Now there is immediate need for more extensive and systematic investigations to make use of this unique renewable marine resource.

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Studies on phytochemical, FTIR analysis and *invitro* antioxidant activity of marine red alga *Hypnea musciformis* (Wulfen) Lamouroux

V. SORNALAKSHMI[1], P.S. TRESINA[2], K. PAULPRIYA[2] AND V.R. MOHAN[2]*

[1]Department of Botany, A.P.C. Mahalakshmi College for Women, Tuticorin - 628 001, Tamil Nadu, India
[2]Ethnopharmacology Unit, Research Department of Botany
V.O. Chidambaram College, Tuticorin - 628 008, Tamil Nadu , India
**Corresponding Authorl : vrmohanvoc@gmail.com*

ABSTRACT

Seaweeds and their organic extracts contain a great variety of bioactive substances with different health effects. In this study, the phytochemical composition and antioxidant activity of various solvent extracts of *Hypnea musciformis* were determined. Fourier transform infrared (FT-IR) analysis was performed to identify the functional group of the crude extracts. Potential antioxidative activities of various solvent extracts of *H. musciformis* were evaluated using four different Reactive Oxygen Species (ROS) scavenging assays including DPPH (1,1-diphenyl-2-pricrylhydrazyl) free radical, hydroxyl radical, superoxide anion, ABTS radical and reducing power assay. Phytochemical analysis showed the presence of flavonoids, terpenoids, glucosides, saponins, phenols, tannins, steroids and xanthoproteins in the methanol and ethanol extracts of *H.musciformis*. The methanol extract showed the highest amount (1.88g100g^{-1}) of total phenolic content and ethanol extract had the highest amount of flavonoid (1.28g100g^{-1}). FT-IR results confirmed the presence of carboxylic acid, aliphatic fluoro compound, phenol or tertiary alcohol, nitroso group, alkenyl, secondary amino salt and hydroxyl group. At 800mg/ml concentration of ethyl acetate 126.16% of DPPH radical scavenging activity was visible. The highest hydroxyl radical scavenging behaviour (119.26%) was seen in methanol extract. The maximum superoxide radical scavenging activity i.e. 143.86% and ABTS radical scavenging activity (113.16%) were observed in ethanol extract of *H. musciformis* (800mg/ml). Of all the solvents tested, ethanol extract revealed a higher reducing activity. Hence, the ethanolic extract of *H. musciformis* can be considered as a natural antioxidant and may be useful in treating diseases caused by oxidation deterioration.

Introduction

Reactive oxygen species (ROS) comprises free radicals such as superoxide, hydroxyl and peroxyl radicals. These radicals play a key role in the manifestation of oxidative stress related diseases like cancer and atherosclerosis (Darely-Usmer and Halliwell, 1996). Many diseases are caused by lipid oxidation in biological membranes and ROS are the important initiators of these reactions (Frei, 1994). Enzymatic scavengers such as catalase, glutathione peroxide and superoxide dismutase are present in the eukaryotic system. These scavengers have a number of cellular defence systems. Seaweed has higher contents of non enzymatic antioxidant compounds, such as ascorbic acid, reduced glutathione, phenols and flavonoids. Hence they have higher antioxidant activity (Wu *et al*., 2010). As a consequence, it is assumed that the antioxidative components in seaweeds are the main cause for their effects on disease protection. As the ROS are implicated in several diseases, antioxidants play an important role in preventing the interaction of reactive oxygen species with biological system (Ames *et al.,* 1993). Many synthetic antioxidants are currently in use, on the other hand, there is a rising evidence of consumer preference for natural antioxidants because of their potentially lower toxicity (Orcic *et al*., 2011). Marine seaweeds consist of high level of antioxidant compounds since it is a valuable antioxidant source (Yan *et al*., 1998; Duan *et al*., 2005; Kuda *et al*., 2005). The aim of the present investigation is to show that seaweeds and its extracts are beneficial to mankind.

The Rhodophyta (red algae) are characterized by the accessory photosynthetic pigments phycoerythrin, phycocyanin and allophycocyanins arranged in phycobilisomes. Some red algae are economically important as

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providers of food and gels (Wilson, 2000). For this reason, extensive farming and natural harvest of red algae occur in many countries. Seaweeds have been known to contain bioactive compounds, but only very few reports are available on *Hypnea musciformis.* Numerous studies have been focused on the proximate compositions, vitamins and minerals of seaweeds (Haque *et al*., 2009; Dhargalkar *et al*., 1980; Manivannan *et al*., 2008; Meenakshi *et al*., 2010). K-carrageenan from *H. musciformis* has potent antioxidant activity (Rafiquzzaman *et al.,* 2016). It has already been proved that *H. musciformis* occurring in southern India and Brazilian coast has possible antioxidant activity with a high phenolic content (Chakraborty *et al.,* 2015). The present study was aimed in evaluating the qualitative and quantitative phytochemical, fourier transform infrared (FT-IR) analysis and in vitro antioxidant activity by employing different model assays viz DPPH, hydroxyl, superoxide and ABTS.

Materials and Methods

Collection of sample

H. musciformis (Wulfen) Lamouroux was collected near the red gate end of the Hare Island, Tuticorin, Tamil Nadu during end of the low tide period. The samples were cut into small fragments and shade dried. The dried samples were powdered using a blender and sieved to get uniform powder by using sieve No.60.

Preparation of plant extract

The coarse powder (100g) of *H. musciformis* was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedures (Saraf, 2010; Shajeela *et al*., 2012; Murugan and Mohan, 2011). The concentrated extracts were used for *in vitro* antioxidant activity. The methanol and ethanol extracts were used for the estimation of total phenolics and flavonoids.

FT-IR analysis

The powdered plant was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 - 400 cm^{-1} (Kareru *et al*., 2008).

Estimation of total phenolic content

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described by McDonald *et al*. (2001) with little modification. The total content and phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of flavonoids

The flavonoid content was determined according to the method given by Eom *et al.* (2007).

DPPH radical scavenging activity

The free radical scavenging activity of all the extracts was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method (Shen *et al*., 2010). The capability to scavenge the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*. (1987).

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al.* (2007).

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al*. (2011).

Reducing power

The reducing power of the extract was determined following the method of Kumar and Hemalatha (2011).

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analysed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for the above parameters were calculated.

Results and Discussion

Qualitative and quantitative phytochemical analysis

Qualitative phytochemical analysis of the methanol and ethanol extracts of *H. musciformis* showed the presence of flavonoids, terpenoids, glucosides, saponins, phenols, tannins, steroids and xanthoproteins. The methanol extract showed the highest amount (1.88g$100g^{-1}$) of total phenolic contentand flavonoid (1.28g$100g^{-1}$). Numerous reports on phenolic compounds have demonstrated their biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer etc (Al-Owaisi *et*

al., 2014, Sulaiman *et al*., 2011). The antioxidant activity of phenolic compounds is mainly due to their reducing properties which allow them to act as metal chelators, absorb and neutralize free radicals (Mishra *et al*., 2010). Among plant secondary metabolites flavonoids are the most potential compounds. They are significant as secondary metaboilites of plants modulating lipid peroxidation that are involved in arthogenesis, thrombosis and carcinogenesis. Many researchers demonstrated that phenolic compounds were one of the most effective antioxidants in marine algae (Namjooyan *et al.,* 2007; Luo *et al*., 2010). Therefore, based on the phytochemical screening results, the total phenolic and flavonoid contents of different extracts of *H.musciformis* were estimated and also its antioxidant potential value were investigated by *in vitro* DPPH, hydroxyl, superoxide and ABTS radical cation scavenging assay methods.

FT-IR Analysis

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The *H. musciformis* powder was passed into the FT-IR and the functional groups of the components were separated based on its peak ratio. The FT-IR spectrum of *H. musciformis* is given in Fig.1. The data on the peak values and the probable functional groups (obtained by FT-IR analysis) present in the *H. musciformis* are presented in Table-1. The functional group analysis results revealed the presence of various characteristic functional groups in *H. musciformis*. The presence of carboxylic acid, aliphatic fluoro compound, phenol or tertiary alcohol, nitroso group, alkenyl, secondary amino salt and hydroxyl group which shows major peaks at 927.49, 1028.83, 1409.61, 1545.09, 1638.53, 2360.27 and 3353.27 respectively has been proved by this test.

Antioxidant activity

The antioxidant activity is system-dependent. Moreover, it depends on the method adopted and the lipid system used as substrate (Singh *et al*., 2006). Hence, the

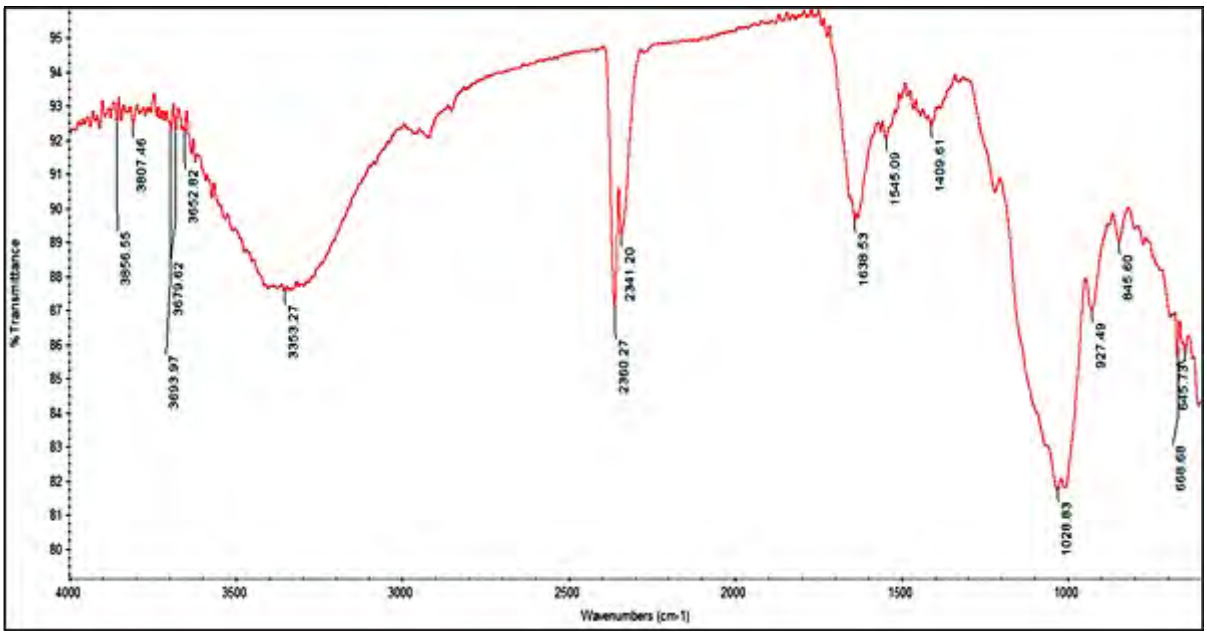

Fig. 1. *Fourier Transform Infrared spectrum of* H. musciformis

Table-1. *FT-IR spectroscopic data of* H. musciformis

Sl.No.	Stretching frequency Cm^{-1}	Functional Groups	Assignment
1	927.49	O-H out of plane bending	Carboxylic acid
2	1028.83	C-F stretching	Aliphatic Fluoro compound
3	1409.61	OH bend	Phenol or tertiary alcohol
4	1545.09	N=O stretching	Nitroso group
5	1638.53	C=C stretching	Alkenyl
6	2360.27	NH_2 asym stretching	Secondary amino salt
7	3353.27	OH stretching	Hydroxyl group

following different methods have been adopted in order to assess the antioxidative potential of *H.musciformis* extracts.

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorbance at 517 nm that can readily undergo reduction by an antioxidant. Due to the ease and convenience of this reaction, it is now used in the free radical-scavenging activity assessment (Brand-Williams *et al.,* 1995). The radical-scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *H. musciformis* is shown in Fig. 2 and expressed as percentage reduction of the initial DPPH absorption by the tested compound. The scavenging effect increased with the concentration of standard and extracts. Highest DPPH radical scavenging activity was observed in ethyl acetate extract among all the solvents tested. The ethyl acetate extract of *H. musciformis* at 800 µg/ml concentration displayed 126.16% scavenging activity. The IC_{50} values of ethyl acetate extract of *H. musciformis* and standard ascorbic acid were 39.13.µg/ml and 34.15µg/ml respectively (Table-2). Ragan and Glombitza (1986) have shown that the radical scavenging activity of seaweeds to be potentially linked to their phenolic contents. However, Siriwardhana *et al*. (2003) and Lu and Foo (2000) reported a high correlation between DPPH radical-scavenging activities and total polyphenolics. Components, such as polysaccharides, pigments, proteins or peptides also impart the antioxidant activity (Siriwardhana *et al*., 2003).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of enzymatic extracts from seaweed was measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton reaction mixture. Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol of *H. musciformis* is shown in Fig. 3. Methanol extract showed a very high potent activity. The methanol extract of *H. musciformis* displayed a scavenging behaviour of 119.26% at 800 µg/ml concentration in hydroxyl radical. The IC_{50} values of methanol extract of *H. musciformis* and standard ascorbic acid were 34.88µg/ml and 27.26µg/ml respectively (Table-2). As the strongest of free radicals, the ability of hydroxyl radical to damage cells extensively is well known (Koppenaol and Liebman, 1984). Some seaweed extracts have displayed positive impact on hydroxyl radical and reached around 60% (Estrada *et al*., 2001; Siriwardhana *et al*., 2003).

Superoxide radical scavenging activity

Several biochemical reactions in viable cells formed Superoxide anion (Fridovich, 1974) and its effect can be magnified because it produces other types of free radicals and oxidizing agents that can induce cell damage (Lui and Ng, 1999). *H. musciformis* extracts were subjected to superoxide radical scavenging assay and the results are shown in Fig. 4. The results in the ethanol extract of *H. musciformis* (800µg/ml) showed the highest quantum of superoxide radical scavenging activity (143.86%) which is higher than the scavenging effect of

Table-2. *IC_{50} value of different solvent extracts of* H.musciformis (*µg/ml)*

Solvent extract	DPPH assay	Hydroxyl assay	Superoxide assay	ABTS assay
Ethanol	37.65	30.16	47.34	35.16
Methanol	33.88	34.88	46.03	32.06
Petroleum Ether	29.16	32.16	28.06	36.11
Benzene	35.63	29.16	44.84	26.84
Ethyl Acetate	39.13	31.65	41.65	30.08
Standard (Ascorbic acid)	34.15	27.26	31.15	-
Standard (Trolox)	-	-	-	28.15

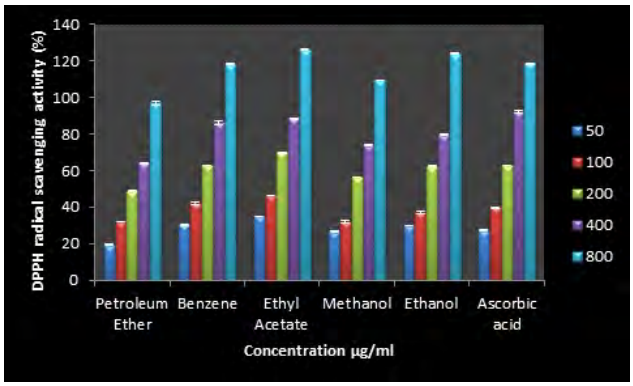

Fig. 2. *DPPH radical scavenging activity of different solvent extracts of* H.musciformis

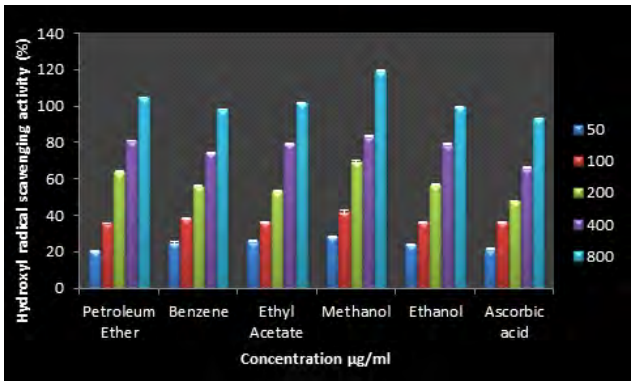

Fig. 3. *Hydroxyl radical scavenging activity of different solvent extracts of* H.musciformis

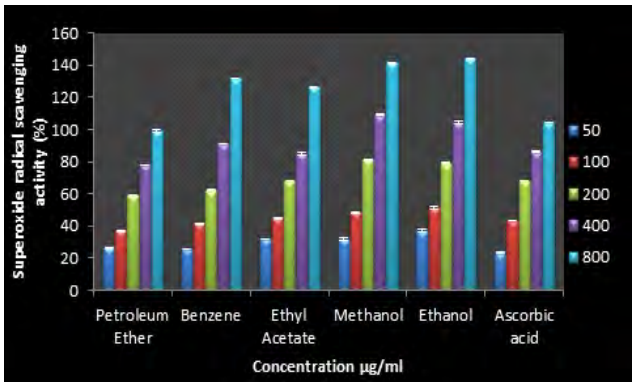

Fig. 4. *Superoxide radical scavenging activity of different solvent extracts of* H.musciformis

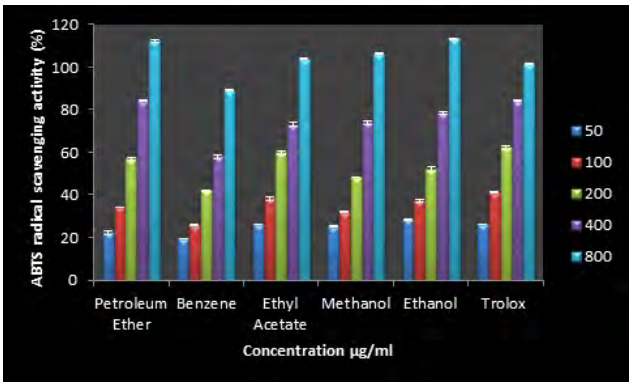

Fig. 5. *ABTS radical cation scavenging activity of different solvent extracts of* H.musciformis

the standard ascorbic acid (104.30 %). The IC_{50} values of ethanol extract of whole plant of *H. musciformis* and standard ascorbic acid were 47.34µg/ml and 31.15µg/ml respectively (Table-2). Studies on superoxide anion scavenging activity of seaweeds have gained interest because of the harmful effect of superoxide anion. Nagai and Yukimoto (2003) recorded a significant superoxide anion scavenging activity for a beverage made of *Hizikia fusiformis,*a brown seaweed and Athukorala *et al*. (2003) reported a highly effective superoxide anion scavenging activity in a edible red seaweed *Grateloupia filicina*.

ABTS radical cation scavenging activity

ABTS radical scavenging activity involves a more drastic that is chemically produced and it is a recent one. It is increasingly utilised to screen complex antioxidant mixtures like plant extracts, beverages and biological fluids. Its efficacy in both the organic and aqueous media and the stability in a wide pH range has caused an interest in the use of ABTS in the estimation of antioxidant activity (Huang *et al*., 2011). *H. musciformis* extracts were subjected to ABTS radical cation scavenging activity and the results are shown in Fig. 5. The ethanol extract exhibited potent ABTS radical cation scavenging activity in a concentration dependent manner. At 800 µg/ml concentration, ethanol extract of *H. musciformis* showed 113.16 % scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 101.26%. IC_{50} values of ethanol extract of *H. musciformis* and standard trolox were 35.16µg/ml and 28.15µg/ml respectively (Table-2).

Reducing power

Figure-6 shows the reducing ability of different solvent extracts of *H. musciformis* comparision to ascorbic acid. The absorbance of the solution increased as the concentration increased. A higher rate of absorbance is an indicator of an increased reducing power. Among the solvents tested, ethanol extract showed a higher reducing activity. The Fe^{3+}-Fe^{2+} transformation was studied in the presence of *H. musciformis* for calculating the reducing activity. The reducing

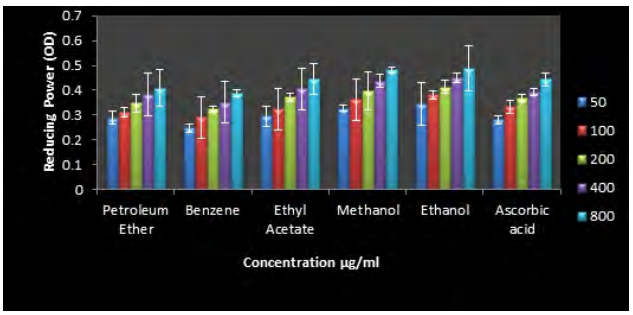

Fig. 6. *Reducing power activity of different solvent extracts of* H.musciformis

capacity of a compound serves as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants are associated to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalyst, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Diplock, 1997; Yildirim *et al*., 2000). It is stated by Devi *et al*. (2008) that the presence of phenolic compound in a seaweed extract has the chances to affect their antioxidant activity. Phenols are one of the main plant necessary chemical constituents. They have the capability of scavenging of free radicals and also this type of solvents are an essential factor for the isolation of antioxidant compounds.

The present investigation showed the excellent radical scavenging effect of various solvent extracts of *H. musciformis.* Thus they could be used in nutraceutical and functional food applications. The detailed investigation on the composition of each component involved is very much necessary to establish appropriate applications which may open new frontiers for human consumption of seaweeds world-wide.

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Season, locality and parts of the thallus influence fucoidan yield and its compositions among the three Gulf of Mannar brown seaweeds

T. ELUVAKKAL AND K. ARUNKUMAR[1]*

Marine Algae Research Division, Post Graduate and Research Department of Botany
Alagappa Government Arts and Science College, Alagappa University, Karaikudi, Tamil Nadu, India
[1]Present address of corresponding author : Department of Plant Science, School of Biological Sciences
Central University of Kerala, RST Campus, Padanakkad - 671 314, Kasaragod, Kerala, India
Corresponding author : rsrnarun@gmail.com

ABSTRACT

In this study, fucoidan yield and its proximate composition(total carbohydrate, fucose and sulphate) extracted from *Sargassum wightii,Dictyota dichotoma* and *Stoechospermum marginatum* during different seasons and at various localities (Pamban-Palk Bay, Rameswaram, Pamban-Gulf of Mannar and Pudumadam, Gulf of Mannar) were recorded. In *Sargassum wightii,* the composition invarious parts was also reported. The yield and its compositions were found to be maximum in the monsoon and the minimum in summer and optimum in the premonsoon and postmonsoon seasons in the three brown seaweeds collected at Pamban-Gulf of Mannar. Significant difference in the fucoidan yield and its compositions were observed due to the influence of environment and seawater quality of respective locality. Significant variations in the fucoidan yield were recorded among the blade, air bladder and stem of *Sargassum wightii*. But the variations in the fucoidan compositions such as total carbohydrate, fucose and sulphate recorded among the blade, air bladder and stem of *Sargassum wightii* were not significant. This suggested that fucoidan composition in various parts of the brown seaweed *Sargassum wightii* was similar. However, insignificantly higher fucoidan content was found in leaf than air bladder and stem. It is due to the soluble form of fucoidan accumulated in the cytoplasm of leaf blade.

Introduction

Fucoidans are a group of sulphated polysaccharides primarily composed of L-fucose with less than 10 % of other monosaccharides. They are widely found in the cell-walls of brown seaweed, but not in other algae or higher plants. The species *Fucus vesiculosus* contains the highest concentration of fucoidans (up to 20 % on a dry weight basis) and isolated algal cell-walls of brown algae contain more than 40 % in dry weight. This polymer can easily be extracted using either hot water or an acid solution. Although the major physiological purposes of fucoidans in the algae are not thoroughly understood, they are known to possess numerous biological properties with potential human health applications (Berteau and Mulloy, 2003). The bioactivity of fucoidan for human health is evidenced as anticoagulant, anti-viral and anti-cancer (Zhuang*et al*.,1995). No toxicological changes were observed when 300 mg/kg body weight per day fucoidan was administered to rats. However, significant blood-cloting times were observed to be prolonged when concentrations were increased three fold (Li *et al*.,2008). Till date, there are several studies related to fucoidan content of brown seaweeds (Castro *et al*., 2015), but on the seasonal variations in the content and composition of fucoidan, only few studies have been made (Parsons, 1994; Honya *et al*., 1999; (Skriptsova *et al*., 2009; Nimura and Mizuta, 2001; Fletcher *et al*., 2017). Fucoidan level also varied depending on the age of the plants (Nimura and Mizuta, 2001; Zvyagintseva *et al*., 2003). There is a correlation between fucoidan content and seasonality (Honya *et al*., 1999).

Works on fucoidan content in relation to seasons and locality as well as distribution among various parts of the thallus are generally few (Fletcher *et al*.,2017) and lacking in Indian brown seaweeds. Therefore, in this study, fucoidan content was

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estimated in three brown seaweeds namely *Dictyota dichotoma, Stoechospermum marginatum* and *Sargassum wightii* collected during four seasons in a year at four different stations along Tamil Nadu coast. Further, fucoidan content in the leaf, air bladder and stem of *Sargassum wightii* was also attempted. *Sargassum wightii, Dictyota dichotoma* and *Stoechospermum marginatum* were chosen as they were reported to have maximum, optimum and minimum fucoidan content respectively among the 11 brown seaweeds screened for fucoidan yield in the earlier study (Eluvakkal *et al*.,2010).

Materials and Methods

Sampling

For seasonal study, fresh, matured, disease free and healthy brown seaweeds [*Dictyota dichotoma* (Hudson) Lamouroux*, Stoechospermum marginatum* (C.Ag.) Kuetz. and *Sargassum wightii* Greville] were collected during the low tide from Pamban (Gulf of Mannar) Tamil Nadu (9°17'N 79°18'E / 9.28°N 79.3°E) during the month of February (post-monsoon), May (summer), August (pre-monsoon) and November (monsoon) covering the four seasons of the year 2010 and 2011. For spatial study, the same three brown seaweeds were collected from Pamban (Palk Bay), Rameswaram, Pamban (Gulf of Mannar) and Pudumadam, Gulf of Mannar during the monsoon season (November) in the year 2011. For studying the fucoidan content, various parts (leaf, air bladder and stem) of *Sargassum wightii* were collected from Pamban (Gulf of Mannar) during the monsoon season (November) in the year 2011. The collected samples were washed thoroughly in seawater followed by tap water and distilled water to remove the macroscopic epiphytes. Then the cleaned samples were air dried in dark for 3 days. Shade dried algal samples were then pulverized into fine powder.

Extraction of crude fucoidan (Chotigeat *et al*., 2004) was made by soaking 100 g of powdered sample in 500 ml of 0.1 N HCl at 95° C for 12 h. The extraction was repeated thrice and the extracts were pooled and filtered through Whatman No.1 filter paper. The filtrate was condensed and dialyzed against water and lyophilized to dryness. The crude fucoidan was measured gravimetrically and expressed on the basis of algal dry weight. Algal dry weight was calculated by keeping known quantity of fresh specimen at 60°C in hot air oven till attaining constant weight. Major composition of fucoidan such as total carbohydrate (Dubois *et al*.,1956), L-fucose (Dische and Shettles, 1948) and sulphate (Verma *et al*., 1977) were estimated. Analysis was carried out in triplicates to give the mean value. One way ANOVA was carried out between variables and significance was tested as per DMRT at 0.05 levels ($P<0.05$).

Results and Discussion

The results obtained on the fucoidan yield and its constituents (total carbohydrate, fucose and sulphate) in the three brown seaweeds *Sargassum wightii, Dictyota dichotoma* and *Stoechospermum marginatum* collected during different seasons at various stations and from different parts of *Sargassum wightii* are presented (Figs. 1 to 4).

Fucoidan and its compositions during different seasons

Yield of fucoidan and its constituents (total carbohydrate, fucose and sulphate) obtained from the three brown seaweeds collected from Pamban (Gulf of Mannar), Tamil Nadu during the four seasons in a year are presented in Fig. 1. Among the three species, maximum fucoidan yield and its constituents were recorded in *Sargassum wightii* followed by *Dictyota dichotoma* and *Stoechospermum marginatum.* Of the four seasons, high fucoidan and its compositions were significantly found during the monsoon season whereas during the postmonsoon and premonsoon seasons the values were optimum and insignificant in all the three species. Low amount of fucoidan was recorded in summer season.

Significant difference in the alginate and laminarian content in brown seaweeds collected at different seasons was registered, whereas there was no such difference in fucoidan (Stewart *et al*., 1961). But in the later studies (Mian and Percival, 1973; Park et *al*., 1993; Usov *et al*., 2001), seasonal variations in the fucoidan content of brown algae were recorded. Patankar *et al.* (1993) reported varied fucoidan content of 1- 20% depending on the species. Koo (1995) recorded different amount of fucoidan in *Laminaria religiosa* (2.7%), *Undaria pinnatifida* (6.7%), *Hijikia fusiforme*(2.5%) and *Sargassum fulvellum*(1.6%). The fucoidan content differed by region, algal species and by the season (Mian and Percival, 1973; Park *et al.,* 1997; Usov *et al.,* 2001). Souchet (2004) correlated variation in the fucoidan content in *Laminaria longicrurissursa* depending on the month of harvest. Lee *et al.* (2006) reported seasonal variations in the fucoidan yield in *Sargassum horneri.*

Eluvakkal *et al*. (2010) observed variations in the yield and constituent of fucoidan among the 11 brown algae collected along the coast of Gulf of Mannar (Pamban), India. The seasonal variations in the fucoidan yield recorded in this study are as reported earlier (Usov *et al.,* 2001; Lee *et al.,* 2006). This study further reported seasonal variations in the fucoidan compositions such as total carbohydrate, fucose and sulphate in the three brown seaweeds *Sargassum wightii, Dictyota dichotoma* and *Stoechospermum marginatum*. The present findings are in agreement with the results obtained in *Undaria pinnatifida* and other brown algae (Duarte *et al.,* 2001). Menshova *et al.* (2012) reported variations in the compositions of polysaccharides such as alginate, laminarin and fucoidan of

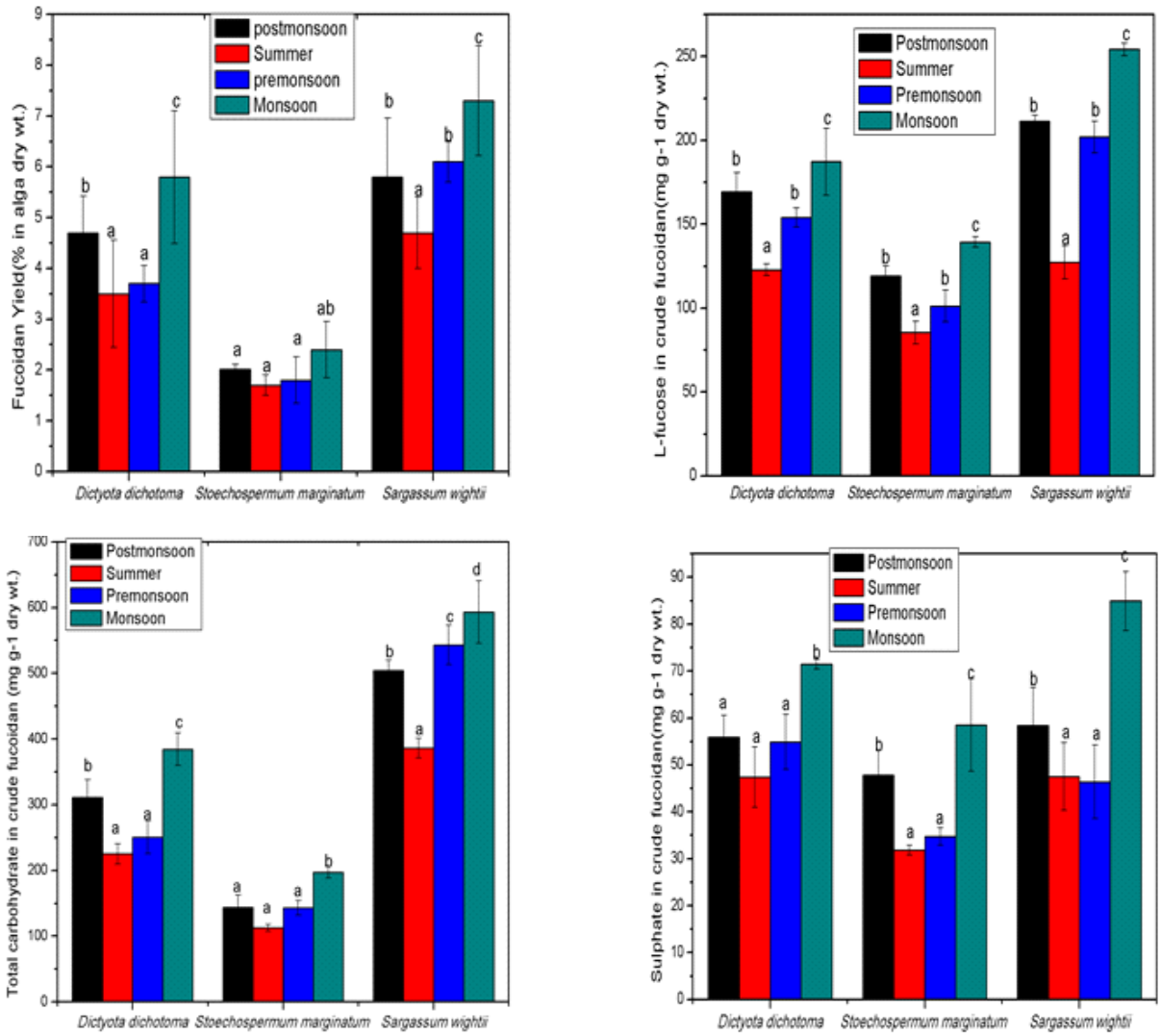

Fig. 1. *Yield, total carbohydrate L-fucose and sulphate content of fucoidan extracted from three brown seaweeds collected from Pamban (Gulf of Mannar), during four seasons in 2010-2011. (Different alphabet within each bar group is significant, P<0.05)*

the brown alga *Padina pavonica* from the Mediterranean Sea. The present observation on the seasonal influence on the variations in the fucoidan and its compositions among the three brown seaweeds collected in the Gulf of Mannar region is similar to the monthly variations in the fucoidans and its compositions recorded in the three brown seaweeds *Fucus serratus, F. vesiculosus* and *Ascophyllum nodosum* collected from the same locality (Fletcher *et al.,* 2017)

Fucoidan and its constituents at different stations

Previous studies reported the differences in the fucoidan yield in some brown seaweeds (Honya *et al.,* 1999; Skriptsova *et al.,* 2009). The variations in the fucoidan content of the brown seaweed *Undaria pinnatifida* collected from two different farms suggested the influence of chemical prosperities of seawater (Skriptsova *et al.,* 2009). There was variation in the amount of fucoidan and its compositions in *Undaria pinnatifida* collected at two coasts of New Zealand (Mak *et al.,* 2013). In the present study, the brown seaweeds *Sargassum wightii, Dictyota dichotoma* and *Stoechospermum marginatum* collected from Pamban (Palk Bay), Rameswaram, Pamban (Gulf of Mannar) and Pudumadam showed significant differences in the fucoidan yield and its compositions such as total carbohydrate, fucose and sulphate level.

Significant difference in the crude fucoidan and its compositions was recorded in *Sargassum wightii* and *Dictyota dichotoma* collected from different coasts, whereas variation in the fucoidan and its compositions observed in *Stoechospermum marginatum* was not significant (Fig.2).This differences in the fucoidan content and its compositions suggested the influence of local environment and seawater chemistry in which seaweeds grow (Menshova *et al*., 2012; Mak *et al.,* 2013). The recent observation made by Fletcher *et al*.

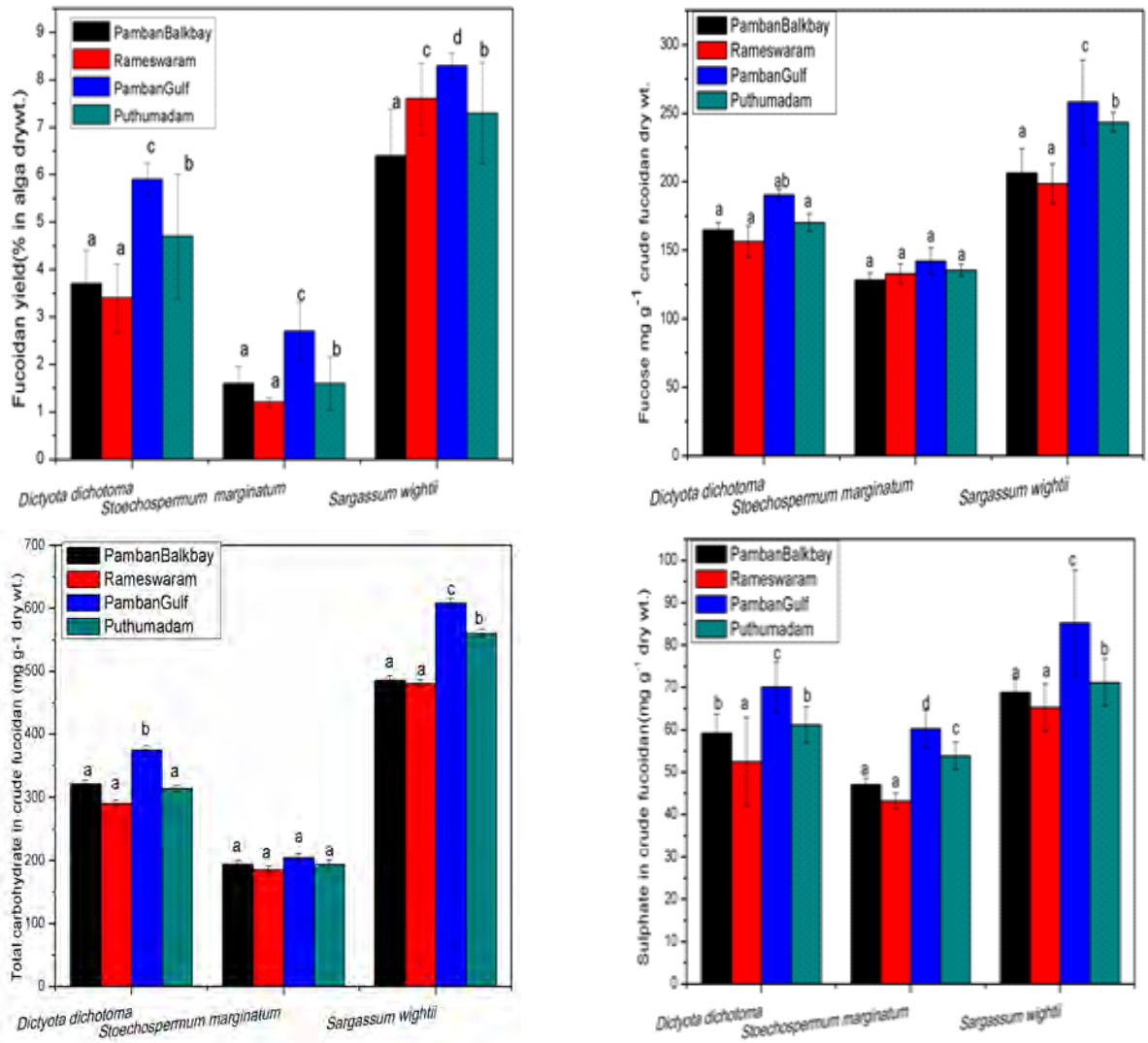

Fig. 2. *Yield, total carbohydrate L-fucose and sulphate content of fucoidan extracted from three brown seaweeds collected at four localities during monsoon season in 2011 (Different alphabet within each bar group is significant, P<0.05)*

(2017) on the structural difference of the fucoidans among the three brown seaweeds *Fucus serratus, F. vesiculosus* and *Ascophyllum nodosum* collected in the same locality and the same species collected during different months suggests that the variations in the fucoidan yield and its compositions during the present study may be due to structural difference in the fucoidans among the three brown seaweeds occurring at different locality and in various seasons in the Gulf of Mannar region.

Fucoidan and its constituents in different parts of *Sargassum wightii*

In this study, crude fucoidan yield (Fig.3) and its compositions such as total carbohydrate fucose and sulphate were recorded in various parts of the brown seaweed *Sargassum wightii* which showed clear distinction into leaf, air bladder and stem (Fig.4). The sulphate content in sporophyllic fucoidan of *Undaria pinnatifida* differed from the two farms (Mak *et al*., 2013). This difference in the sulphate content was proportional to the percentage yield of sporophyll-derived fucoidan in *Undaria pinnatifida* due to the formation and maturation of sporophylls (Skriptsova *et al*., 2009). Inconsistency in the blade derived sulphate content suggests that the majority of the endogenous changes of *U. pinnatifida* during sporogenesis occur in the reproductive region of the alga. Similarly earlier study also showed that the sulphate content in blade-derived fucoidan from *Laminaria japonica* was stable during its growing season (Honya *et al.,* 1999).

Monthly changes in the uronic acid content of fucoidan may also be affected by the sporogenesis of *Undaria pinnatifida* (Honya *et al.,* 1999; Skriptsova *et al.,* 2009). In this study, *Sargassum wightii* showed higher amount of fucoidan yield in leaf than air bladder and stem which showed insignificant difference, whereas the observed difference in the

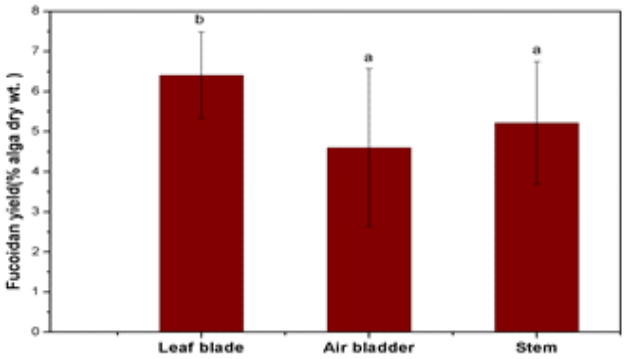

Fig. 3. *Fucoidan yield in different parts of the brown seaweed* Sargassum wightii. *(Different alphabet between bar is significant, P<0.05)*

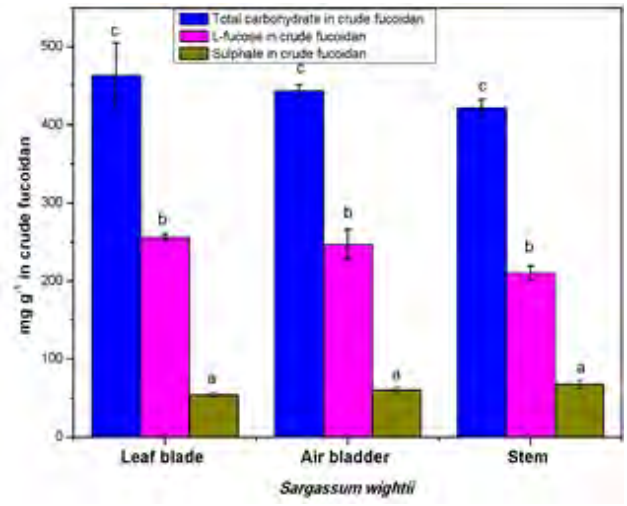

Fig. 4. *Fucoidan compositions in various parts of the brown seaweed* Sargassum wightii. *(Different alphabet within each bar group is significant, P < 0.05)*

fucoidan compositions such as total carbohydrate, fucose and sulphate were not significant among the leaf, air bladder and stem. This suggested that the accumulation of fucoidan among the various parts of the plant was same as in brown seaweed *Sargassum wightii.* The soluble form of fucoidan accumulated in the cytoplasm of the leaf influenced insignificantly higher amount than air bladder and stem.

The present study shows that the seasonal variation in the fucoidan yield and its compositions such as total carbohydrate, fucose and sulphate was maximum during the monsoon, minimum during the summer and optimum during the premonsoon and postmonsoon seasons in the three brown seaweeds viz. *Sargassum wightii,Dictyota dichotoma* and *Stoechospermum marginatum* collected from Pamban (Gulf of Mannar) of Tamil Nadu. The significant difference in the fucoidan yield and its compositions of the three brown seaweeds collected from Pamban (Palk Bay), Rameswaram, Pamban (Gulf of Mannar) and Pudumadam of Tamil Nadu (India) suggested the influence of environment and seawater chemistry. Significant variations in the fucoidan yield among the leaf, air bladder and stem of *Sargassum wightii* were recorded but variations in the fucoidan compositions like total carbohydrate, fucose and sulphate among the blade, air bladder and stem were not significant. The soluble form of fucoidan accumulated in the cytoplasm of the leaf insignificantly influenced higher amount than in air bladder and stem.

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In silico docking and interaction analysis of bioactive marine compound Fucoidan against the Anti - apoptosis involving factors

S. SIVASANKARA NARAYANI*[1], S. SARAVANAN[1], S. CHRISTOPHER BABU[2], M.S.RAMASAMY[3] AND K. MEENAKSHI SUNDARAM[3]

[1]*CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Portonovo -608 502, Chidambaram*

[2]*Department of Biotechnology, Bishop Heber College, Tiruchy-620 017*

[3]*Indian Systems of Medicine –Natural products laboratory, MIT campus of Anna University, AUKBC Research Centre, Anna University, Chennai-600044*

**Corresponding author: sivasan91@gmail.com*

ABSTRACT

In an effort to develop potent anticancer drug from marine flora, the present investigation was made to evaluate the inhibition effect of fucoidan against cancer associated protein by computational molecular docking studies. Docking studies were performed for fucoidan with protein involved in cancer by autodock 1.5.4. Molecular docking studies of fucoidan with cancer associated protein exhibited binding interactions. The results showed that the best stability of interaction was observed in Ubiquitin - fucoidan with least energy of about -6.06 Kcal/mole. The molecular docking studies could contribute for the further development of the drug for the prevention and treatment of cancer. This study concludes that marine natural products with interesting biological properties and structural biodiversity may serve as valuable lead drug candidates for the treatment of human ailments including cancer of different types in close proximity to future.

Introduction

Computational biology, bioinformatics and cheminformatics have the potential of speeding up the discovery process, reduces the costs and also of changing the way drugs are designed. There are several softwares helping to design drugs in different approaches to identify novel compounds. One user friendly method is the docking of the drug molecule with the target receptor (Manikandan *et al.*, 2009). Brown algae represent a rich and easily regenerated source of polysaccharides with structural interests and biological activity. Fucoidans are extracted from brown algae. Fucoidans are water soluble (Horn *et al.*, 1999; Moen *et al.,* 1999) high molecular weight sulfated polysaccharides which are widely dispersed in the cell walls of brown seaweed. Fucoidans have antineoplastic, anticoagulant, anticomplementary, antivirus activities including anti –HIV infection, herpes and hepatitis viruses (Nagumo and Nishino, 1996; Chevolot *et al.*, 1999; Zvyaginsteva *et al.,* 2000; Schaeffer and Krylov, 2000; McClure *et al.,* 1992) and also the crude extracts of brown algae is known to exert broad range of biological activities such as antibacterial, antiviral, antipyretic, analgesic, anti-inflammatory, antiedema and others. Extensive review of literature concludes that both the terrestrial and marine medicinal plants have known to exert potent biological activity. However, when compared with terrestrial plants very little research has been carried out so far on the anticancer studies with marine plants. Hence, this study aimed to evaluate the *in silico* cancer inhibition effect of fucoidan.

3-phosphoinositide dependent protein kinase-1, also known as PDPK1 is a protein which in humans is encoded by the *PDPK1* gene. It is implicated in the development and progression of melanomas (Scorteagagna, 2014). An important role for PDPK1 is in the signaling pathways activated by several growth factors and hormones including insulin signaling. Ubiquitin is a small protein that is found in almost all cellular tissues in humans and other eukaryotic organisms, which helps to regulate the processes of other proteins in the body. Through a process known as ubiquitination, an ubiquitin molecule can

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bind to a substrate protein, changing the way it functions. This can lead to a number of different outcomes. It is most widely recognized for its role of apoptosis of proteins, earning it the title of the molecular "Kiss of Death" for protein. Although it also plays a major part in several cellular processes related to the regulation of proteins, ubiquitin can affect apoptosis (cell death), cell division and multiplication, degeneration of neurons and muscular cells, DNA transcription and repair (Miranda and Sorkin, 2007).

The BAD protein is a pro-apoptotic member of the Bcl-2 gene family which is involved in initiating apoptosis BAD is a member of the BH 3 only family, a subfamily of the BCl-2 family (Adachi and Imai, 2006). It does not contain a c- terminal transmembrane domain for outer mitochondrial membrane and nuclear envelope targeting, unlike most other members of the Bcl -2 family(Hsu *et al.*, 1997). After activation, it is able to form a heterodimer with anti-apoptotic proteins and prevent them from stopping apoptosis. Mutation in the human tumour suppressor adenomatous polyposis coli (APC) gene is the major causes of hereditary and sporadic colorectal cancers (Kinzler and Vogelstein, 1996; Rustgi, 2007). APC provides a structural platform on which many interaction partners bind, including APC-stimulated guanine nucleotide exchange factor (GEF; Asef) (Kawasaki *et al.*, 2000).

Materials and Methods

Computational and software requirements

Molecular docking studies was performed using MGL tool, a molecular docking software installed in a single machine running on Intel core TM2 Duo processor with 4GB RAM and 500GB hard disk. Some non - commercial available docking analysis done with the help of auto dock non commercial tool MGL tool, Cygwin, Discovery studio Visualiser, Binary files, Java (Syed *et al.,* 2013).

Target protein Identification and Preparation

Three Dimensional structure of cancer associated proteins (Ubiquitin (1AAR), Phosphoinositidae – dependent protein kinase (3RWQ), Complex of BCl-xl with peptide from BAD (1G5J) and crystal structure of APC Complex with ASAF (3NMX) were obtained from www.rcsb.org/pdb. The protein was preprocessed separately by deleting the substrate cofactors as well as crystallographically observed water molecules (water without H bonds), hydrogen bond optimization was done.

Ligand Identification and Preparation

The 2D structure of Fucoidan was obtained from http:// pubchem.ncbi.nlm.nih.gov/compound/92023653 (Fig.1) Energy minimization was done by Avagadro software (Fig. 2). Open file and then have to read molecule, after that edit the molecule by add polar hydrogens, kollman charges and save it as target. Kollman charges are template values for each amino acid that were derived from the corresponding electrostatic potential using quantum mechanics. Open ligand and set number of torsions and aromatic carbons, aromaticity criterion enter angle in Degrees: 7.5'and save it as ligand. Open Grid and have to Set Map Types. We have to set X,Y,Z dimension as 60x60x60. For docking click target and open and select ligand .Using Cygwin for Molecular Docking. Open Cygwin and type the commands in Cygwin window.

Analyzing Results

For analysing results have to go for autodock and open docked file and finally retrieving Ligand-Enzyme interaction complex .pdb file and open complex.pdb in Discovery Studio Visualizer. Have to select all other complexes and delete them except the best. (*In our case Complex model 10 was best as conformation 10 was showing best results in our case). Analyze the ligand Interactions and click Show ligand Binding Site Atoms. Right Click on Complex and Click Label aminoacid and 1 letter and ID insertion code and save as image files.By using ligplus software got interaction images.

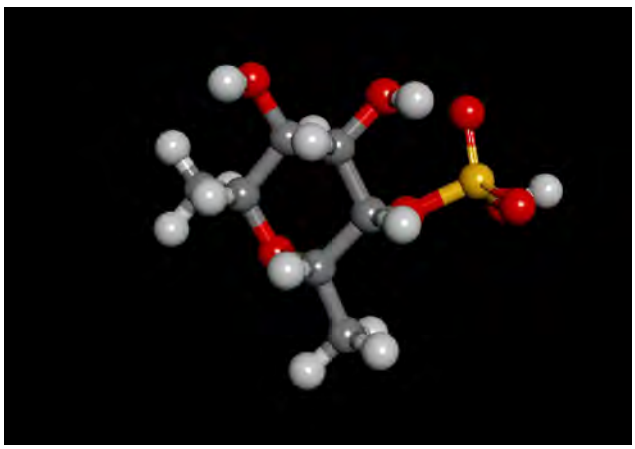


Fig. 1. *Structure of fucoidan*

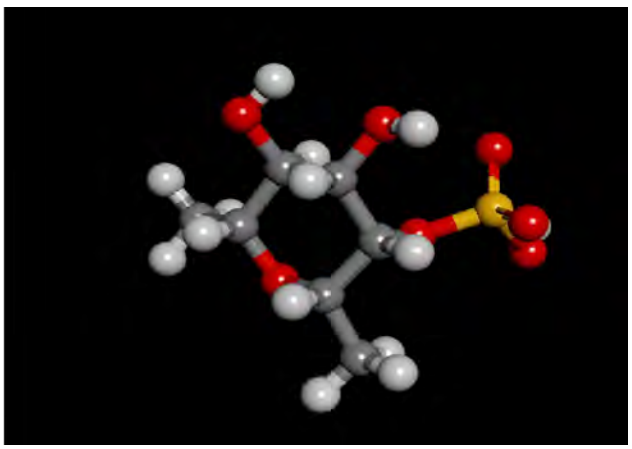


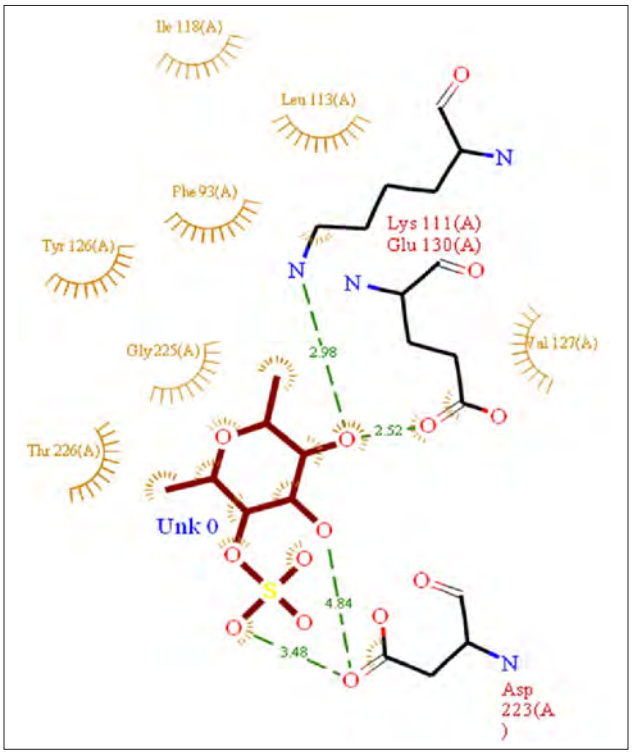
Fig. 2. *Energy minimised*

Table-1. *Protein – Ligand Interaction*

Sl. No	Ligand	Target Protein	Binding energy (kcal / mol)	Inhibition constant (µm)	Inter molecular energy (kcal/mol)	Ref RMS	Distance	No. of hydrogen bonds invoved in bonding	Amino acids involved in hydrogen bonding
1	Fucoidan	3RWQ	-5.46	99.72	-5.06	54.38	2.98 2.52 4.84 3.48	2	Lysine, Glutamic acid
2		1AAR	-5.27	136.05	-6.06	20.39	2.83 2.84 2.73 2.53 3.21 2.46	3	Alaninne, Lysine, Threonine
3		1G5J	-3.57	2.4	-3.57	4.84	2.87 2.73 2.89	1	Alanine
4		3NMX	-5.08	187.91	-5.18	36.2	3.27 2.84 2.63 2.91	2	Threonine, Glutamic acid

Results and Discussion

The molecular formula of sulphated fucoidan is found to be $(C_6H_{10}O_7S)n$ with the molecular weight 242.24686g/mol. The docking simulation technique was performed using autodock tool 1.5.6 with polysaccharide fucoidan, derived from the cell wall of brown algae was docked against target human Ubiquitin, Phosphoinositidae – dependent protein kinase 1g5j (Complex of BCl-xl with peptide from BAD) and 3NMX (crystal structure of APC Complex with ASef). Docking results , binding energy, inhibition constant, intermolecular energy, Ref RMS and amino acids involved in hydrogen bonding were analysed. Docking results and binding interactions were analysed (Table-1). Very good stability was observed in 1AAR - 6.06Kcal/mol.



Fig, 3. *Interaction between Phosphoinositidae – dependent protein kinase (3WQ) and Fucoidan*

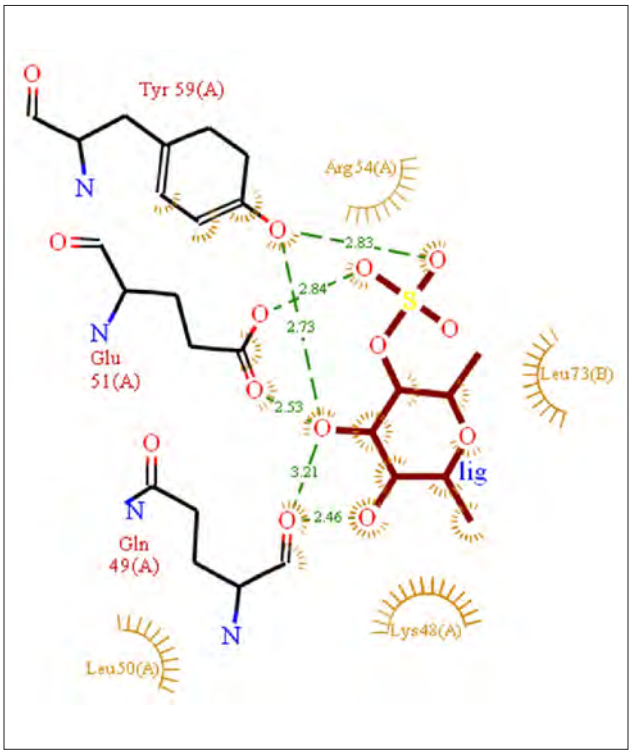


Fig. 4. *Interaction between Ubiquitin (1AAR) and Fucoidan*

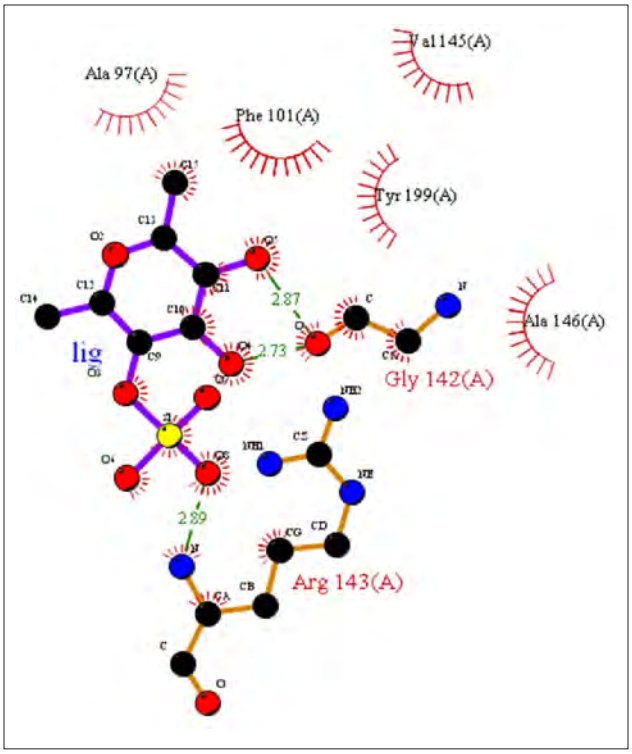


Fig. 5. *Interaction between Complex of BCl-xl with peptide from BAD (1g5j) and fucoidan*

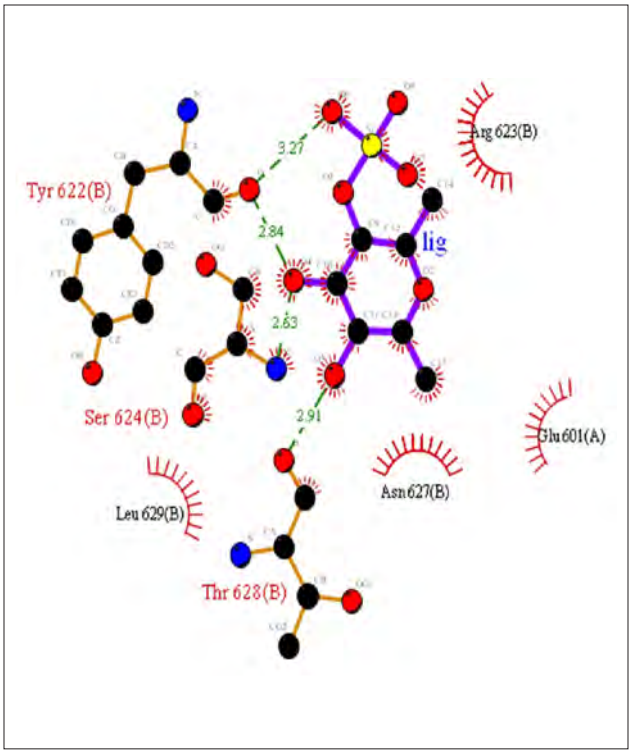


Fig. 6. *Interaction between Crystal structure of APC with Asef (3NMX) and fucoidan*

Marine floral resources need to be extensively investigated for their identification of potent bioactive compounds which are hidden due to lack of indepth research on marine resources (Angela *et al*., 2012). This study concludes that marine natural products with interesting biological properties and structural diversity may serve as valuable lead drug candidate for the treatment of human ailments including cancer. This study may provide an insight for exploitation of drugs from marine compounds against different types of cancer of different types in close proximity of future.

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Feasibility of seaweed cultivation at new locations in the coastal waters of Tamil Nadu

C. PERIYASAMY[1], P.V. SUBBA RAO[2] K.T. SELVAVINAYAGAM[3] AND P. ANANTHARAMAN[4]

[1]Department of Botany, Pasumpon Muthuramalinga Thevar College
Melaneelithanallur - 627 953, Tirunelveli, Tamil Nadu, India
[2]Aquaculture Foundation of India, Madurai - 625 003, Tamil Nadu, India
[3]Research Department of Botany, Pasumpon Muthuramalinga Thevar Memorial College
Kamuthi, Ramanathapuram, Tamil Nadu, India
[4]CAS in Marine Biology, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India
**Corresponding author : aqua.afi@gmail.com; raokappaphycus@gmail.com*

ABSTRACT

The coastal waters in six districts of Tamil Nadu namely Thiruvallur, Kancheepuram, Villupuram, Cuddalore, Nagapattinam and Kanniyakumari were surveyed during January to May 2014 for identifying suitable sites for seaweed cultivation. Hydrological parameters viz. seawater temperature, salinity, nitrate, phosphate, turbidity and water movement were recorded along with nature of substratum, nature of sea coast and seaweed occurrence. In all these six districts, 75 villages are recommended for seaweed cultivation. Seaweeds like *Kappaphycus alvarezii*, *Gracilaria edulis, G. verrucosa* and *G.dura* are recommended for cultivation. By cultivating *Kappaphycus alvarezii* alone, 1000 people would get benefitted with lucrative income.

Introduction

Seaweeds have been used for food, feed and fodder from time immemorial and they also form a source of phytochemicals like agar, algin and carrageenan (McHugh, 2003). Among the 9000 species reported in the World (Khan *et al.,* 2009), 255 species of seaweeds are utilised commercially - 145 species for food and 110 species for phycocolloid production (agar, algin and carrageenan) (Zemke-White and Ohno, 1999). Seaweeds are cultivated in 47 countries in the world (Zemke-White and Ohno, 1999) and 30 countries have introduced carrageenan farming seaweeds (*Eucheuma, Kappaphycus, Chondrus, Sarcothalia,* and *Griffithsia*) to evaluate their potential biomass production (Neish, 2003). However, warm water *Eucheuma* seaweeds viz. *Kappaphycus alvarezii* and *Eucheuma denticulatum* have been cultivated substantially and commercially (FAO, 2013). *Kappaphycus alvarezii* alone has been introduced in 26 countries (Mandal *et al*., 2010) and 1,83,000 tons (dry) in the world are produced through aquaculture (Bixler and Porse, 2011). In India 4210 tons (wet) of *K. alvarezii* was cultivated till 2010 (Krishnan and Narayanakumar, 2013).

At present only 8 seaweeds are intensively cultivated, namely *Laminaria japonica and Undaria pinnatifida* (brown algae), *Porphyra, Eucheuma, Kappaphycus* and *Gracilaria* (red algae), *Monostroma* and *Enteromorpha* (green algae) to meet the demand both for food and phycocolloid production (Wikfors and Ohno, 2001). According to the available data in 2012, 33 countries and territories worldwide harvested 23.78 million tons (26.297 % of total aquaculture production) of aquatic plants mostly seaweeds from aquaculture, while capture was only 1.1 million tons (4.626 % of the total aquatic plants production) (FAO, 2014). *Kappaphycus* and *Eucheuma* production level also surpassed that of Japanese kelp (*Laminaria japonica*) production in 2010 (FAO, 2014). Major carrageenan seaweed producing countries include Indonesia (60.5%), Philippines (31.9%), Malaysia (3.7%), United Republic of Tanzania (2.3%), China (1.1%) and other countries (0.5%) (FAO, 2013).

In India, 1153 species of seaweeds, including forms and varieties have been reported (Subba Rao and Mantri, 2006) and forms only 12.81% of 9000 species reported in the World (Khan *et al.,* 2009). Of these some seaweeds like *Gelidiella acerosa, Gracilaria edulis,* species of *Sargassum* and *Turbinaria* are used for production of phycocolloids (agar and alginate). As the natural resources of some of these the seaweeds (*Gelidiella acerosa* and *Gracilaria edulis*) are not enough to meet the industrial demand for the production of phycocolloids, cultivation technologies for these seaweeds have been developed. Late Prof. V. Krishnamurthy was the first

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to start *Gracilaria edulis* cultivation in India at Marine Algal Research Station (MARS), Mandapam during his tenure as Discipline Co-Ordinator of Marine Algae Discipline, Central Salt and Marine Chemical Research Institute (CSMCRI), Bhavnagar and this cultivation was done in 1970 in the lagoon waters at the Northern side of Krusadai Island in Gulf of Mannar waters of Bay of Bengal, Southeast coast of India (Krishnamurthy *et al*., 1977). Because of this Late Prof. Krishnamurthy is the Father of Seaweed Cultivation in India. Subsequently Scientists from Central Salt and Marine Chemical Research Institute (CSMCRI), Bhavnagar - a unit of Council of Scientific and Industrial Research (CSIR) and Central Marine Fisheries Research Institute (CMFRI) - a unit of Indian Council of Agriculture Research (ICAR) at its Regional Center in Mandapam Camp have been involved for the development of cultivation technologies for economic seaweeds.

Since 1970, attempts were made to evaluate experimental cultivation of agarophytes *Gelidiella acerosa* (Subbaramaiah *et al*., 1975; Patel *et al*., 1986)*, Gracilaria edulis* (Raju and Thomas, 1971), carrageenophytes *Hypnea valentiae* (Rama Rao *et al*., 1985); *H. musciformis* (Ganesan *et al*., 2006) and alginophyte *Sargassum wightii* (Subba Rao *et al*., 1984) at different locations on southeast coast of India using various culture techniques. Now viable cultivation technologies are available for the agarophytes *Gelidiella acerosa, Gracilaria edulis* and carrageenophyte *Kappaphycus alvarezii. Gelidiella acerosa* yields crop of 4 tones (dry)/ha/yr in two harvests while *Gracilaria edulis* yields 20 tones (dry)/ha/yr in three harvests in long line rope method and 30 tones (dry)/ha/yr in five harvests in SRFT (Single Rope Floating Raft Technique) method (Subba Rao *et al*., 2004). *Kappaphycus alvarezii* gives a crop yield of 40 tones (dry)/ha/yr in eight harvests (Eswaran *et al.,* 2006, Subba Rao and Mantri, 2006). *Kappaphycus alvarezii* was introduced in September 1995 at Thonithurai (Mandapam near Pamban Bridge) in Gulf of Mannar waters of Bay of Bengal, southeast coast of India by Dr. P. V. Subba Rao, the former Scientist in Charge, Marine Algal Research Station (MARS), Mandapam Camp, Tamil Nadu.

Among all the technologies developed *Kappaphycus alvarezii* cultivation technology has been commercialised providing self-employment for thousands of costal fisher folk in Tamil Nadu with earning of Rs.15000 to 16000/-per person per month (Periyasamy *et al.,* 2014). Commercial farming of this seaweed has been going on only in Tamil Nadu *i.e.* along the coastal waters of Ramanathapuram, Pudukkottai, Tanjore and Tuticorin districts.The demand for *Kappaphycus* and other seaweeds has been increasing day by day. Hence there is need to expand the seaweed farming at new locations. International Fund for Agricultural Development - Post Tsunami Sustainable Livelihood Program (IFAD - PTSLP), Chennai sanctioned a project for this purpose to Aquaculture Foundation of India (AFI), Madurai. Under this programme, investigation was carried out by the Scientists of AFI during January 2014 to May 2014 to locate suitable places for seaweed cultivation in coastal waters of Tamil Nadu in Thiruvallur, Kancheepuram, Villupuram, Cuddalore, and Nagapattinam districts in the Palk Bay side of Bay of Bengal and Kanniyakumari district in Gulf of Mannar side of Bay of Bengal, Indian Ocean side and Arabian Sea side. For this purpose data have been collected on the seaweed vegetation, nature of sea coast; nature of sea bottom and seawater parameters viz. surface seawater temperature, salinity and nutrients (nitrate and phosphate**).**

Survey of places in six coastal districts

The present survey was done during January to May 2014 to find out at suitable sites for seaweed farming in the coastal waters of Thiruvallur, Kanchipuram, Villupuram, Cuddalore, Nagapattinam and Kanniyakumari Districts.

Thiruvallur District

Thiruvallur District is the northern end of 13 maritime districts of Tamil Nadu and it is located to the south of Andhra Pradesh. It has a coastline of 27.9 km on the Palk Bay side. The major coastal villages are 29. Majority of the people depend on fishing and some people work in the nearby towns. This district consists of two blocks and 13 coastal panchayats.

In the 29 coastal villages surveyed, seawater temperature varied from 30^0C to 32^0C. The highest temperature (32^0C) was recorded at five sites and lowest temperature (30^0C) at16 sites. The salinity varied from 30‰ to 33‰. The highest salinity (33‰) was recorded at only one place and lowest (30 ‰) at 9 places. The nitrate content varied from 3.13 ± 0.81 µ. mol/ L to 0.91 ± 0.76 µ. mol/ L. The highest nitrate content (3.13 ± 0.81 µ. mol/ L) was recorded in the coastal waters near L & T Units and the lowest (0.91 ± 0.76 µ. mol/ L) in the waters of Kasikoil Kuppam village. The phosphate content varied from 2.61 ± 0.33 µ. mol/ L to 0.95 ± 0.17 µ. mol/ L. The highest phosphate content (2.61 ± 0.33 µ. mol/ L) was registered in the waters of Ernavore Kuppam village and the lowest (0.95 ± 0.17 µ. mol/ L) in the waters of Mugathuvaran Kuppam village.(Table-1). Ideal water motion was recorded at the first 11 sites (Thoniyur to Kavarachi) whereas in all other sites the water motion recorded was more than 25cm/sec. Clear seawater was found in 17 sites (Thoniyur to Kavarachi and Palakai Thitti to Nallathanni Odai Kuppam) and in the other 12 sites it was turbid. Seaweeds were found growing in 24 sites. The sea bottom at most of the sites was sandy and the seacoast was sandy in 12 places and rocky in 17 places.

Kancheepuram District

Kancheepuram District is located to the south of Chennai and north of Villupuram district of Tamil Nadu. It has a

Table-1. Various parameters recorded at different sites in Thiruvallur District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (‰)	Nitrate ($\mu.mol\ L^{-1}$)	Phosphate ($\mu.mol\ L^{-1}$)	Water Motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Thoniyur	30	31	1.14 ± 0.04	1.01 ± 0.71	Moderate	Clear	Sandy	Sandy	Yes
2. Koonakuppam	30	31	1.56 ± 0.03	2.32 ± 0.38	Moderate	Clear	Sandy	Sandy	Yes
3. Sembas Pillai Kuppam	31	30	1.19 ± 0.02	2.01 ± 0.72	Moderate	Clear	Sandy	Sandy	Yes
4. Thirumalai Nagar	32	31	2.11 ± 0.08	1.91 ± 0.33	Moderate	Clear	Sandy	Sandy	Yes
5. Light House	30	31	2.01 ± 0.12	1.86 ± 0.45	Moderate	Clear	Sandy	Sandy	Yes
6. Nadu Kuppam	31	30	2.12 ± 0.04	1.91 ± 0.23	Moderate	Clear	Sandy	Sandy	Yes
7. Arangam	32	30	2.01 ± 0.01	1.88 ± 0.12	Moderate	Clear	Sandy	Sandy	Yes
8. Viravan Kuppam	30	30	1.89 ± 0.91	1.90 ± 0.44	Moderate	Clear	Sandy	Sandy	Yes
9. Kora Kuppam	30	30	1.69 ± 0.19	1.64 ± 0.76	Moderate	Clear	Sandy	Sandy	Yes
10. Kamunkalli	30	30	1.72 ± 0.34	1.82 ± 0.34	Moderate	Clear	Sandy	Sandy	Yes
11. Kavarachi	30	30	1.61 ± 0.23	1.50 ± 0.12	Moderate	Clear	Sandy	Sandy	Yes
12. L & T Units	31	30	3.13 ± 0.81	1.41 ± 0.02	Very High	Turbid	Sandy	Sandy	No
13. Nedun Kuppam	32	32	1.03 ± 0.19	1.61 ± 0.76	Very High	Turbid	Sandy	Rocky	No
14. Thalan Kuppam	30	32	0.98 ± 0.32	1.72 ± 0.59	Very High	Turbid	Sandy	Rocky	No
15. Ennore Kuppam	31	31	0.94 ± 0.12	0.98 ± 0.21	Very High	Turbid	Sandy	Rocky	No
16. Mugathuvaran Kuppam	30	32	1.09 ± 0.35	0.95 ± 0.17	Very High	Turbid	Sandy	Rocky	No
17. Kattu Kuppam	30	32	1.11 ± 0.42	1.29 ± 0.61	Very High	Turbid	Rocky	Rocky	Yes
18. Periya Kuppam	30	31	1.32 ± 0.33	1.02 ± 0.52	Very High	Turbid	Rocky	Rocky	Yes
19. Chinna Kuppam	31	32	1.02 ± 0.72	2.13 ± 0.27	Very High	Turbid	Rocky	Rocky	Yes
20. Ernavore Kuppam	30	32	1.00 ± 0.21	2.61 ± 0.33	Very High	Turbid	Rocky	Rocky	Yes
21. Indhira Gandhi Kuppam	31	33	0.98 ± 0.32	1.89 ± 0.44	Very High	Turbid	Rocky	Rocky	Yes
22. Kasi Koil Kuppam	30	32	0.91 ± 0.76	1.75 ± 0.81	Very High	Turbid	Rocky	Rocky	Yes
23. Kasi Viswanathar Kuppam	30	32	1.21 ± 0.66	1.83± 0.85	Very High	Turbid	Rocky	Rocky	Yes
24. Palakai Thitti	30	32	1.31 ± 0.43	0.99 ± 0.44	Very High	Clear	Rocky	Rocky	Yes
25. Thiruvetriyur	31	32	1.02 ± 0.91	1.45 ± 0.62	Very High	Clear	Rocky	Rocky	Yes
26. Ondi Kuppam	30	32	1.31 ± 0.24	1.59 ± 0.66	Very High	Clear	Rocky	Rocky	Yes
27. Thrisnan Kuppam	32	30	1.02 ± 0.40	1.81 ± 0.23	Very High	Clear	Rocky	Rocky	Yes
28. Power Kuppam	31	31	1.11 ± 0.32	1.56 ± 0.61	Very High	Clear	Rocky	Rocky	Yes
29. Nallathanni Odai Kuppam	32	32	1.06 ± 0.76	1.67 ± 0.54	Very High	Clear	Rocky	Rocky	Yes

coastline of 87.2 km on the Palk Bay side. Total major coastal villages along the coast are 38. The majority of the people's occupation is fishing and agriculture. Some people go for jobs to the nearly places. This district has five blocks and 24 coastal panchayats.

In the 38 coastal villages surveyed, seawater temperature varied from 30^{0}C to 34^{0}C. The highest temperature (34^{0}C) was recorded at Kalpakkam site and lowest (30^{0}C) at 15 sites. The salinity varied from 30‰ to 33‰. The highest salinity (33 ‰) was recorded at Kalpakkam and Memmari Kuppam and lowest (30 ‰) at 9 sites. The nitrate content varied from 1.31 ± 0.42 µ. mol/ L to 0.76 ± 0.11 µ. mol/ L. The highest nitrate content (1.31 ± 0.42 µ. mol/ L) was recorded in waters of Ilanthoppu Kuppam village and the lowest (0.76 ± 0.11 µ. mol/ L) in the waters of Vettiyankani Kuppam village. The phosphate content varied from 2.11 ± 0.34 µ. mol/ L to 0.69 ± 0.11 µ. mol/ L. The highest phosphate content (2.11 ± 0.34 µ. mol/ L) was recorded in the waters of Alambarak Kuppam village and the lowest (0.69 ± 0.11 µ. mol/ L) in the waters of Umarik Kuppam village (Table-2). Ideal water motion was recorded at only seven sites (Kovalam, Semmanseri Kuppam, Pudiya Kalpakkam, Pudupattinam, Uyalli Kuppam, Panniyur Chinna Kuppam and Panniyur Periya Kuppam). Clear seawater was seen at 22 sites. Sea bottom was sandy in most of the places and it was rocky in other places. The sea coast was sandy in 26 places and rocky in 6 places.

Villupuram District

Villupuram District is located to the south of Kancheepuram District and north of Cuddalore District of Tamil Nadu coast. It has a coastline of 40.7 km on the Palk Bay side. Total major coastal villages 20. Majority of the people are involved in fishing and agriculture. Some people go for other jobs. There are two blocks and 8 coastal panchayats in Villupuram District.

Seawater temperature varied from 30^{0}C to 32^{0}C. The highest temperature (32^{0}C) was recorded at 6 sites and lowest temperature (30^{0}C) at 8 sites. The salinity varied from 30‰

Table-2. Various parameters recorded at different sites in Kancheepuram District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (‰)	Nitrate (µ.mol L^{-1})	Phosphate (µ.mol L^{-1})	Watern Motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Kottivakkam	30	32	0.95 ± 0.18	1.32 ± 0.60	Very High	Turbid	Rocky	Rocky	Yes
2. Palavakkam	30	32	1.21 ± 0.77	0.95 ± 0.33	Very High	Turbid	Rocky	Rocky	Yes
3. Siru Neelangarai	30	32	1.09 ± 0.28	0.89 ± 0.14	Very High	Turbid	Rocky	Rocky	Yes
4. Peru Neelangarai	31	32	0.85 ± 0.42	1.00 ± 0.09	Very High	Turbid	Rocky	Rocky	Yes
5. Vettiyankani Kuppam	30	32	0.76 ± 0.11	1.11 ± 0.31	Very High	Turbid	Rocky	Rocky	Yes
6. Enjan Kuppam	31	31	0.79 ± 0.21	1.02 ± 0.16	Very High	Turbid	Rocky	Rocky	Yes
7. Panniyur Kuppam	32	31	0.94 ± 0.17	0.99 ± 0.24	Very High	Turbid	Sandy	Sandy	Yes
8. Nainar Kuppam	31	31	0.99 ± 0.42	0.89 ± 0.09	Very High	Turbid	Sandy	Sandy	Yes
9. Retti Kuppam/ Kanathur	30	31	0.85 ± 0.45	0.69 ± 0.19	Very High	Turbid	Sandy	Sandy	Yes
10. Karikattu Kuppam	30	31	0.81 ± 0.13	0.79 ± 0.42	Very High	Clear	Sandy	Sandy	Yes
11. Kovalam	31	32	0.88 ± 0.11	1.02 ± 0.53	Moderate	Clear	Sandy	Sandy	Yes
12. Semmanseri Kuppam	30	32	0.92 ± 0.41	1.11 ± 0.21	Moderate	Clear	Sandy	Sandy	Yes
13. Pudiya kalpakkam	32	31	0.95 ± 0.32	1.23 ± 0.19	Moderate	Clear	Sandy	Sandy	Yes
14. Memmeri Kuppam	32	33	0.99 ± 0.21	1.02 ± 0.08	Very High	Clear	Sandy	Sandy	Yes
15. Kattu Kuppam	30	31	0.89 ± 0.11	1.51 ± 0.22	Very High	Clear	Sandy	Sandy	Yes
16. Pattipulam Kuppam	30	32	1.02 ± 0.29	1.03 ± 0.08	Very High	Clear	Sandy	Sandy	Yes
17. Ilanthoppu Kuppam	31	32	1.31 ± 0.42	1.15 ± 0.28	Very High	Clear	Sandy	Sandy	Yes
18. Salan Kuppam	31	32	1.00 ± 0.15	1.00 ± 0.10	Very High	Clear	Sandy	Sandy	Yes
19. Deveneri Kuppam	31	31	0.98 ± 0.31	0.99 ± 0.14	Very High	Clear	Rocky	Sandy	Yes
20. Mahapallipuram	31	31	1.03 ± 0.54	0.89 ± 0.04	Very High	Turbid	Rocky	Sandy	Yes
21. Vempurusam Kuppam	33	31	1.11 ± 0.21	0.78 ± 0.15	Very High	Turbid	Rocky	Sandy	No
22. Kottilmedu Kuppam	33	31	0.95 ± 0.22	0.91 ± 0.25	Very High	Turbid	Rocky	Sandy	No
23. Kalpakkam	34	33	0.85 ± 0.31	0.77 ± 0.08	Very High	Turbid	Rocky	Sandy	No
24. Umarik Kuppam	32	32	0.99 ± 0.34	0.69 ± 0.11	Very High	Turbid	Rocky	Sandy	No
25. Sattarasu	32	32	1.02 ± 0.43	0.98 ± 0.34	Very High	Turbid	Rocky	Sandy	No
26. Pudupattinam	32	31	1.19 ± 0.22	0.99 ± 0.23	Moderate	Clear	Rocky	Sandy	No
27. Uyalli Kuppam	31	31	0.91 ± 0.83	0.91 ± 0.44	Moderate	Clear	Sandy	Sandy	No
28. Angalak Kuppam	31	30	0.98 ± 0.21	0.78 ± 0.38	Very High	Clear	Sandy	Sandy	No
29. Paliyanadu Kuppam	31	30	0.99 ± 0.32	1.02 ± 0.43	Very High	Clear	Sandy	Sandy	No
30. Pudunadu Kuppam	31	30	1.02 ± 0.24	1.36 ± 0.16	Very High	Clear	Sandy	Sandy	No
31. Velampur	31	30	1.18 ± 0.22	0.89 ± 0.09	Very High	Clear	Sandy	Sandy	No
32. Perunthuravu	31	30	1.07 ± 0.17	0.90 ± 0.32	Very High	Clear	Sandy	Sandy	Yes
33. Paramangani Kuppam	30	30	0.98 ± 0.11	1.00 ± 0.41	Very High	Clear	Sandy	Sandy	Yes
34. Thalaithali Kuppam	30	30	0.93 ± 0.09	1.05 ± 0.13	Very High	Clear	Sandy	Sandy	Yes
35.Chinna Kuppam	30	31	1.06 ± 0.01	0.95 ± 0.14	Very High	Clear	Sandy	Sandy	Yes
36. Panniyur Periya kuppam	30	30	0.99 ± 0.15	1.20 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
37. Kadappakkam	30	30	0.94 ± 0.21	1.31 ± 0.52	Moderate	Clear	Sandy	Sandy	Yes
38. Alambarak Kuppam	30	32	1.02 ± 0.31	2.11 ± 0.34	Very High	Turbid	Sandy	Sandy	Yes

to 33‰. The highest salinity (33 ‰) was recorded at only one site (Kaipeni Kuppam) and lowest salinity (30 ‰) at 5 sites. The nitrate content varied from 1.32 ± 0.34 µ. mol/ L to 0.74 ± 0.17 µ. mol/ L. The highest nitrate content (1.32 ± 0.34 µ. mol/ L) was recorded in waters of Pudu Kuppam village and the lowest value (0.74 ± 0.17 µ. mol/ L) in waters of Pommiyar Palayam Kuppam. The phosphate content varied from 1.61 ± 0.31 µ. mol/ L to 0.71 ± 0.09 µ. mol/ L. The highest phosphate content (1.61 ± 0.31 µ. mol/ L) was recorded in the waters of Muttukadu/ Thalanguda village and the lowest content (0.71 ± 0.09 µ. mol/ L) in the waters of Manja Kuppam village (Table-3). Ideal water motion was recorded at only two sites namely Muttukadu/ Thalanguda and Pudukuppam. Clear seawater was recorded at only one site ie. Muttukadu/Thalanguda. The sea bottom is sandy in all the sites and there is possibility of deposition of silt on the seaweeds to be cultured and tempering of their growth.

Cuddalore District

Cuddalore District is also one among the 13 maritime districts of Tamil Nadu. It is located to south of Villupuram District and north of Nagapattinam District. It has 57.50 km long coastline on the Palk Bay side. Total major coastal villages are 31. Majority of the people are involved in fishing activities and agriculture works. This district has four blocks and 26 coastal

Table-3. Various parameters recorded at different sites in Villupuram District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (ppt)	Nitrate (µ.mol L)	Phosphate (µ.mol L)	Water Motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Muttukadu/ Thalanguda	30	32	0.95 ± 0.14	1.61 ± 0.31	Moderate	Clear	Sandy	Sandy	No
2. Alagan Kuppam	30	32	1.02 ± 0.31	0.98 ± 0.13	Very high	Turbid	Sandy	Sandy	No
3. Vasan Kuppam	30	32	1.31 ± 0.09	0.99 ± 0.42	Very high	Turbid	Sandy	Sandy	No
4. Kaipeni Kuppam	31	33	1.15 ± 0.06	0.81 ± 0.32	Very high	Turbid	Sandy	Sandy	No
5. Ekkiyar Kuppam	32	31	1.11 ± 0.21	0.88 ± 0.23	Very high	Turbid	Sandy	Sandy	No
6. Pudu Kuppam	30	31	1.32 ± 0.34	0.99 ± 0.08	Very high	Turbid	Sandy	Sandy	No
7. Komunjavadi Kuppam	30	31	0.95 ± 0.12	1.21 ± 0.07	Very high	Turbid	Sandy	Sandy	No
8. Anumanthai Kuppam	32	31	0.99 ± 0.31	1.10 ± 0.18	Very high	Turbid	Sandy	Sandy	No
9. Chetti Nagar Kuppam	32	32	1.09 ± 0.24	0.81 ± 0.13	Very high	Turbid	Sandy	Sandy	No
10. Notchi Kuppam	32	32	1.16 ± 0.33	0.77 ± 0.05	Very high	Turbid	Sandy	Sandy	No
11. Manja Kuppam	32	32	1.29 ± 0.41	0.71 ± 0.09	Very high	Turbid	Sandy	Sandy	No
12. Koonimedu Kuppam	32	32	1.00 ± 0.08	0.91 ± 0.03	Very high	Turbid	Sandy	Sandy	No
13. Mudaliyar Kuppam	31	30	0.99 ± 0.18	1.11 ± 0.18	Very high	Turbid	Sandy	Sandy	No
14. Anucha Kuppam	31	30	0.94 ± 0.09	1.09 ± 0.08	Very high	Turbid	Sandy	Sandy	No
15. Pudu Kuppam	31	30	0.91 ± 0.21	1.15 ± 0.12	Moderate	Turbid	Sandy	Sandy	No
16. Pillai Chavadi Kuppam	31	30	0.85 ± 0.33	1.41 ± 0.19	Very high	Turbid	Sandy	Sandy	No
17. Pommiyar Palayam Kuppam	31	30	0.74 ± 0.17	1.21 ± 0.28	Very high	Turbid	Sandy	Sandy	No
18. Mudaliyar Chavadi	30	31	0.77 ± 0.21	1.15 ± 0.31	Very high	Turbid	Sandy	Sandy	No
19. Thanthiran Kuppam	30	31	0.89 ± 0.43	1.21 ± 0.12	Very high	Turbid	Sandy	Sandy	No
20. Nadu Kuppam	30	31	0.88 ± 0.16	1.09 ± 0.06	Very high	Turbid	Sandy	Sandy	No

panchayats.

The seawater temperature varied from 30°C to 32°C. The highest temperature (32°C) was recorded at 14 sites and lowest temperature (30°C) at 7 sites. The seawater salinity varied from 30‰ to 32‰. The highest salinity (32‰) was recorded at 18 sites and lowest (30 ‰) at 2 sites (MGR Thittu and Mulli Thurai). The nitrate content varied from 2.31 ± 0.09µ. mol/ L to 0.87 ± 0.09 µ. mol/ L. The highest nitrate content (2.31 ± 0.09 µ. mol/ L) was recorded in waters of Thalanguda village and the lowest (0.87 ± 0.09 µ. mol/ L) in waters of Kumarap Pettai village. The phosphate content varied from 2.22 ± 0.09 µ. mol/ L to 0.79 ± 0.06 µ. mol/ L. The highest phosphate content (2.22 ± 0.09 µ. mol/ L) was recorded in the waters of Mudasal Odai village and the lowest (0.79 ± 0.06 µ. mol/ L) in the waters of Velicom Pettai village (Table-4). Ideal water motion was recorded at 23 sites. Among these 23 sites, water motion was very ideal for seaweed cultivation at the first 9 sites. Clear seawater was recorded at 25 sites. Three seaweeds were recorded during the survey. Sea bottom was sandy with intermittent rocks.

Nagapattinam District

Nagapattinam District is located to south of Cuddalore District and north of Thiruvallur District of Tamil Nadu coast. It has a long coast line of 124.90 km on the Palk Bay side. Total major coastal villages are 47. Many people depend on fishing and South Nagai people depend on agriculture.There are 7 blocks and 29 coastal panchayats in this district.

The seawater temperature varied from 30°C to 32°C. The highest temperature (32°C) was recorded at 23 sites and lowest (30°C) at 4 sites. The salinity varied from 30‰ to 32‰. The highest salinity (32‰) was recorded at 30 sites and lowest (30 ‰) at 3 sites. The nitrate content varied from 2.36 ± 0.20 µ. mol/ L to 0.54 ± 0.04 µ. mol/ L. The highest nitrate content (2.36 ± 0.20 µ. mol/ L) was recorded in the waters of Chinna Kottiyamedu village and the lowest (0.54 ± 0.04 µ. mol/ L) in the waters of Pusbavanam village. The phosphate content varied from 3.22 ± 0.34 µ. mol/ L to 0.37 ± 0.09 µ. mol/ L. The highest phosphate content (3.22 ± 0.34 µ. mol/ L) was recorded in the waters of Perumal Pettai village and the lowest (0.37 ± 0.09 µ. mol/ L) in the waters of Kottiyamedu village (Table-5). Ideal water motion was recorded at only four sites (Mahendrapalli, Kottur, Maniyanthur and Kodiyakkarai) whereas at all other sites the water motion recorded was more than 50cm/sec except at Thirumullaivasal where it was 30cm/ sec. Clear seawater was recorded at all 47 sites. Three species of seaweeds were recorded during the survey. Sea bottom and sea coast were sandy at many sites.

Kanniyakumari District

Kanniyakumari District is the southern end of Tamil Nadu coast. It is located to the south of Thirunelvelli district and also to the south of Kerala. It is coastline is 11.5 km long in the Gulf of Mannar side and 60 km in the south and west coast

Table-4. Various parameters recorded at different sites in Cuddalore District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (‰)	Nitrate (μ.mol L^{-1})	Phosphate (μ.mol L^{-1})	Water motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Thalanguda	32	31	2.31 ± 0.09	1.29 ± 0.18	Moderate	Clear	Sandy	Sandy	Yes
2. Devanampattinam	32	31	2.11 ± 0.42	1.11 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
3. Sonank Kuppam	32	31	2.17 ± 0.13	1.51 ± 0.03	Moderate	Clear	Sandy	Sandy	Yes
4. Singara Thoppu	31	31	1.98 ± 0.09	1.12 ± 0.23	Moderate	Clear	Sandy	Sandy	Yes
5. Kori Akkarai	31	32	1.11 ± 0.12	1.34 ± 0.17	Moderate	Clear	Sandy	Sandy	Yes
6. Thaikkal	31	32	1.00 ± 0.31	1.27 ± 0.18	Moderate	Turbid	Sandy	Sandy	Yes
7. Sothi Kuppam	32	32	1.19 ± 0.16	1.39 ± 0.11	Moderate	Turbid	Rocky	Rocky/ Sandy	Yes
8. Raja Pettai	32	32	1.11 ± 0.19	0.95 ± 0.09	Moderate	Turbid	Sandy	Sandy	Yes
9. Chitra Pettai	32	32	1.09 ± 0.21	0.89 ± 0.04	Moderate	Turbid	Sandy	Sandy	Yes
10. Velicom Pettai	32	32	1.12 ± 0.08	0.79 ± 0.06	Heavy	Turbid	Sandy	Sandy	Yes
11. Damannam Pettai	32	32	1.32 ± 0.10	0.98 ± 0.09	Heavy	Turbid	Sandy	Sandy	Yes
12. Nakkam Pettai	32	32	1.33 ± 0.08	0.87 ± 0.12	Heavy	Clear	Sandy	Sandy	Yes
13. Periya Kuppam	32	32	1.00 ± 0.13	0.91 ± 0.08	Heavy	Clear	Sandy	Sandy	Yes
14. Ayyam Pettai	32	32	0.99 ± 0.18	0.97 ± 0.13	Heavy	Clear	Sandy	Sandy	Yes
15. Rettiyar Pettai	31	32	0.91 ± 0.11	0.94 ± 0.19	Moderate	Clear	Sandy	Sandy	Yes
16. Kumarap Pettai	31	31	0.87 ± 0.09	0.94 ± 0.10	Moderate	Clear	Sandy	Sandy	Yes
17. Indhra Nagar	31	31	1.11 ± 0.18	1.02 ± 0.13	Moderate	Clear	Sandy	Sandy	Yes
18. Madava Pallam	31	31	1.25 ± 0.24	1.00 ± 0.08	Moderate	Clear	Sandy	Sandy	Yes
19. Annappan Pettai	31	31	0.98 ± 0.30	1.11 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
20. Samiyar Pettai/	31	31	0.99 ± 0.14	1.22 ± 0.33	Moderate	Clear	Sandy	Sandy	Yes
21. Pudu Kuppam	31	31	0.87 ± 0.10	1.13 ± 0.08	Moderate	Clear	Sandy	Sandy	Yes
22. Pudu Pettai	30	32	0.93 ± 0.19	1.41 ± 0.07	Moderate	Clear	Sandy	Sandy	Yes
23. Chinnur	30	32	1.07 ± 0.31	0.99 ± 0.13	Moderate	Clear	Sandy	Sandy	Yes
24. Anna Koil	30	32	0.97 ± 0.22	1.32 ± 0.17	Heavy	Clear	Sandy	Sandy	Yes
25. Parangipettai	30	32	1.49 ± 0.09	2.13 ± 0.29	Heavy	Clear	Sandy	Sandy	Yes
26. Mudasal Odai	30	32	1.52 ± 0.17	2.22 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
27. MGR Thittu	30	30	1.39 ± 0.22	1.75 ± 0.11	Moderate	Clear	Sandy	Sandy	Yes
28. Mulli Thurai	30	30	1.51 ± 0.34	1.47 ± 0.33	Moderate	Clear	Sandy	Sandy	Yes
29. Chinna Vaikkal	32	32	1.33 ± 0.18	1.65 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
30. Pillmedu/ TS Pettai	32	32	1.54 ± 0.12	1.29 ± 0.42	Moderate	Clear	Sandy	Sandy	Yes
31. Kodiyam Palayam	32	32	1.21 ± 0.31	1.45 ± 0.08	Heavy	Clear	Sandy	Sandy	Yes

(Indian Ocean side and Arabian Sea side) with a total distance of 71.5 km. Total major coastal villages on the coast are 47. Majority of the people depend on fishing and agriculture as well. This district is having five blocks and 13 coastal panchayats.

The seawater temperature varied from 32^{0}C to 34^{0}C. The highest temperature (34^{0}C) was recorded at Enayam site and lowest (32^{0}C) at more than 60% (28 sites) of the total sites. The salinity varied from 31‰ to 33‰. The highest salinity (33‰) was recorded at 21 sites with lowest (31 ‰) at 2 sites (Kurum Panai and Kela Midalan). The nitrate content varied from 1.22 ± 0.03 µ. mol/ L to 0.39 ± 0.10 µ. mol/ L. The highest nitrate content (1.22 ± 0.42 µ. mol/ L) was recorded in the waters of Kottilpadu village and the lowest (0.39 ± 0.10 µ. mol/ L) in the waters of Leepuram village. The phosphate content varied from 2.22 ± 0.09 µ. mol/ L to 0.69 ± 0.19 µ. mol/ L. The highest phosphate content (2.22 ± 0.09 µ. mol/ L) was recorded in the waters of Thengai Pattinam village and the lowest (0.69 ± 0.19 µ. mol/ L) in the waters of Leepuram village (Table-6). The water motion recorded was more than 25cm/ sec at all sites. The seawater was turbid in all the 47 sites surveyed.

Summary

In the coastal waters of six districts, the seaweed occurrence in the natural habitat and washed away to shore was abundent except in Villupuram district where the availability of the seaweed is very less in some places and nil in many places . Chlorophyceae members (green algae) are very common in all the coastal waters. But in Kanniyakumari District Chlorophyceae, Phaeophyceae and Rhodophyceae members are also rich and seaweed collectors are collecting the seaweeds for industrial usage Except in Kanniyakumari, in all other five districts the seaweed diversity is very less. In overall the sea bottom was either sandy or rocky that favorus seaweed cultivation. The shores were sandy interspersed with rocks at most of the places.

Among these six coastal districts, the overall seawater temperature varied from 30^{0}C to 34^{0}C. For good growth of seaweed the optimum temperature would be from

Table-5. Various parameters recorded at different sites in Nagapattinam District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (‰)	Nitrate (μ.mol L^{-1})	Phosphate (μ.mol L^{-1})	Water motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Kodiyampalayam	30	32	1.09 ± 0.15	2.36 ± 0.50	Moderate	Clear	Sandy	Sandy	Yes
2. Mahendrapalli	30	32	1.11 ± 0.12	1.54 ± 0.12	Moderate	Clear	Sandy	Sandy	Yes
3. Kottur	30	32	0.98 ± 0.09	0.46 ± 0.07	Moderate	Clear	Sandy	Sandy	Yes
4. Palayaru	31	32	1.30 ± 0.13	0.59 ± 0.06	Moderate	Clear	Rocky	Sandy	Yes
5. Madava Medu	31	32	1.36 ± 0.23	0.72 ± 0.03	Moderate	Clear	Rocky	Sandy	Yes
6. Kottiyamedu	31	32	1.46 ± 0.05	0.37 ± 0.09	Moderate	Clear	Sandy	Sandy	Yes
7. Chinna Kottiyamedu	31	32	2.36 ± 0.20	0.42 ± 0.04	Moderate	Clear	Sandy	Sandy	Yes
8. Kooliyaru	31	32	2.04 ± 0.08	0.48 ± 0.06	Moderate	Clear	Sandy	Sandy	Yes
9. Thoduva	31	32	0.94 ± 0.22	1.47 ± 0.06	Moderate	Clear	Sandy	Sandy	Yes
10. Thirumullaivasal	31	32	1.02 ± 0.28	3.15 ± 0.04	Moderate	Clear	Sandy	Sandy	Yes
11. Kela Mookkarai	31	31	1.00 ± 0.16	2.78 ± 0.05	Moderate	Clear	Sandy	Sandy	Yes
12. Mela Mookkarai	31	32	1.18 ± 0.08	2.58 ± 0.03	Heavy	Clear	Sandy	Sandy	Yes
13. Chavadi Kuppam	31	32	1.35 ± 0.10	1.41 ± 1.05	Heavy	Clear	Sandy	Sandy	Yes
14. Chinna Aundipatty	32	31	0.94 ± 0.06	1.90 ± 0.01	Heavy	Clear	Sandy	Sandy	Yes
15. Periya Aundipatty	32	32	1.46 ± 0.12	0.94 ± 0.06	Heavy	Clear	Sandy	Sandy	Yes
16. Pudu Kuppam	32	32	1.14 ± 0.06	0.84 ± 0.07	Heavy	Clear	Sandy	Rocky	Yes
17. Pumbukar	32	32	1.09 ± 0.08	0.75 ± 0.05	Heavy	Clear	Sandy	Rocky	Yes
18. Vanagiri	32	32	1.31 ± 0.02	0.57 ± 0.04	Heavy	Clear	Sandy	Rocky	Yes
19. Chinnamedu	32	32	2.14 ± 0.06	0.45 ± 0.06	Heavy	Clear	Sandy	Rocky	Yes
20. Chinna Kudi	32	30	1.59 ± 0.06	0.41 ± 0.04	Heavy	Clear	Sandy	Rocky	Yes
21. Thalampettai	32	30	0.72 ± 0.05	0.48 ± 0.04	Heavy	Clear	Sandy	Rocky	Yes
22. Pudupettai	32	30	0.81 ± 0.02	1.21 ± 0.13	Heavy	Clear	Rocky	Rocky	Yes
23. Perumal pettai	32	32	0.77 ± 0.06	3.22 ± 0.34	Heavy	Clear	Rocky	Rocky	Yes
24. Vellakkoil	32	32	1.27 ± 0.08	2.95 ± 0.25	Heavy	Clear	Rocky	Rocky	Yes
25. Kuttiyadurai	32	32	0.88 ± 0.05	2.28 ± 0.25	Heavy	Clear	Rocky	Rocky	Yes
26. Tharangampadi	32	31	1.01 ± 0.14	1.33 ± 1.00	Heavy	Clear	Rocky	Rocky	Yes
27. Chandrapadi	32	31	1.28 ± 0.09	2.04 ±0.08	Heavy	Clear	Rocky	Rocky	Yes
28. Vanjur	32	32	1.04 ± 0.08	1.05 ± 0.06	Heavy	Clear	Rocky	Rocky	Yes
29. Nagore	31	32	0.94 ± 0.06	0.91 ± 0.02	Heavy	Clear	Rocky	Rocky	Yes
30. Samunthapettai	32	32	0.91 ± 0.02	0.73 ± 0.02	Heavy	Clear	Rocky	Sandy	Yes
31. Nambiyar Nagar	31	31	1.49 ± 0.03	0.62 ± 0.04	Heavy	Clear	Rocky	Sandy	Yes
32. Annattu Theru	31	32	1.77 ± 0.08	0.58 ± 0.04	Heavy	Clear	Rocky	Sandy	Yes
33. Nagai	32	32	0.93 ± 0.05	0.51 ± 0.03	Heavy	Clear	Rocky	Rocky	Yes
34. Ketchakuppam	32	32	0.95 ± 0.05	0.72 ± 0.06	Heavy	Clear	Rocky	Rocky	Yes
35. Akkarapettai	32	31	0.83 ± 0.06	1.40 ± 0.13	Heavy	Clear	Rocky	Rocky	Yes
36. Velankanni	32	31	1.48 ± 0.05	2.95 ± 0.09	Heavy	Clear	Rocky	Rocky	Yes
37. Seruthur	32	31	1.05 ± 0.07	2.98 ± 0.18	Heavy	Clear	Rocky	Rocky	Yes
38. Kameshwaram	32	31	1.28 ± 0.05	2.25 ± 0.20	Heavy	Clear	Sandy	Rocky	Yes
39. Vadakadu	32	31	1.10 ± 0.01	1.39 ± 0.93	Heavy	Clear	Sandy	Sandy	Yes
40. Villunthamavadi	31	31	1.22 ± 0.03	1.72 ± 0.59	Heavy	Clear	Sandy	Sandy	Yes
41. Vanavar Mahadevi	31	31	0.70 ± 0.04	0.98 ± 0.21	Heavy	Clear	Sandy	Rocky	Yes
42. Vellaipallam	31	31	0.57 ± 0.09	0.95 ± 0.17	Heavy	Clear	Sandy	Sandy	Yes
43. Pusbavanam	31	31	0.54 ± 0.04	1.29 ± 0.61	Heavy	Clear	Sandy	Sandy	Ye
44. Akkara Pallivasal	31	32	0.79 ± 0.05	1.02 ± 0.52	Moderate	Clear	Sandy	Sandy	Yes
45. Arkattuthurai	31	32	0.85 ± 0.03	2.13 ± 0.27	Moderate	Clear	Sandy	Sandy	Yes
46. Maniyanthur	31	32	1.12 ± 0.03	2.10 ± 0.71	Moderate	Clear	Sandy	Sandy	Yes
47. Kodiyakkarai	30	32	1.00 ± 0.12	1.59 ± 0.21	Moderate	Clear	Sandy	Sandy	Yes

27°C to 34°C and most of the seaweeds survive from 25°C to 35°C. The seawater temperature may increase one or two degrees during the summer. Even then the temperature is ideal for the seaweed cultivation at all places of the six coastal districts. The overall salinity varied from 30‰ to 33‰. For good growth of seaweed the optimum salinity would be from 27‰ to 33‰ and the seaweed can survive in the salinity ranging from 25‰ to 35‰. The salinity may decrease during the monsoon period but there will not be much variation. So the salinity is ideal for the seaweed cultivation. The nitrate content varied

Table-6. Various parameters recorded at different sites in Kanniyakumari District

Name of the coastal villages	Seawater Temp. (0°C)	Salinity (‰)	Nitrate (µ.mol L^{-1})	Phosphate (µ.mol L^{-1})	Water motion	Seawater clarity	Nature of sea bottom	Nature of sea coast	Occurrence of seaweeds
1. Rasthakadu	32	33	0.91 ± 0.02	1.01 ± 0.09	Heavy	Turbidity	Sandy & Rocky	Sandy lagoon	Yes
2. Vattakottai	32	33	0.57 ± 0.03	0.99 ± 0.24	Heavy	Turbidity	Rocky	Sandy	Yes
3. Arokkiapuram	32	33	0.59 ± 0.04	0.89 ± 0.09	Heavy	Turbidity	Sandy & Muddy	Stone wall & Sandy	Yes
4. Leepuram	32	32	0.39 ± 0.10	0.69 ± 0.19	Heavy	Turbidity	Sandy & Rocky	Stone wall	Yes
5. Mai Gnanapuram	32	33	0.58 ± 0.06	0.79 ± 0.42	Heavy	Turbidity	Sandy & Rocky	Sandy	Yes
6. Chinna Muttam	32	33	0.72 ± 0.07	1.02 ± 0.53	Heavy	Turbidity	Sandy & Muddy	Stone wall	Yes
7. Kanniyakumari	32	32	0.52 ± 0.03	1.50 ± 0.12	Heavy	Turbidity	Sandy & Rocky	Stone wall & Sandy	Yes
8. Kovalam	32	33	0.41 ± 0.12	1.41 ± 0.02	Heavy	Turbidity	Sandy	Sandy & Concrete wall	Yes
9. Kela Manakudi	32	32	0.49 ± 0.09	1.61 ± 0.76	Heavy	Turbidity	Sandy	Stone wall & Sandy	Yes
10.Mela Manakudi	32	33	0.42 ± 0.06	1.72 ± 0.59	Heavy	Turbidity	Sandy	Sandy	Yes
11. Pallam	32	33	0.95 ± 0.05	0.98 ± 0.21	Heavy	Turbidity	Sandy	Sandy	Yes
12. Puthen Thurai	32	33	0.86 ± 0.06	0.95 ± 0.17	Heavy	Turbidity	Sandy & Rocky	Stone Wall	Yes
13. Kasavan Puthen Thurai	32	32	0.86 ±0.06	1.29 ± 0.61	Heavy	Turbidity	Sandy & Rocky	Sandy	Yes
14. Polikarai	32	32	0.69 ± 0.03	1.02 ± 0.52	Heavy	Turbidity	Sandy	Sandy	No
15. Periyakadu	33	32	0.49 ± 0.08	1.09 ± 0.08	Heavy	Turbidity	Sandy	Stone Wall	Yes
16. Rajakamangalam	33	32	0.56 ± 0.08	1.15 ± 0.12	Heavy	Turbidity	Sandy	Sandy	No
17. Azhikkal	33	32	0.73 ± 0.08	1.41 ± 0.19	Heavy	Turbidity	Sandy	Sandy	No
18. Pilla Thoppu	33	32	0.42 ± 0.02	1.21 ± 0.28	Heavy	Turbidity	Sandy	Sandy	No
19. Mela Thurai	33	32	0.43 ± 0.04	1.15 ± 0.31	Heavy	Turbidity	Sandy	Sandy	No
20.Muttam	33	33	0.48 ± 0.06	1.21 ± 0.12	Heavy	Turbidity	Clay, Sandy & Rocky	Rocky	Yes
21. Kadia pattinam	32	33	0.73 ± 0.08	0.95 ± 0.09	Heavy	Turbidity	Sandy & Rocky	Rocky	Yes
22. Chinna Valai	33	32	0.47 ± 0.06	0.89 ± 0.04	Heavy	Turbidity	Sandy	Stone Wall	Yes
23. Periya Valai	33	32	1.05 ± 0.07	0.79 ± 0.06	Heavy	Turbidity	Sandy	Stone Wall	Yes
24. Puthoor	33	32	1.08 ± 0.07	0.98 ± 0.09	Heavy	Turbidity	Sandy	Stone Wall	Yes
25. Kottilpadu	33	32	1.22 ± 0.03	0.87 ± 0.12	Heavy	Turbidity	Sandy	Stone Wall	Yes
26. Colachal	32	33	0.70 ± 0.04	0.91 ± 0.08	Heavy	Turbidity	Sandy & Rocky	Sandy & Rocky	Yes
27. Kodi Munai	33	32	0.57 ± 0.09	0.97 ± 0.13	Heavy	Turbidity	Sandy	Sandy	No
28. Vaniyak Kudi	33	32	0.54 ± 0.04	0.94 ± 0.19	Heavy	Turbidity	Sandy	Sandy	Yes
29. Kurum Panai	32	31	0.79 ± 0.05	0.94 ± 0.10	Heavy	Turbidity	Sandy	Sandy	Yes
30. Kela Midalan	32	31	0.85 ± 0.03	1.02 ± 0.13	Heavy	Turbidity	Sandy	Sandy	Yes
31. Nadu Thurai	32	33	0.55 ± 0.06	1.00 ± 0.08	Heavy	Turbidity	Sandy	Concrete Wall	No
32. Chinna Thurai II	32	32	0.47 ± 0.05	1.11 ± 0.09	Heavy	Turbidity	Sandy	Sandy	No
33. Mela Midalam	32	32	0.65 ± 0.05	1.22 ± 0.33	Heavy	Turbidity	Sandy	Stone wall & Sandy	Yes
34. Enayam	34	33	0.61 ± 0.13	1.13 ± 0.08	Heavy	Turbidity	Sandy	Sandy	Yes
35. E. Chinna Thurai	32	33	1.20 ± 0.02	1.41 ± 0.07	Heavy	Turbidity	Sandy & Rocky	Concrete Wall	Yes
36. E. Puthen Thurai	32	33	1.11 ± 0.02	0.99 ± 0.13	Heavy	Turbidity	Sandy & Rocky	Concrete Wall	Yes
37. Raman Thurai	33	32	0.98 ± 0.04	1.32 ± 0.17	Heavy	Turbidity	Sandy	Stone & Concrete Wall	Yes
38. Mullur Thurai	33	32	0.93 ± 0.11	2.13 ± 0.29	Heavy	Turbidity	Sandy	Stone & Concrete Wall	Yes
39. Thengai Pattinam	33	32	0.89 ± 0.20	2.22 ± 0.09	Heavy	Turbidity	Sandy	Stone Wall	Yes
40. Eramanan Thurai	32	33	0.88 ± 0.07	1.32 ± 0.34	Heavy	Turbidity	Sandy & Rocky	Stone Wall	Yes
41. Poo Thurai	32	33	0.91 ± 0.11	0.95 ± 0.12	Heavy	Turbidity	Sandy & Rocky	Stone Wall	Yes
42. Thoothur	32	33	0.85 ± 0.04	0.99 ± 0.31	Heavy	Turbidity	Sandy & Rocky	Stone Wall	Yes
43. Chinna Thurai I	32	33	1.00 ± 0.08	1.09 ± 0.24	Heavy	Turbidity	Sandy & Rocky	Stone Wall	Yes
44. Eraivi Puthen Thurai	32	33	1.06 ± 0.02	1.16 ± 0.33	Heavy	Turbidity	Sandy & Rocky	Sandy & Rocky	Yes
45. Vallavilai	33	32	1.01 ± 0.09	1.29 ± 0.41	Heavy	Turbidity	Sandy	Sandy	No
46. Marthandan Thurai	33	32	0.99 ± 0.04	1.00 ± 0.08	Heavy	Turbidity	Sandy	Sandy	No
47. Nirodi	33	32	1.01 ± 0.05	0.99 ± 0.18	Heavy	Turbidity	Sandy	Sandy	No

from 3.13 ± 0.81 µ. mol/ L to 0.39 ± 0.10 µ. mol/ L.. The phosphate content varied from 3.22 ± 0.34 µ. mol/ L to 0.37 ± 0.09 µ. mol/ L. Nitrate and phosphate contents are sufficient for good growth of seaweeds. The environmental parameters were recorded during the present survey. Nutrient variation may happen during northeast monsoon, which could be positive sign for seaweed cultivation. Ideal water motion for seaweed cultivation using the existing bamboo raft/ monoline method is less than 25cm/ sec. If the water motion is more than 25cm/ sec, it would be difficult to cultivate the seaweed with the existing

technique. Among these six coastal districts, the overall water motion recorded was more than 25cm/ sec at most of the sites. At a few sites the water motion was less than 25cm/ sec. The water motion may increase during the monsoon period which should be the real challenge for seaweed cultivation in all the six coastal districts. Turbidity was recorded at a few sites and at most of the sites the seawater was very clear. There will not be any possibility of much variation in turbidity in all other months. So, the seawater turbidity will not be a problem for seaweed cultivation in all the six coastal districts. With the prevailing environmental conditions and nature of habitat, seaweed cultivation could be carried out using tubular and net bag monoline methods instead of raft and monoline methods (Periyasamy *et al*., 2017a).

Comparison of surveyed sites with existing seaweed culture locations

For selecting a farming location, one has to understand the basic elements required for seaweed farming and farming site characters. Basic elements required for farming seaweed are seawater temperature, salinity, water motion and light intensity. Seawater temperature affects the plants directly by disturbing the physiological processes or indirectly impacts the surrounding environment. For example temperature affects the water motion, generating wind, waves and currents and farm productivity. For example, *Kappaphycus* could grow well at different temperatures - from 22.8 to 29.2^{0}C (Ohno and Orosco, 1987), from 27 to 32^{0}C (Njoman *et al*., 1987) and from 25 to 30^{0}C (Neish, 2003). In India, *Kappaphycus* could yield good biomass at 30 ± 3^{0}C (Subba Rao *et al*., 2008; Periyasamy *et al*., 2014, 2015; Sureshkumar *et al*., 2016). In general, at high temperature of above 35^{0}C, the seaweed will not survive much. Ohno and Orosco (1987) and Mairh *et al*. (1986) reported that *Kappaphycus* transferred from the central Philippines to Tosa Bay, Japan was dead, when the temperature fell below 20^{0}C. Thus seawater temperature is one of the most important factors influencing the growth of *Kappaphycus*.

Water motion helps to clean plants, bring fresh nutrients, remove metabolites and apply hydraulic forces that stimulate plant growth. In San Bernardino Strait, Philippines, where rapidly flowing water produced *Kappaphycus* plants as much as two meters long and with major branches more than 2cm across (Neish, 2003). Azanza - Corrales *et al*. (1996) reported the importance of water motion in the seeding or natural sporulation experiments done in *Eucheuma* and *Kappaphycus* farms at Philippines. Water motion is an important factor to be taken into account during the selection of farm sites and crop logging (Periyasamy *et al*., 2015; 2016).

The salinity and nutrition are critically important for seaweed growth. At most successful farm sites the salinity seemed to be in the range of 30 to 35 ‰ (Neish 2003; Subba Rao *et al*., 2008; Periyasamy *et al*., 2016). Nutrients such as phosphate, nitrate, nitrite and silicates also play an important role for *Kappaphycus* growth. Among the macronutrients, nitrogen is crucial for productive farming and this nutrient might be a limiting factor in sea cultures (Neish, 2008). In environments with low or erratic nutrient supply, surge ammonium uptake was described for *K. alvarezii* as a strategy to avoid nitrogen limitation of growth (Dy and Yap, 2001)

The basic elements of seaweed growth dictate that successful farm systems must have the following features as advocated by Neish (2003) and Periyasamy *et al*. (2017b). (1)Large surface area exposed to sunlight having optimum characteristics, (2) Effective even water flow to and from all plants in the system, (3) Even dispersion of plants throughout the farm sites, (4) Amenable to frequent cropping, cleaning and adhering so weeds, pests, disease and fouling organisms cannot overrun seaplant cultures, (5) Rugged enough to withstand the substantial hydraulic forces of moving water and wind, (6) Located in places with environmental conditions as close to ideal as possible for the crops being grown, (7) Minimal fixed and variable production costs, (8) Protected from weather and sea conditions beyond farm habitats structural limits, (9)Secure from human interferences and (10) Ultimately the only way to find out whether a given site supports vigorous plant growth is to plant test plots and expand where plants grow best Keeping the above parameters in view seaweed cultivation sites have been recommended.

Recommended sites for seaweed cultivation

All the surveyed coastal villages in the districts of Thiruvallur, Kancheepuram, Villupuram, Cuddalore, Nagapattinam and Kanniyakumari were found to have favourable waters possessing the required seawater temperature, salinity, nutrients and water motion for seaweed cultivation and the following sites are recommended for taking up seaweed cultivation on commercial scale.

In Thiruvallur District, first 11 villages (Thoniyur, Koonakuppam, Sembas Pillai Kuppam, Thirumalai Nagar, Light House, Nadu Kuppam, Arangam, Viravan Kuppam, Kora Kuppam, Kamunkalli and Kavarachi) are promising sites as they have good facility like space for drying and other activities.

In Kancheepuram District, although water motion is very heavy (more than 25cm/ sec) at all 38 coastal villages, 12 villages (Kovalam, Pudupattinam, Uyalli Kuppam, Angalak Kuppam, Paliyanadu Kuppam, Pudunadu Kuppam, Velampur, Perunthuravu, Paramangani Kuppam, Thalaithali Kuppam, Panniyurperiya kuppam and Kadappakkam) are potential sites for seaweed cultivation.

In Villupuram District, water motion is very heavy (more than 25cm/ sec) at all 20 coastal villages. However cultivation can be done in 10 villages (Manja Kuppam,

Koonimedu Kuppam, Mudaliyar Kuppam, Anucha Kuppam, Pudu Kuppam, Pillai Chavadi Kuppam, Pommiyar Palayam, Mudaliyar Chavadi, Thanthiran Kuppam and Nadu Kuppam).

In Cuddalore District, water motion is very heavy (more than 25cm/ sec) at all 31 coastal villages. Except water motion all other parameters are favourable. Cultivation may be done in 12 villages (Devanampattinam, Sonank Kuppam, Singara Thoppu, Rettiyar Pettai, Indhra Nagar, Madava Pallam, Annappan Pettai, Samiyar Pettai, Pudu Kuppam, Pudu Pettai, Chinnur, Pillmedu/ TS Pettai)where other suitable environmental conditions are prevailing.

In Nagapattinam District, water motion is very heavy (more than 25cm/ sec) at all 47 coastal villages. Except water motion, all other environmental parameters are favourable. Cultivation may be done at 15 villages (Kodiyampalayam, Mahendrapalli, Kottur, Palayaru, Madava Medu, Kottiyamedu, Chinna Kottiyamedu, Kooliyaru, Thoduva, Thirumullaivasal, Kela Mookkarai, Akkara Pallivasal, Arkattuthurai, Maniyanthur and Kodiyakkarai).

In Kanniyakumari District, water motion is very heavy (more than 25cm/ sec) at all 47 coastal villages. Except water motion all other parameters are favourable. Cultivation may be done in 15 villages (Arokkiapuram, Leepuram, Chinna Muttam, Kovalam, Kela Manakudi, Mela Manakudi, Pallam, Periyakadu, Rajakamangalam, Azhikkal, Pilla Thoppu, Mela Thurai, Muttam, Colachal and Enayam).

Some of the promising seaweeds to be adopted for cultivation along with *Kappaphycus alvarezii* are *Gracilaria edulis, G. verrucosa, G.dura , G. salicornea; Hypnea musciformis, H. valentiae; Ulva lactuca, U. reticulate, Enteromorpha flexuosa; Gelidiella acerosa* and *Acanthophora spicifera*. Among the six coastal districts, seaweed cultivation may be done in 75 villages. In these 75 villages, the potential space available may be taken as 100 hectares on a safer side. If *Kappaphycus* cultivation is done, possibility of output will be 50 tons (dry)/ hectare year. The expected output from these six coastal districts will be 5000 tons (dry)/year worth of Rs. 20 crores (when cost is Rs. 40 per kg). Employment to 10 beneficiaries directly may be provided per hectare. So in all the six districts, employment to 1000 beneficiaries may be provided with lucrative income. The present study provides self employment generation to the coastal people.

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Feeding deterrent activity of marine algae from southern coast of India

DEEPA S. RAJAN[1] and K. PADMAKUMAR[2]*

[1]*Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom Thiruvananthapuram - 695581, Kerala, India*
e-mail : deepa.anand.tvpm@gmail.com
[2]*Centre for Marine Biodiversity, Kerala University Campus, Kariavattom Thiruvananthapuram - 695581, Kerala, India*
Corresponding author : k.padmakumar@gmail.com

ABSTRACT

Feeding deterrent activity of the extracts of marine algae from southeast and southwest coast of India was evaluated using the gold fish, *Carassius auratus.* Among the 41 species of algae assayed (13 species of Chlorophyceae; 10 species of Phaeophyceae and 18 species of Rhodophyceae), 22 species showed considerable feeding deterrence for gold fish. Only 3 species *(Sargassum myriocystum, Grateloupia lithophila* and *Galaxaura* sp.) showed very high deterrent activity (>90%). Six algal species showed above 80% deterrence. Sixteen species exhibited >50% deterrence. Two species *(Hypnea musciformis* and *Padina tetrastromatica)* exhibited >40% deterrence. Seven species of algae were found inactive. When considering Chlorophyceae, among the 13 species assayed, 6 species exhibited high deterrence (>80%). In Phaeophyceae, among the 10 species assayed, only 1 species *(Sargassum myriocystum)* exhibited very high activity. Eighteen species from Rhodophyceae were assayed for feeding deterrent activity. Among them, four species exhibited high activity (>80%).

Introduction

Predation is a significant cause of mortality in natural communities and shows strong effects on community structure (Paine, 1994; Carpenter, 1997; Hixon, 1997). Population dynamics of both producers and consumers were influenced by resistance to predation (Lubchenco and Gaines, 1981; Crawley, 1983; Rhoades, 1983) and it is a heritable trait subject to natural selection (Rousi *et al.,* 1991). The predator-prey and the internal or external parasite-host interactions are the important types of interactions which make a constant chance for extinction of prey populations. So, any adaptation of the prey capable of reducing the impact of the predatory pressure would be favoured. It is no wonder that most of the organisms have developed elaborate anti-predatory systems (Brakeman and Daloze, 1986).

Although there are several mechanisms to prevent predation, including behavioural or structural defenses, chemical based deterrence seems widespread in marine environments (Paul, 1992; Hay 1996; McClintock and Baker, 2001). For marine plants such as benthic macro algae (seaweeds), most evidence for the chemical mediation of ecological interactions comes from studies of plant/herbivore interactions (Hay and Fenical, 1988; Hay and Steinberg, 1992), where it is clear that algal secondary metabolites play an important role as defenses against herbivory. It was reported that marine algae are defended from herbivory by a variety of secondary metabolites (Paul *et al.,* 2001), which includes polyphenolics, acetogenins, terpenes, amino-acid-based and halogenated compounds and these compounds can influence palatability. There are evidences that these metabolites deter feeding by marine herbivores in the field and in laboratory assays (Hay, 1984a, 1984b; Van Alstyne *et al.,* 2001; Van Alstyne and Houser, 2003). These compounds are thought to reduce digestibility, affect nervous system or cardiac functions, or be otherwise toxic or unpalatable to herbivores.

Materials and Methods

Forty one algal species were collected from intertidal and subtidal regions from Tamil Nadu (Kilakarai, Pudumadam, Mandapam, Pamban, Rameswaram, Kanyakumari) and Kerala

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(Vizhinjam and Kovalam). The algae were cleaned and washed in sea water and then in fresh water to remove extraneous mater, dried in shade and further in oven at 40±2ºC, powdered and soaked in ethanol/methanol. The soaked solvents were drained after three days, filtered and evaporated to dryness under reduced pressure at 40±2º C. The dried crude extracts were kept frozen (-20ºC) until further use. Voucher specimens were preserved in 5% formalin for taxonomic characterization.

Feeding deterrent assay

Antifeedant activity of the extracts of algae, seagrasses and sponges were evaluated using the gold fish, *Carassius auratus*. The fishes were exclusively fed with a formulated pellet feed (100 g contains, 66 g fish meal, 10 g ground nut oil cake, 14 g tapioca powder, and 10 g rice bran) and acclimatized to laboratory condition. Fishes of uniform size was used throughout the assay. Experiments were conducted by placing one fish in each tank of 1000 ml aerated fresh water. Six replicates were conducted for assaying each extracts and a control was run parallel. Known concentration (20 mg/ml) of the extract was prepared, dissolving in ethanol and volumetrically applied to 100 mg feed. After impregnation, the ethanol was evaporated at 40ºC. The treated pellet was dropped in to fish tanks. An extract was assumed to give deterrent reaction when the treated feed was ejected immediately after taken up into the mouth or piece of untreated feed was first consumed and refused to feed even after for several hours. From the observations, percentage of deterrence was calculated and data were statistically analysed for the significance.

Results

Table 1, 2 and 3 show the feeding deterrent activity of the extracts of marine algae. Among the 41 species of algae assayed, 22 species showed considerable feeding deterrence for gold fish. When considering Chlorophyceae, among the 13 species assayed, 6 species exhibited high deterrence (>80%). *Caulerpa sertularioides* exhibited highest activity and *Ulva recticulata* did not show any activity. Six species (*Caulerpa sertularioides, C. peltata, C. taxifolia, Ulva compressa, Bryopsis plumosa,* and *Cladophoropsis* sp. exhibited >"80% deterrence (Table-1).

In Phaeophyceae, among the 10 species assayed, only 1 species (*Sargassum myriocystum*) exhibited very high activity. Five species (*Sargassum ilicifolium, Colpomenia sinuosa, Padina gymnospora, Zonaria crenata* and *Hydroclathrus clathratus*) showed moderate activity. Two species (*Sargsssum longifolium and S. wightii*) did not exhibit any deterrence (Table-2). Eigteen species from Rhodophyceae were assayed for feeding deterrent activity. Among them four species (*Grateloupia lithophila, Galaxaura sp., Laurencia poiteaui* and *Gelidium pusillum*) exhibited high activity (≥ 80%). Two species (*Portieria hornemannii* and *Laurencia flagelliformis*,) exhibited no activity (Table-3).

Discussion

The feeding deterrent properties involve olfaction and taste, whereby an organism is avoided by a predator or receives a low food preference. These behavioural responses in the predator can be chemically mediated by secondary compounds either produced by the prey itself (Eisner, 1970; Gerhart, 1983) or sequestered by the prey from its own food (Brower *et al.,* 1970; Eisner *et al.,* 1974; Schulte *et al.,* 1980; Thompson *et al.,* 1982*;* Carte and Faulkner, 1983; Jensen, 1984). Herbivory is an important factor in determining the structure of benthic algal assemblages in marine communities. Algal natural products have been assumed to function as defenses against herbivores in the marine environment (Ogden and Lobel, 1978; Lobel and Ogden, 1981; Norris and Fenical, 1982; Hay, 1984a; Lewis, 1985).

The gold fish *Carassius auratus* can easily be trained to feed under experimental conditions. The organism is discriminating in its feeding behavior and sensitive to feeding deterrents. As the fish is a fresh water species, it will most likely to have had no exposure to or contact with the marine organisms in recent evolutionary period and would not have evolved adaptations of resistance or tolerance to marine derived metabolites. There are previous reports that several members of Chlorophyta are deterrent or unpalatable to various herbivores (Paul and Hay, 1986; Wylie and Paul, 1988; Meyer *et al.,* 1994). Among this, the Order Bryopsidales ranks first for the deterrent activity. Several species in this order including *Caulerpa, Halimeda* etc. were found to be deterrent in artificial assays (Paul and Van Alstyne, 1992). Reports from several authors indicate that *Ulva* and *Cladophora* sp. from the order Ulvales are less palatable to the predators (Paul and Hay, 1986; Van Alstyne *et al.,* 2001; Van Alstyne and Houser, 2003).

In the present investigation, among 13 species from Chlorophyceae, 6 species exhibited high deterrence (≥ 80%). The results indicate that *Caulerpa sertularioides* exhibits highest activity (88.89%) deterrence. Other species showing high deterrence are *Caulerpa peltata, C. taxifolia, Bryopsis plumosa* (all are from the order Bryopsidales), *Ulva compressa,* and *Cladophoropsis* sp. (order Ulvales). Other two species of *Caulerpa* (*C. cupressoides* and *C. fergusonii*) exhibited moderate activity. *C. scalpelliformis* and *C. microphysa* showed only negligible activity. Lowe (1974) and Lemee *et al.,* (1996) reported that different species of *Caulerpa* are deterrent to sea urchins. Wylie and Paul (1988) reported that *Caulerpa* spp. was avoided by the surgeon fish *Zebrasoma flavescens*. They also suggested that *Bryopsis* contain galactopeptides which are deterrent in nature. In contrast to the above reports, Paul and Hay (1986) and Paul *et al.* (1990) opined that several species of *Caulerpa* are consumed by many reef fishes.

Table 1. Feeding Deterrent Activity of Chlorophyceae

S.No.	Seaweed	Control-Eaten Mean±Error	Treated- Eaten Mean ± Error	't' Value	Significance at 1% level	Percentage Deterrence
1	*Bryopsis plumosa*	97.22 ± 6.80	19.44 ± 40.02	3.83	Significant	80.56
2	*Caulerpa cupressoides*	83.33 ± 25.82	33.33 ± 38.01	2.17	Not significant	66.67
3	*Caulerpa fergusonii*	100 ± 0	25 ± 2 2.97	6.53	Significant	75.00
4	*Caulerpa microphysa*	94.44 ± 8.61	63.89 ± 40.023	1.49	Not significant	36.13
5	*Caulerpa peltata*	97.22 ± 6.80	16.67 ± 21.08	7.27	Significant	83.33
6	*Caulerpa scalpelliformis*	100 ± 0	96 ± 8.94	1	Not significant	4.00
7	*Caulerpa sertularioides*	100 ± 0	11.11 ± 13.61	13.06	Significant	88.89
8	*Caulerpa taxifolia*	100 ± 0	19.44 ± 6.80	23.68	Significant	80.56
9	*Cladophoropsis* sp.	100 ± 0	13.89 ± 12.55	13.73	Significant	86.11
10	*Ulva compressa*	100 ± 0	19.44 ± 34.02	4.73	Significant	80.56
11	*Ulva intestinalis*	100 ± 0	29.99 ± 13.94	11.22	Significant	70.01
12	*Halimeda macroloba*	94.44 ± 8.67	33.33 ± 29.81	3.93	Significant	66.67
13	*Ulva reticulata*	100 ± 0	100 ± 0	0	Not significant	0.00

Table 2. Feeding Deterrent Activity of Phaeophyceae

S.No.	Seaweed	Control-Eaten Mean±Error	Treated- Eaten Mean ± Error	't' Value	Significance at 1% level	Percentage Deterrence
1	*Colpomenia sinuosa*	100 ± 0	46.67 ± 46.25	2.58	Not significant	53.33
2	*Hydrclathrus clathratus*	100 ± 0	33.33 ± 51.64	2.58	Not significant	66.67
3	*Padina gymnospora*	88.89 ± 13.61	27.78 ±27.22	4.017	Significant	72.22
4	*Padina tetrastromatica*	88.89± 27.22	52.78± 34.21	1.66	Not significant	47.22
5	*Sargassum ilicifolium*	92±17.88	28±30.33	4.06	Significant	72.00
6	*Sargassum longifolium*	100±0	100±0	0	Not significant	0.00
7	*Sargassum myriocystum*	100±0	4± 8.94	24	Significant	91.10
8	*Sargassum wightii*	100±0	100±0	0	Not significant	0.00
9	*Turbinaria conoides*	90±22.36	85±33.54	0.277	Not significant	15.00
10	*Zonaria crenata*	100±0	44.44 ± 37.52	2.96	Not significant	55.56

Table-3. Feeding Deterrent Activity of Rhodophyceae

S.No.	Seaweed	Control-Eaten Mean±Error	Treated- Eaten Mean ± Error	't' Value	Significance at 1% level	Percentage Deterrence
1	*Amphiroa fragilissima*	100 ± 0	63.69 ± 40.02	1.80	Not significant	36.31
2	*Centroceras clavulatum*	100 ± 0	41.67 ±20.41	5.716	Significant	58.33
3	*Ceramium* sp.	97.22 ± 6.80	27.78 ± 13.70	9.13	Significant	72.22
4	*Cheilosporum spectabile*	100 ± 0	91.67 ± 13.94	1.20	Not significant	8.33
5	*Galaxaura* sp.	100 ± 0	8.33 ± 13.94	13.15	Significant	91.67
6	*Gelidiopsis variabilis*	83.33 ± 18.26	63.89 ± 40.02	0.88	Not significant	36.11
7	*Gelidium pusillum*	94.44 ± 8.61	19.44 ± 19.50	7.042	Significant	80.56
8	*Gracilaria corticata*	100 ± 0	100 ± 0	0	Not significant	0.00
9	*Gracilaria pudumadamensis*	100 ± 0	22.22 ± 20.19	7.71	Significant	77.78
10	*Gracilariopsis sjoestedtii*	100 ± 0	33.33 ± 57.64	2.58	Not significant	66.67
11	*Grateloupia lithophila*	88.89 ± 20.18	2.778 ± 6.80	8.09	Significant	97.22
12	*Grcilaria corticata* var. *cylindrica*	100 ± 0	30.56 ± 35.62	3.90	Significant	69.44
13	*Hypnea musciformis*	100 ± 0	52.78 ± 30.58	3.09	Not significant	47.22
14	*Laurencia obtusa*	94.44 ± 8.61	63.89 ± 19.49	2.87	Not significant	36.11
15	*Laurencia poiteaui*	100 ± 0	16.67 ± 40.82	4.08	Significant	83.33
16	*Laurencia flagelliformis*	100 ± 0	100 ± 0	0	Not significant	0.00
17	*Portieria hornemannii*	100 ± 0	100 ± 0	0	Not significant	0.00
18	*Spyridia insignis*	100 ± 0	27.78 ± 13.61	10.61	Significant	72.22

It was reported that *Ulva* spp. were avoided by sea urchins *Strongylocentrotus droebachiencis* (Van Alstyne and Houser, 2003), *Echinometra lacunter* (Erickson *et al.,* 2006), *P. lividus* (Lemee *et al.,* 1996) and *L. variegatus* (Lowe, 1974), the spot tail pinfish *Diplodus holbrookii* (Hay *et al.,* 1987) and *A. longimania* (Duffy and Hay, 1994). It is observed from the present study that *Ulva reticulata* did not show any deterrence. In the present investigation, *Halimeda macroloba* showed moderate deterrence (66.67%). In a previous study conducted by Paul and Van Alstyne (1992), it was found that halimedatrial and halimeda tetraacetate were deterrent towards herbivorous fishes. According to Schupp and Paul (1994), fishes *Scarus sordius* and *Ctenocheatus striatus* were deterred by *Halimeda* diterpenes where as *Naso lituratus* and *Signus spinus* were not. The deterrence observed in the present study may be due to the presence of deterrent metabolites such as halimedatrial or halimeda tetra acetate.

There are many reports suggesting that crude extracts and isolated metabolites from different red algal species deterring the different types of herbivores (Ogden, 1976; Crews *et al.,* 1984; Hay *et al.,* 1987). Hay *et al.,* (1987) reported that isolaurenterol and elatol, isolated from *Laurencia* spp. detered grazing by sea urchin *Diadema*. The antifeedant activity of *Portieria* was reported by Crews *et al.* (1984). It is observed from the present study that among 18 species of algae assayed from the Rhodophyceae, *Grateloupia lithophila* exhibited highest (97.22%) deterrence towards the test organism followed by *Galaxaura* sp. (91.67%). Two more species, *Laurencia poiteaui* and *Gelidium pusillum* showed high activity (e"80% deterrence). Contrary to the previous studies, *Portieria* exhibited no deterrent activity. Similarly, *L. flagelliformis*, and *Gracilaria corticata* were not deterrent. Two other species of *Gracilaria,* (*G. pudumadamensis*, and *G. corticata* var. *cylidrica*), *Gracilariopsis sjoestedtii*, *Spyridia insignis* and *Ceramium* sp. exhibited moderate activity.

When considering Phaeophyceae, the present study indicates that the extract of *Sargassum myriocystum* alone among the 10 species assayed exhibited high activity (91.1%). *S. ilicifolium, Colpomenia sinuosa, Padina tetrastromatica, Zonaria crenata* and *Hydroclathrus clathratus* exhibited moderate activity ($\geq$50% deterrence). *S. longifolium* and *S. wightii* did not show any deterrence. The deterrent effect of algae from Phaeophyceae was well documented by different authors (Steinberg, 1988; Hay *et al.,* 1987; Cronin *et al.,* 1997; Cruz-Reviera and Hay, 2003). Eventhough so many bioactive metabolites are derived from *Sargassum* spp., the feeding deterrent activity of crude extract was not much studied. It is inferred from the present study that marine algae possess metabolites to deter potential herbivores or predators. Such secondary metabolites help these organisms to establish and survive in the highly competitive environment.

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Hydrobiological investigations of sediment inhabiting diatoms of Manakudy estuary (South India)

H. PRABAVATHY[1] AND M. MATHEVAN PILLAI[2]*

[1]*Department of Botany, Vivekananda College, Agestheeswaram - 629 701, Tamil Nadu, India*

[2]*P.G. and Research Centre, Department of Botany, S.T. Hindu College, Nagercoil - 629 002, Tamil Nadu, India*

**Corresponding author : mavethe@gmail.com*

ABSTRACT

Temporal variations of the sediment inhabiting diatom assemblages were investigated for two years covering four intertidal regions of Manakudy estuary in Kanniyakumari District of Tamil Nadu. The associated physico-chemical parameters were also measured on every sampling. During the study period, a total of 128 species of sediment inhabiting diatoms belonging to 50 genera and 21 species were of common occurrence and dominance of pennate (74) diatom species over the centric (54) diatom taxa from the four stations. The studied diatom taxa in all the four stations were alike. The most common genera were *Achnanthes, Amphora, Amphiprora, Ceratulina, Chaetoceros, Cymbella, Diploneis, Eunotia, Gyrosigma, Hemidiscus, Nitzschia* and *Pleurosigma.* The physico-chemical parameters showed spatial and temporal variations. Temperature, salinity and nutrients are the controling factors influencing the distribution of intertidal sediment inhabiting diatoms.

Introduction

Sediment inhabiting diatom population of estuarine predominantly consist of benthic diatoms. The seasonal changes in the intertidal sediment inhabiting diatom characteristics are directly controlled by primary ecological factors such as rainfall, temperature, salinity, dissolved oxygen, pH and nutrients. Hence, it becomes important to determine the ecological factors that would affect the growth of sediment inhabiting diatoms. Studies pertaining to the ecology of marine sediment inhabiting diatoms are meagre when compared to those on ecology of marine planktonic diatoms (Jeyachandran, 1989; Mathevanpillai, 1994; Anantharaman, 1994; Kannan *et.al.,* 1997; Mathevanpillai, 2000; Mathevanpillai and Sukumaran, 2004; Sindhudevi, 2004; Sunitha, 2005; Ampli, 2009; Prabavathy *et.al.,* 2010; Mathevanpillai *et al.,* 2012 and Alphose Jenitrasanthi, 2012; Prabavathy, 2013, Brintha, 2014;). There were only very limited studies on the ecology of sediment inhabiting diatoms of the Indian coasts. Hence, the present study was made to evaluate the influence of various meteorological and physicochemical parameters on the sediment inhabiting diatom assemblages of the Manakudy estuarine environment and no such studies have been reported so far from this place.

Materials and Methods

Description of the study area

The present study was carried out in the Manakudy estuary (Lat. $8^0$05' N; Long. $77^0$32' E), located in Kanniyakumari District of Tamil Nadu State. The estuary is formed near the mouth of Pazhayar River at its confluence with the Arabian Sea. The artificial mangrove forest developed in the upper reaches of the estuary has its own influence on the estuarine biota. Various human activities such as salt industry, coir industry, lime shell and sand mining and waste disposal on the banks of the estuary are seen. Based on the South west monsoon, it was divided into pre-monsoon, monsoon, summer and post-monsoon seasons for convenience and easy interpretation of results.

Description of the sampling stations

The present study was carried out at four stations in the estuary. Station I is the mangrove area, 3.5 km away from the sea towards the river Pazhayar. This station is characterized by mangrove plants mainly *Rhizophora* sp. *and Avicenia* sp. with their extensive root systems, litter fall and clayey sediments. Station-II is located 900 m away from Station- I towards the sea and close to the salt pans. Station-III is 1.25 km away from Station-II. This station is dynamic with

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retting ponds of the coir industry, with hydrogen sulphide gas, have much contaminating effect on the estuarine ecosystem. The bar mouth with disposal of sewage, and domestic wastes are major factors in Station-IV. Sampling stations were chosen at a distance of about half to one kilometre away from each other.

Rainfall data were obtained from the Meteorological Department at Nagercoil. Collection of interstitial water sample was done by following the procedure outlined by Hackney and Delacruz (1978) at monthly intervals for a period of two years (July 2008 to June 2010) from four different stations. After collection at each station, samples were stored in a portable ice-cooled box and labeled and transported to the laboratory for analysis in the same day. The temperature (air and water) was determined using a mercury thermometer. pH of interstitial water was determined by using an Elico pH meter (LI10). Salinity was estimated using a hand refractometer (Erma, Japan). For the analyses of nutrients, interstitial water samples were filtered using a Millipore filtering system and analyzed for dissolved inorganic nitrite, nitrate, reactive silicate and phosphate adopting the standard methods described by Strickland and Parsons (1972). Parameters such as turbidity, sodium, potassium, ammonia, chloride, fluoride and sulphate were estimated by following the standard method described in APHA (2000). Sediment samples containing the diatoms were collected from the intertidal areas at low tides following the methods described by Amspoker and McIntyre (1978). Quantification of the sediment inhabiting diatoms was made using the procedure described by Sridharan (1979). The diatom taxa were identified by refering the classical works of Boyer (1926-1927), Hustedt (1927, 1930 and 1966), Cupp (1943), Cleve-Euler (1951-1955), Hendey (1964), Subramanian (1946), Desikachary (1986, 1987 and 1988), Mathevan Pillai (1994) and Anantharaman (1994) among others. The simple correlation co-efficient (r) analyses have been employed for the statistical interpretation of data obtained during present study period.

Results and Discussion

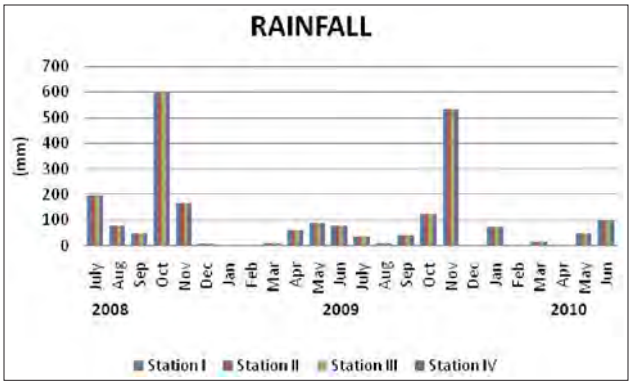
Monthly variations in meteorological and physicochemical parameters *viz.* rainfall, air temperature, water temperature, salinity, water pH, dissolved oxygen, turbidity, dissolved inorganic phosphate, nitrite, nitrate, reactive silicate, calcium, magnesium, sodium, potassium, ammonia, chloride, fluoride, sulphate, iron, manganese and their influence on the distribution of intertidal sediment inhabiting diatom assemblages for a total period of two years from July 2008 to June 2010 at four different stations of the Manakudy estuary are given in Figure 1 to 3.

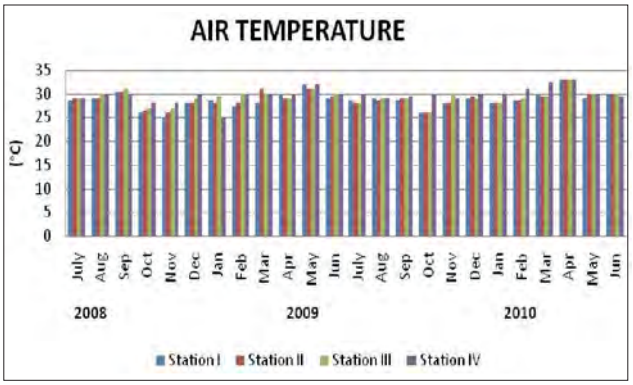
A total of 128 species (Table-1) of sediment inhabiting diatoms belonging to 50 genera were recorded from the four stations. Station I, II, III and IV recorded 78, 67, 68 and 65 diatom species respectively. The most common genera were *Achnanthes, Amphora, Amphiprora, Ceratulina, Chaetoceros, Cymbella, Diploneis, Eunotia, Gyrosigma, Hemidiscus, Nitzschia* and *Pleurosigma.* There is a clear dominance of pennate (74) over centric (54) diatoms at all the studied stations. This may be attributed to their elongated nature with which they can attach themselves better on sediments (Mathevanpillai, 1994; Kannan *et al*., 1997).

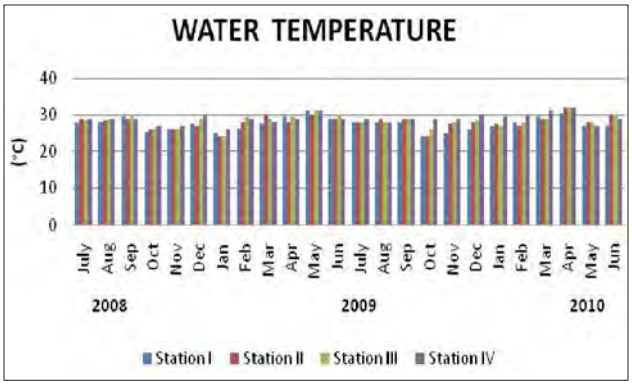
Population density of sediment inhabiting diatoms was low (7354 cells g^{-1}) during the summer season and maximum (20999 cells g^{-1}) during the premonsoon season at all the stations. The minimum population density was due to heavy sunshine and reduced nutrient level. This indicated that many benthic diatoms could be removed from their habitats due to high temperature (air, water and sediments) since they could not balance the heat caused by the sun (Jayachandran, 1989; McIntyre 1996; Prabavathy *et al.*, 2010).

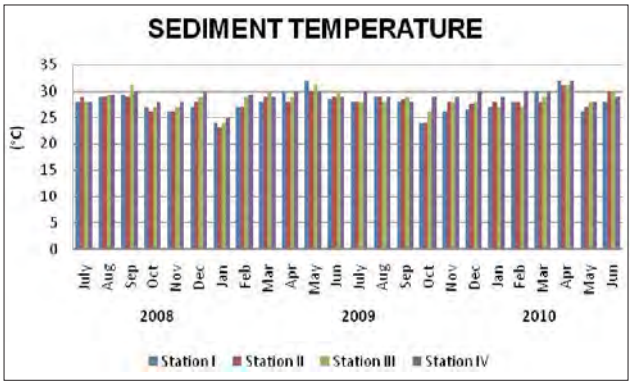
In the present study during the summer season, when temperature was comparatively higher, the sediment inhabiting diatom population density was low at all studied stations (Station I - 10125 - 26875 cells g^{-1}, Station II - 5188 - 17250 cells g^{-1}, Station III - 11875 - 23188 cells g^{-1}, Station IV - 9375 - 17000 cells g^{-1}). This is in agreement with the findings of Rajesh *et al*. (2000) who reported the key role of temperature in the distribution and establishment of sediment inhabiting diatom communities; Salinity is regarded as one of the controling factors influencing the distribution of sediment inhabiting diatom species in estuarine areas (Aberle Wilshire, 2006). Low salinity (6.7 $‰$ at Station-I, 13 $‰$ at Station-II, 11.3 $‰$ at Station-III and 14.3 $‰$ at Station-IV) was recorded during the early postmonsoon season from all the studied stations. Station IV recorded more salinity than other stations as it is situated near the esturaine mouth adjecent to the sea and the other stations are situated towards the upstream 0.5 to 1 km North of Station-IV between each stations.

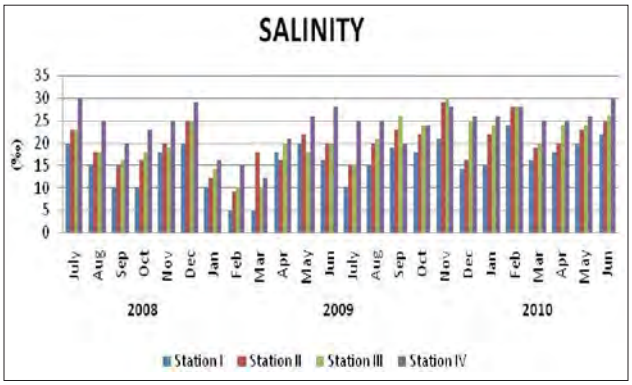
Some of the sediment inhabiting diatoms, *Achnanthes brevipes, Amphora coffeaeformis, Amphora holsatica , Amphora marina, Amphiprora gigantea, Ceratulina bergonii, Chaetoceros curvisetus, Coscinodiscus gigas, Cymbella marina, Cymbella cistula, Diplonies bombus, D. ovalis, D. subovalis, Eunotia pseudolunaris, Gyrosigma scalproides, Hemidiscus cuneiformis, Nitzschia commutata, N. longissima, N.obtusa, N. vermicularis and Pleurosigma angulatum* were found to be distributed over a wide range of salinity (5 - 30 %) at all the studied stations. This indicates that these diatoms are euryhaline in nature. There was a wide range of fluctuation in water pH during the study period and showed no marked variation between various sesons and years. Higher pH value recorded at the studied stations during the summer seaseon would have resulted as a cause of the redox changes in the sediments and water column apart from the influence of

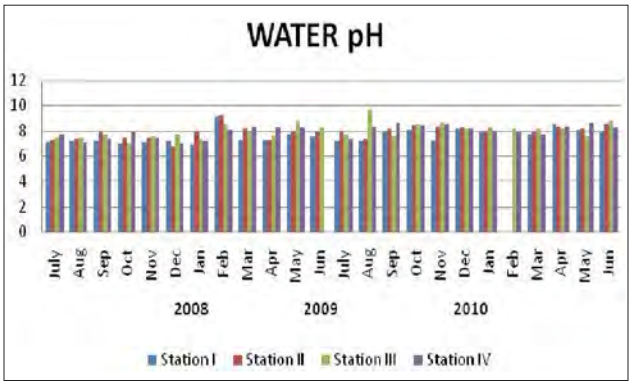

RAINFALL
(mm)
2008
2009
2010
Station I
Station II
Station III
Station IV

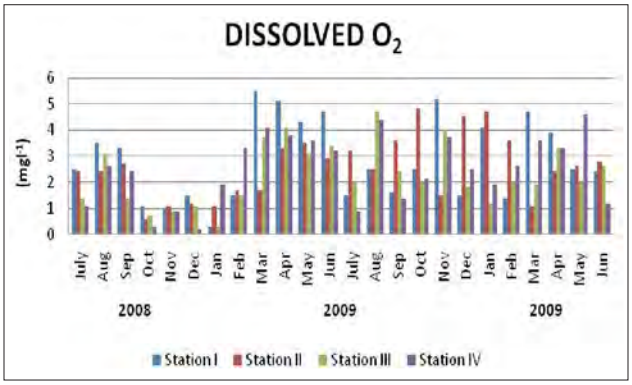

AIR TEMPERATURE
(°C)
2008
2009
2010
Station I
Station II
Station III
Station IV


WATER TEMPERATURE
(°C)
2008
2009
2010
Station I
Station II
Station III
Station IV


SEDIMENT TEMPERATURE
(°C)
2008
2009
2010
Station I
Station II
Station III
Station IV


SALINITY
(‰)
2008
2009
2010
Station I
Station II
Station III
Station IV


WATER pH
2008
2009
2010
Station I
Station II
Station III
Station IV


DISSOLVED O2
(mgl-1)
2008
2009
2009
Station I
Station II
Station III
Station IV

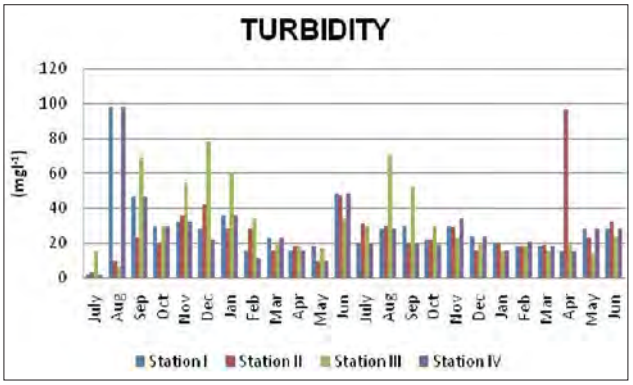
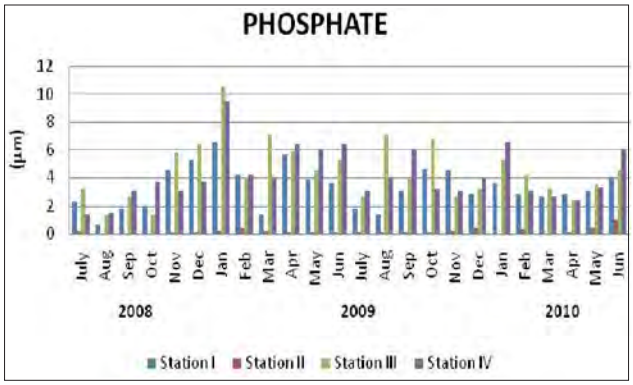
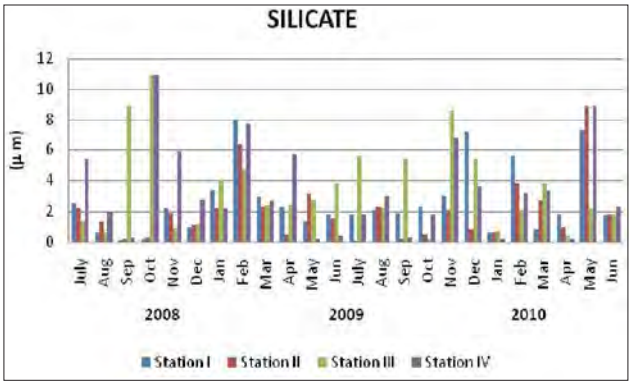
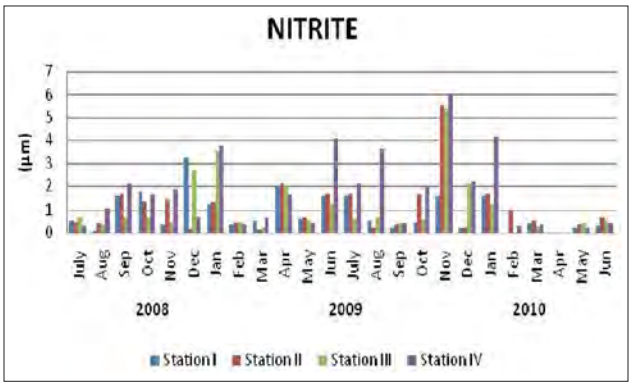
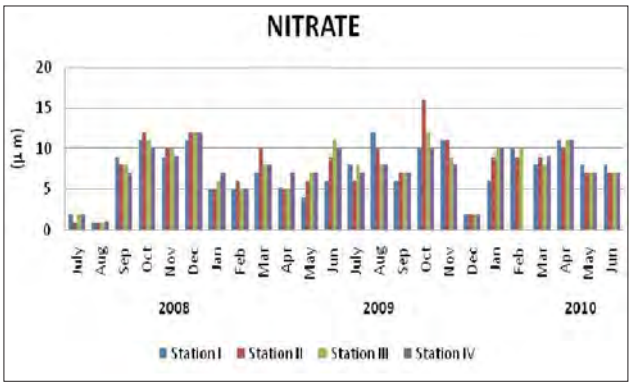

TURBIDITY
(mgl-1)
Station I
Station II
Station III
Station IV

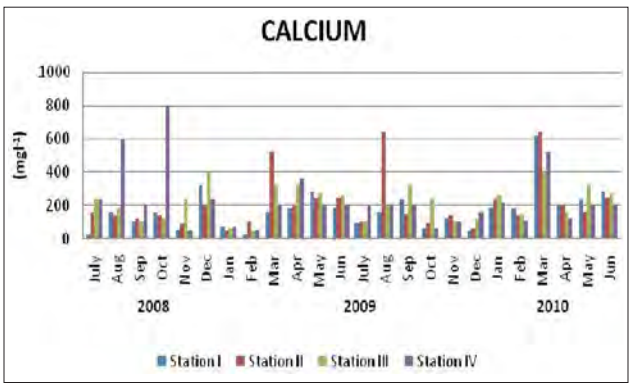
Figure 1.

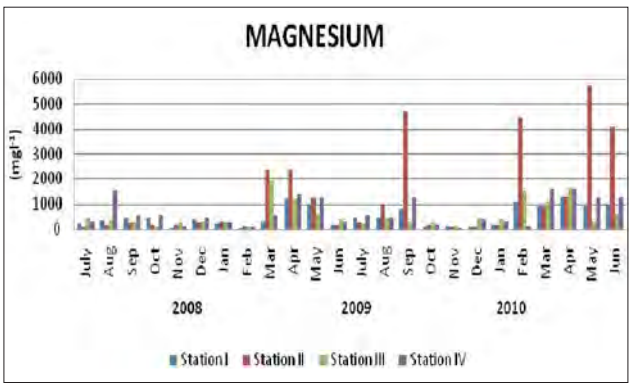


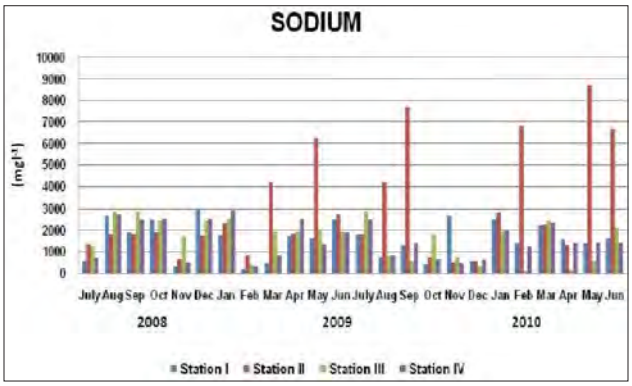












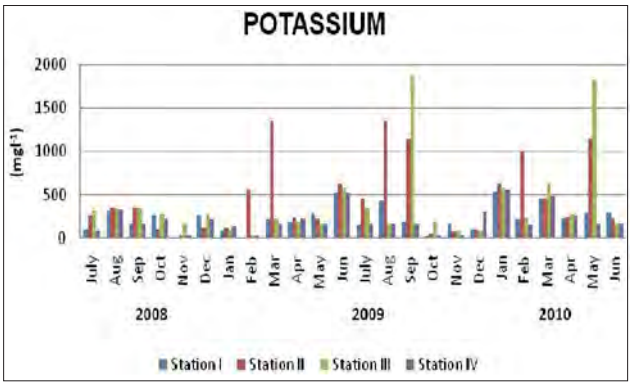


Figure 2.

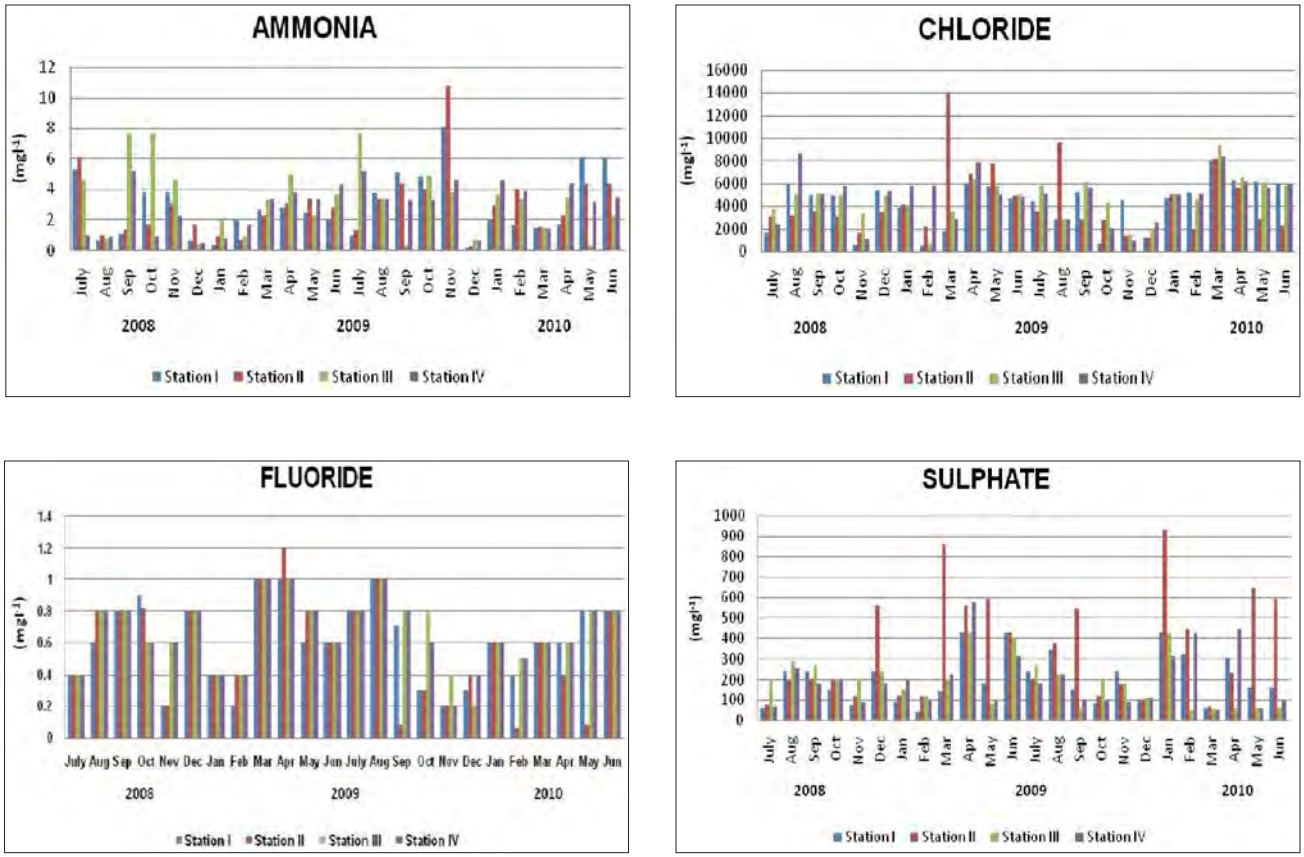


Figure 3.

Table-1. List of sediment inhabiting diatom species recorded at Stations-I to IV

S.No.	Name of the species	I	II	III	IV	TPD Cells g^{-1}
1	*Achnanthes brevipes Ag.*	P	P	P	P	219999
2	*A. coarctata Breb.*	P	-	P	-	1938
3	*A. inflata Kutz*	P	P	P	-	5625
4	*Amphiprora gigantea Grun.*	P	P	P	P	7138
5	*Amphora coffeaeformis Ag.*	P	P	P	P	8250
6	*A. decussata Grun.*	P	-	-	-	1500
7	*A granulata Grun.*	-	-	-	P	250
8	*A holsatica Hust.*	P	P	P	P	4500
9	*A marina W.Sm.*	P	P	P	P	3500
10	*A ovalis Kutz*	P	-	-	P	13375
11	*Amphora sp.*	-	P	P	P	2313
12	*Anomoeoneis sphaerophora (Kutz) Pfi.*	P	P	P	-	11875
13	*Aulacoseira granulata (Ehr) Sim.*	C	-	C	C	1063
14	*Aulacodiscus orbiculatus Sp.nov.*	C	-	-	C	4750
15	*Auliscus reticulatus Grev.*	-	C	-	-	875
16	*Bacillaria paradoxa Gmelin.*	P	P	P	-	22188
17	*Bacteriastrum varians Lauder*	-	-	C	-	750
18	*Biddulphia aurita (Lyngbye) Breb.*	-	C	C	-	2688
19	*B. reticulata Roper.*	C	C	-	C	625
20	*B. sinensis (Grev.) Grun.*	-	C	-	C	4875
21	*Ceratulina bergonii Peragallo*	C	C	C	C	4500

22	*Chaetoceros coarctatus Lauder*	P	-	-	-	125
23	*C. curvisetus Cl.*	P	C	C	C	438
24	*C.lorenzianus Grun.*	-	-	-	C	63
25	*C. peruvianus Btw.*	C	C	-	-	1513
26	*C. socialis Lauder*	-	C	-	-	63
27	*Climacodium frauenfeldianum Grun.*	C	-	-	C	3500
28	*Climacosphenia elongata Bailey*	P	-	P	P	15063
29	*Coscinodiscus centralis Ehr.*	-	-	-	C	5250
30	*C. concinnus W.Sm*	C	-	C	-	1813
31	*C. excentricus Hust.*	C	-	-	C	5313
32	*C. gigas Ehr.*	C	C	C	C	1938
33	*C. granii Gough.*	-	-	C	C	7750
34	*C. lineatus Ehr.*	C	-	-	C	1500
35	*C. marginatus Ehr.*	C	-	-	-	625
36	*C. reniformis Castr.*	-	C	-	-	3250
37	*C. rothii (Ehr) Grun.*	-		C	C	22813
38	*Cyclotella meneghiniana Kutz.*	-	C	C	-	875
39	*C. striata (Kutz) Grun.*	C	C	C	-	32375
40	*C. stylorum Btw.*	C	-	C	C	1188
41	*C. subovalis Btw.*	-	-	C	-	1563
42	*Cymbella cistula (Hemp.) Grun.*	P	P	P	P	2313
43	*Chustedtii Krasske*	P	-	-	-	750
44	*C. marina Cast.*	P	P	P	P	1438
45	*C. turgida (Greg.)Cl.*	-	-	P	P	938
46	*Diploneis bombus Ehr.*	P	P	P	P	3250
47	*D. suborbicularis (Greg.) Cl.*	P	P	P	P	2563
48	*D. ovalis (Hilse) Cl.*	P	-	-	-	4000
49	*D. subovalis Cl.*	P	P	P	P	59813
50	*Eucampia cornuta (Cl.) Grun.*	-	-	C	C	1438
51	*Eunotia pseudolunaris Sp.nov.*	P	P	P	P	47875
52	*Fragilaria brevistriata Grun.*	P	-	-	-	563
53	*F. intermedia Grun.*	P	P	-	-	1750
54	*F. oceanica Cl.*	-	-	P	P	375
55	*F. pinnata Ehr.*	-	-	P	P	14313
56	*Fragilaria sp*	-	P	P	-	1625
57	*Gyrosigma balticum (Ehr.) Rabh.*	P	P	-	P	8313
58	*G. scalproides (Rabh) Cl.*	P	P	P	P	1625
59	*Hemidiscus hardmannianus (Greville)*	C	C	C	C	188
60	*H. cuneiformis Wallich*	-	C	-	-	4688
61	*Lauderia annulata Cl.*	-	-	C	-	438
62	*Leptocylindrus danicus Cleve.*	C	-	-	-	938
63	*L. minimus Grun.*	-	-	-	C	2563
64	*Licmophora abbreviata Agardh*	-	P	-	P	2813
65	*Lithodesmium undulatum Ehr.*	C	-	-	C	1625
66	*Mastogloia braunii Grun.*	P	P	-	-	1063
67	*M. smithii Thw.*	-	-	P	P	1188
68	*Melosira sulcata (Ehr.)Kutz*	-	-	C	C	2250
69	*Menusigma angulatum Kutz*	-	-	-	C	813.
70	*Navicula angusta Ehr.*	-	P	-	-	3313
71	*N. cincta (Ehr.)Kutz*	P	-	-	P	3250
72	*N. cuspidata Kutz*	P	-	-	P	2250
73	*N. digitoradiata (Greg.) W.Sm.*	-	P	P	-	1188
74	*N. gracilis Ehr.*	P	P	-	P	8250
75	*N. granulata Bail.*	-	P	P	P	5375
76	*N. lacustris Ehr.*	P	-	-	-	813

77	*N. lyra Ehr.*	P	-	-	-	688
78	*N. praetexta Ehr.*	-	P	-	-	188
79	*N. sp.*	P	P	-	-	1063
80	*Nitzschia angustata (W. Sm.) Grun.*	P	-	P	P	1875
81	*N. commutata Grun.*	P	P	P	P	3250
82	*N. granulata Grun.*	P	-	-	-	2188
83	*N. longissima (Breb.)Ralfs*	P	P	P	P	10250
84	*N. obtusa W.Sm.*	P	P	P	P	6250
85	*N. palea (Kutz.) W.Sm.*	P	-	-	-	1438
86	*N. punctata Grun.*	P	-	-	-	2188
87	*N. vermicularis (Kutz.) Hantsch*	P	P	P	P	6938
88	*Paralia sulcata (Ehr.) Cl.*	-	C	C	C	438
89	*Pinnularia ampigua Cl.*	P	-	P	P	5375
90	*P. angulatum Cl.*	-	-	-	P	315
91	*P. interrupta W.Sm.*	P	P	-	P	7375
92	*P. raeana (Castr.) De Toni.*	-	-	P	-	63
93	*Plagiogramma staurophorum(Greg.)He*	-	-	-	P	750
94	*Pleurosigma angulatum W.Sm.*	P	P	-	-	6563
95	*P. aestuarii Grun.*	P	P	P	P	3250
96	*P. elongatum W.Sm.*	P	-	P	-	250
97	*P. normanii Ralfs*	-	P	P	-	6250
98	*P. pulchellum Ralfs*	P	-	-	-	125
99	*P. rhombeum (Grun.) Cl.*	P	P	-	-	625
100	*P. salinarum Grun.*	P	-	P	-	125
101	*Podosira montagnei Kutz.*	-	C	-	C	1375
102	*Pyxilla gracilis Temp. (Forti.)*	-	P	-	-	750
103	*Rhabdonema punctatum Har & Bailey*	-	P	-	-	563
104	*Rhaphoneis discoides Sp.nov.*	P	P	P	-	1313
105	*Rhizosolenia alata Btw.*	C	-	-	C	625
106	*R. cylindrus Cl.*	C	-	C	-	188
107	*R. imbricata Btw.*	-	C	-	-	1938
108	*R. setigera Btw.*	C	-	-	C	688
109	*R. styliformis Btw.*	C	-	-	-	750
110	*R. discoides Btw.*	-	C	-	C	438
111	*Sceletonema costatum (Greville) Cl.*	-	C	C	-	1875
112	*Surirella striatula Grun.*	-	-	P	P	750
113	*Synedra ulna Ehr.*	P	P	P	-	17313
114	*Thallassiosira eccentrica Ehr.*	C	C	C	-	250
115	*Thallassiosira sp.*	-	-	C	-	8750
116	*Thallassionema nitzschioides Grun.*	P	P	P	-	35075
117	*Thallassiothrix frauenfeldi Grun.*	P	P	P	-	3563
118	*T. longissima Cl. & Grun*	P	P	P	-	16688
119	*Trachyneis antillarum Cl.*	-	-	P	-	1250
120	*T. distinctum Jan.in.A.S.*	-	C	-	-	313
121	*T. dubium Btw.*	-	-	-	C	938
122	*T. favus Ehr.*	C	-	-	-	438
123	*T. robertsianum Grev.*	-	C	-	C	313
124	*T. arcticum Btw.*	C	-	-	-	188
125	*T. reticulum Ehr.*	C	-	-	-	2938
126	*T. subovalis Ehr.*	-	-	C	-	250
127	*T. zonulatum (Grev.)Mann.*	-	-	C	-	188
128	*Tropidoneis lepidoptera (Greg.)Cl.*	-	-	P	-	63
	Centric diatom	25	23	24	27	
	Pennate diatom	53	43	44	38	
	Total	78	66	68	65	

fresh water (Sukumaran,2009). While the lower pH recorded during the monsoon season was due to the dilution of sea water by the fresh water flow at the stations. The dissolved oxygen concentration was higher during summer seasons at stations might be due to biological activity of microphytobenthos along with the dissolved gases (Mathevanpillai, 1994); Adeyemo *et al.* 2008; Jahankir Sarker *et al.,* 2009. The lower dissolved oxygen concentration recorded during the monsoon seasons mainly due to waste water that had entered the estuaries through drainages. Turbidity in the interstitial water was higher during the monsoon season and lower during the premonsoon season. The monsoonal maximum was due to the runoff materials transported by the monsoonal rainfall leading to increase in the concentration of turbidity during the period of study as suggested by Fathima Suba (2004) in the Manakudy estuarine environment.

The dissolved nutrients concentration was maximum during monsoon and minimum during pre-monsoon seasons This variable is dependent on a multiple factors such as local hydrographical condition, input of fresh water, tidal variation and human activities (Ault *et al*., 2000; Chandrasekhar *et al*., 2000; and Mathevan Pillai and Prabavathy 2012; Kocum *et al.,* 2002 and Underwood and Barnett, 2006) which alter the nutrients and also the distribution of sediment inhabiting diatoms. During the present study period, inorganic phosphate content showed positive significance with population density ($r=0.58$, $P<0.05$) of Station-I population density ($r=0.55$, $P<0.05$) of Station-III and population density ($r = 0.57$, $P<0.05$) of Station-IV. While the reactive silicate of interstitial water showed a positive significant correlation with the population density at Station-I ($r = 0.50$, $p<0.05$), II ($r = 0.53$, $p<0.05$) and IV ($r = 0.51$, $p<0.05$).

Higher nitrite content was observed during monsoon season and the lower was during summer in all the studied stations. Simple correlation coefficient for nitrite showed the positive significance with population density ($r = 0.53$, $P<0.05$) of Station-I, ammonia ($r = 0.79$, $P<0.05$) (Station-II), potassium, iron, manganese and population density. The maximum nitrate content was observed in the monsoon season at all the stations. This was due to the addition of nitrogenous nutrients mainly by fresh water and terrestrial runoff as suggested by Chandran (1982), Joanita (2005) and Sarker *et al.* (2005). Low concentration of nitrate was observed during premonsoon season except Station-III. Such monsoonal and premonsoonal patterns of nitrate distribution were reported earlier by Jeyachandran (1989) from the Pitchavaram mangroves.

In general, the inorganic salts like calcium, magnesium sodium, potassium sulphate and chloride were higher during monsoon and early post monsoon season, and their distribution patterns were irregular between stations and seasons. Simple correlation coefficient for magnesium showed positive significance ($p<0.05$) with population density ($r = 0.65$, $P<0.05$) at (Station-IV). While potassium showed positive significance ($p<0.05$) with population density($r = 0.56$, $p<0.05$) at Station-II and population density($r = 0.52$, $p<0.05$) at Station-IV and sulphate showed positive significance ($p<0.05$) with population density($r = 0.58$, $p<0.05$) at Station-I and population density($r = 0.65$, $p<0.05$) at Station-IV.

Total hardness in the interstitial waters of the study area is mainly governed by the content of calcium and magnesium which are largely combined with bicarbonate and carbonate and with sulphate, chloride and other anions of minerals. Manakudy estuary is nutrient rich and its total hardness values ranged from 76 to 3688 mg l^{-1}. Kaur *et al.* (1996) and Meenakshi Singh *et al.,* (2010) reported that high values of hardness were probably due to the regular addition of large quantities of sewage, detergents and municipal waste in the water body from the nearby residential localities. It showed highly positive relationship with rainfall ($r = 0.57$, $p<0.05$) at Station-IV.

The topographic features of Manakudy estuary have changed after tsunami resulting the loss of sediments and the distribution of sediment inhabiting diatom species. Hence, monitoring of the coastal environment and documentation of physicochemical parameters are necessary. The present baseline information of the intertidal diatoms distribution and ecological characters will be useful for further ecological assessment and long term monitoring of the coastal ecosystems of Manakudy estuary.

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Community structure and distribution of phytoplankton in the solar salt-works of Puthalam, Kanyakumari District, Tamil Nadu

L. LEENA SUMAM AND M. MATHEVAN PILLAI*
P.G. and Research Centre, Department of Botany, S.T. Hindu College, Nagercoil - 629 002, Tamil Nadu, India
**Corresponding author : mavethe@gmail.com*

ABSTRACT

In the present study, the distribution of phytoplankton in the solar saltpan of Puthalam was investigated. Sixty species of phytoplankton belonging to 4 different classes were identified during the study period. A specific pattern was seen in the distribution of the phytoplankton species. Of the four different classes, twenty eight species belong to Bacillariophyceae, twenty two species to Cyanophyceae, six species to Chlorophyceae and four species to Dinophyceae. Phytoplankton diversity and richness values increased when the number of species increased. While the dominance index was higher, the evenness index was lower and *vice versa*. In crystallizer pond, the unicellular alga *Dunaliella salina* was found during the period of study.

Introduction

Solar salt-works or salt pans consist of a number of interconnected ponds where salt is produced from sea brine or subsoil brine by means of evaporation using solar energy as well as wind energy. In the evaporating ponds, salinity is maintained within fixed limits. Depending on the salinity of water, these ponds provide home to different types of microorganisms. Thus, the ponds that maintain their unique ecosystems are a source of biological resources (Zeno, 2009). The evaporation of water leads to the concentration of nutrients in the abiotic environment of saltpans, providing the conducive atmosphere for the multiplication of phytoplankton that can survive in specific environments (Dolapsakis *et al.,* 2005; Oren, 2009). A characteristic flora adapted to the prevailing saline conditions is present in each saltpan. In the initial ponds, where salinity is low, algal diversity is more when compared to the high saline crystallizer pond, where only the halophilic algae are seen (Rodriguiz – Valere, 1988; Evagelepoulos *et al.,* 2008).There was a rapid decrease in the number of species with the increase of salinity. The microorganisms in the solar salt works can function effectively and constitute a peculiar biological system which has a profound influence on the physicochemical process of salt production (Korovessis and Lekkas, 2006). The distribution of phytoplankton follows a specific pattern in a salt pan which are the primary producers in that ecosystem. The physical process of salt production is very much dependent on the biological system present in every pond of the solar salt-works (Krumbein, 1985). The biological system in the saltpan can help or harm salt production (Davis, 1993). The present investigation was aimed to identify phytoplankton present in the different salinity ponds and study their community structure.

Materials and Methods

The study area was the solar salt works of Puthalam situated at latitude of 8.1048^{0}N and longitude of 77.4666^{0}E of Kanniyakumari District, Tamilnadu. Puthalam saltpan receives water from the Manakudy estuary/subsoil brine and covers an area of about 250 acres of land. Each saltpan was divided into four sites based on the salinity of water: Site I (source), where salinity is between 3 and 5^{0}Be, Site II (reservoir) where salinity ranged from 6 to 15^0 Be Site III (condenser) where salinity was from 15 to 20^{0}Be and Site IV (crystallizer) where salinity ranged between 20 and 30^0. Samples were collected at monthly intervals for a period of one year from October 2010 to September 2011.Soil samples were taken from the surface of

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the ponds for algal identification (Amspoker and McIntire, 1978). The collected samples were preserved in 4% formaldehyde solution for further study. Phytoplankton were observed under a compound microscope and identified by refering classical works of Hustedt (1927, 1930-1966), Cupp (1943),Subramanian(1946), Prescott (1962),Hendey (1964) and Desikachary (1986, 1987 and 1988) among others. For enumerating the phytoplankton, all the cells in the slide were counted and multiplied it by an aliquot factor (Sridharan, 1979). Species diversity index (H¢) species richness (SR), evenness index (J¢) and Dominance index (S¢) were calculated using the formula of Shannon and Weaver (1949), Gleason (1922) and Pielou (1966) respectively.

Results and Discussion

Puthalam saltpans are one of the hypersaline extreme environments. They are considered as coastal aquatic ecosystems consisting of a range of habitat types depending on the salinity levels. The ecosystems are characterized by higher species diversity in low salinity ponds and lower species diversity in high salinity ponds. During the study period, sixty species of phytoplankton were identified in the ponds of Puthalam salt-works of Kanniyakumari District (Table-1).Of the 60 species of phytoplankton, 28 belong to Bacillariophyceae, 22 to Cyanophyceae, 6 to Chlorophyceae and 4 to Dinophyceae. The number of phytoplankton varied with stations and seasons. Twenty three species of Bacillariophyceae, 18 species of Cyanophyceae, 6 species of Chlorophyceae, 4 species of Dinophyceae were observed in Station-I (source). In Station-II (reservoir), 22 species of Bacillariophyceae, 21 species of Cyanophyceae, 5 species of Chlorophyceae, 4 species of Dinophyceae were recorded. In Station-III (condenser), 10 species of Bacillariophyceae, 11 species of Cyanophyceae and 1 species of Chlorophyceae were identified. In Station-IV (crystallizer), only 1 species of Chlorophyceae was found.

The species composition of phytoplankton varied with

Table-1. *List of recorded taxa at the four sampling station of Puthalam solar salt works*

Sl. No	Name of the species	Station I	II	III	IV
	BACILLARIOPHYCEAE				
1.	*Achnanthes brevipes* Ag.	+	+	+	-
2.	*A. hauckiana* Grun.	-	-	+	-
3.	*Amphora coffeaeformis* Ag.	-	-	+	-
4.	*Bacillaria paradoxa*	+	+	-	-
5.	*Biddulphia obtusa* (Kutz.)Ralfs	+	+	-	-
6.	*B. rhombus* (Ehr.) W.Sm.	+	+	-	-
7.	*Coscinodiscus marginatus* Ehr.	+	+	-	-
8.	*C. radiatus* Ehr.	+	+	-	-
9.	*Cyclotella striata* (Kutz.) Grun.	-	-	+	-
10.	*Cymbella aspera* (Ehr.)Cl.	+	+	-	-
11.	*Diploneis interrupta* (Kutz.)Cl.	+	+	-	-
12.	*Fragilaria constricta* Ehr	+	+	-	-
13.	*Gyrosigma balticum* (Ehr.)Rabh.	+	+	-	-
14.	*Mastogloia euxina* Cl.	+	+	-	-
15.	*Navicula henneydei* W.Sm.	-	-	+	-
16.	*N.lyra* Ehr.	+	+	+	-
17.	*Nitzschia closterium* (Ehr.)W.Sm.	+	+	+	-
18.	*N.gracilis* Ehr.	+	+	+	-
19.	*N.longissima* (Breb.) Ralfs	+	+	-	-
20.	*N.lorenziana* Grun.	+	+	-	-
21.	*Pleurosigma elongatum* W.Sm.	+	+	-	-
22.	*Surirella fastuosa* Ehr.	+	-	-	-
23.	*Synedra ulna* (Nitz)Ehr.	+	+	-	-
24.	*Thalassionema eccentrica* Ehr.	-	-	+	-
25.	*T.nitzschoides* Grun.	+	+	-	-
26.	*Thalassiothrix longissima* Cl. (Grun.)	+	+	+	-
27.	*Triceratium favus* Ehr.	+	+	-	
28.	*T. reticulatum* Ehr.	+	+	-	-
	CYANOPHYCEAE				
29.	*Anabaena subcylindrica* B.	+	+	-	-
30.	*Aphanothece microscopica* Nug.	+	+	-	-
31.	*Calothrix geitonos* Skuja	+	+	-	-
32.	*Chroococcus turgidus* (Kutz.)Nug.	+	+	+	-
33.	*Lyngbya aestuarii* Liebm.ex Gom.	+	+	-	-
34.	*Microcystis littoralis* (Hansg.)Forti	+	+	-	-
35.	*Myxosarcina burmensis* Skuja.	+	+	-	-
36.	*Oscillatoria chlorina* Kutz. ex Gom.	+	+	+	-
37.	*O.limosa* Ag. ex Gom.	+	+	-	-
38.	*O.salina* Biswas	+	+	+	-
39.	*O.subbrevis* Schm.	+	+	-	-
40.	*O.tenuis* Ag. ex Gom.	+	+	+	-
41.	*Phormidium fragile* (Men.)Gom.	-	+	+	-
42.	*P.tenue* (Men.)Gom.	+	+	+	-
43.	*P.valderianum* (Delp.)Gom.	+	+	+	-
44.	*Plectonema terebrans* Born. ex Gom.	+	+	-	-
45.	*Spirulina labyrinthiformis* (Men.)Gom.	-	-	+	-
46.	*S.major* Kutz.ex Gom.	-	+	+	-
47.	*S.subsalsa* Oerst. ex Gom.	-	+	+	-
48.	*Stichosiphon sansibaricus* (Heron.) Drouet et Daily	+	+	-	-
49.	*Synechococcus aeruginosus* Nug.	+	+	+	-
50.	*Synechococcus elongatus* Nug.	+	+	-	-
	CHLOROPHYCEAE				
51.	*Chlamydomonas globosa* (Snow.)	+	-	-	-
52.	*Chlorella ellipsoidea* (Gerneck.)	+	+	-	-
53.	*Cladophora glomerata* (L.)Kuetz	+	+	-	-
54.	*Closterium acerosum* (Sch.) Ehr.	+	+	-	-
55.	*Cosmarium granatum* (Breb.)	+	+	-	-
56.	*Dunaliella salina* Teadar.	+	+	+	+
	DINOPHYCEAE				
57.	*Ceratium hirundinella* (O.F.M.)Duj.	+	+	-	-
58.	*Exuviella sp.* Lohmann	+	+	-	-
59.	*Gymnodinium uberrimum* (Allon.)Kof.et.Sw.	+	+	-	-
60.	*Peridinium aciculiferum* (Lemm.)	+	+	-	-

seasonal changes. During monsoon, all the four groups of algae showed abundance when compared to the other seasons because of the run-off water and availability of nutrients. In the summer season, the temperature and salinity increase which have an adverse effect on the species diversity of phytoplankton in the saltpan ecosystem. During the period of study, the class Bacillariophyceae was dominant over other algal classes during monsoon and postmonsoon seasons while during summer and premonsoon seasons, the halophilic algae of Chlorophyceae and Cyanophyceae were dominant. Similar patterns of variation were also reported by Radhika *et al.* (2011), Santhanakrishnan *et al.* (2015) in the saltpans of Tuticorin and Umarani *et al*. (2016) in the saltpan of Swamithoppe. At Station-I, there was an abundance of microalgae compared to other stations, where the salinity of water was low. In the higher saline stations, the salt tolerant species such as *Dunaliella salina, Amphora coffeaeformis, Achnanthes hauckiana, Spirulina subsalsa, Oscillatoria salina, O.chlorina* were observed during the course of study. The biological systems in the solar salt-works consisting mainly of phytoplankton are greatly influenced by the salinity changes. Higher salinity has a negative effect on species diversity and species richness (Williams, 1998). Thus, it can be concluded that the number of species decreased rapidly and significantly with increasing salinity in the ponds. (Dolapsakis *et al.,* 2005; Madkour and Gaballah,2012).

Population density of different algal groups varied with each station. The algae belongs to Bacillariophyceae were maximum at Station-I, whereas it was minimum at Station-IV. It was the same with Cyanophyceae, Chlorophyceae and Dinophyceae. The marked decrease in the number of phytoplankton with increasing salinity was also reported by Nagasathya and Thajuddin (2008 a,b) in the saltpans of southeastern coast of India. Only the tolerant species can thrive in environments with high environmental stress. This may be the reason for the survival of the salt tolerant species in the final ponds with extreme conditions (Mustafa *et al.*, 1999). During the study period, the population density of *Dunaliella* in crystallizer pond (Station-IV) varied greatly according to geographic location, nutrient status and management of the saltpans. Oren (2009) reported that the green alga *Dunaliella* is the sole primary producer in the crystallizer that lives in association with dense communities of heterotrophic halophilic Archaea that colour the brine red.

Community structure of an ecosystem can be assessed on the basis of species diversity and species richness. Species diversity, richness, evenness and dominance index of phytoplankton varied from station to station in a solar saltpan (Table-2). During the study period, the phytoplankton species diversity varied from 0.96 to 3.2, richness 0.16 to 1.17, evenness 0.94 to 0.99 and dominance index 24.9 to 68.2. The community structure of phytoplankton species serves as an important tool in solar salt-work studies, as it would function as a yardstick to identify biological differences between identical habitats. During the course of study, diversity and richness values have increased as the number of species increased. While the dominance index was higher, the evenness index was lower and *vice versa.* Similar patterns of variation in the community structure were also reported by Kannan *et al*. (1997) in the Tamil Nadu coastal

Table-2. *Seasonal mean values of phytoplankton population density and community structure (species diversity, richness, evenness and dominance index) recorded at four stations (Station-I to IV) of Puthalam salt works from October 2010 to September 2011*

Station	Monsoon - 2010		Post-Monsoon - 2011		Summer		Pre-monsoon	
	PD	SD	PD	SD	PD	SD	PD	SD
				Bacillariophyceae				
I	2875	3.2	2417	3.2	2021	3.1	2042	3.2
II	2438	3.0	1688	2.8	1375	2.5	1479	2.8
III	1792	2.8	1230	2.3	688	1.5	813	1.7
IV	1584	2.6	688	1.7	104	0.97	417	1.8
				Cyanophyceae				
I	2833	3.0	2125	2.7	2021	3.0	2167	3.3
II	2375	2.9	1542	2.4	1250	2.5	1500	2.8
III	1792	2.7	1146	2.2	667	1.7	729	1.7
IV	1604	2.7	792	1.8	292	0.96	500	2.0
				Chlorophyceae				
I	729	1.5	833	1.5	479	1.2	438	0.99
II	604	1.4	271	1.0	250	...	230	...
III	396	0.98	459	0.99	396	...	438	...
IV	293	1.0	479	...	355	...	375	...
				Dinophyceae				
I	354	0.99	354	0.99	229	0.98	250	0.97
II	354	0.99	335	0.99	250	1.0	157	...

Table-2. *Continued...*

Station	SR	SE	DI	SR	SE	DI	SR	SE	DI	SR	SE	DI
						Bacillariophyceae						
I	1.1	0.98	27.7	1.1	0.99	25.8	1.0	0.99	27.9	1.1	0.99	26.8
II	0.94	0.94	33	0.81	0.99	32	0.69	0.99	36.7	0.86	0.99	30.8
III	0.85	0.98	32.2	0.6	0.98	48.8	0.36	0.98	68.2	0.39	0.96	49.5
IV	0.80	0.97	33.3	0.4	0.99	61.9	0.17	0.97	...	0.39	0.99	60.1
						Cyanophyceae						
I	0.97	20.99	28.7	0.78	0.97	30.9	0.96	0.99	27.9	1.2	0.99	24.7
II	0.9	0.98	31.4	0.6	0.96	37.4	0.35	0.98	41.8	0.86	0.99	30.8
III	0.76	0.98	39.5	0.56	0.99	48.3	0.36	0.97	68.1	0.35	0.99	55.1
IV	0.77	0.98	36.4	0.39	0.99	60.3	0.19	0.96	...	0.46	0.99	45.5
						Chlorophyceae						
I	0.29	0.97	65.7	0.29	0.97	58.1	0.21	0.98	62.6	0.17	0.99	...
II	0.25	0.99	65.9	0.17	0.99	...	...	...	...	...	...	...
III	0.17	0.98	...	0.16	0.99	...	...	...	...	...	...	...
IV	0.17	0.99	...	...	...	...	...	...	...	...	...	...
						Dinophyceae						
I	0.17	0.99	...	0.17	0.99	...	0.17	0.98	...	0.17	0.97	...
II	0.17	0.98	...	0.17	0.99	...	0.17	0.98	...	...	...	...

PD-Population density (cells g^{-1}), SD-Species diversity, SR-Species richness, SE-Species evenness and DI-Dominance index

regions. It is evident that the community structure of phytoplankton is influenced by the unique environmental conditions present in the saltpans. The detailed study of the different types of phytoplankton and the influence of physicochemical parameters present in the saltpans will help to understand the effect of the biological system on the salt production process.

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Endophytic fungi associated with marine macroalga *Sargassum wightii*

R. KRISHNA PRIYA[1] and K. PADMAKUMAR[2]*

[1]*Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom Thiruvananthapuram - 695581, Kerala, India*

[2]*Centre for Marine Biodiversity, Kerala University Campus, Kariavattom Thiruvananthapuram - 695581, Kerala, India*

**Corresponding author : k.padmakumar@gmail.com*

ABSTRACT

Marine macroalga *Sargassum wightii* was collected from Kovalam and Kanyakumari and screened for the presence of fungal endophytes. A total of eight endophytic fungi belonging to seven genera were isolated from the alga. *Aspergillus pencillioides*, *Aspergillus* sp., *Eurotium amstelodami*, *Curvularia* sp., *Cladosporium* sp., *Pestalotiopsis* sp., *Cheatomium globosum*, *Fusarium* sp. were the endophytic fungi isolated from *Sargassum wightii*. Of these eight fungi, *Cheatomium globosum* and *Eurotium amstelodami* were isolated for the first time from *Sargassum wightii*. This study was focused on the diversity of endophytic fungi associated with *Sargassum wightii* along the south west coast of India.

Introduction

Endophytes are organisms that reside within the plant tissue either for a part of its life or entire life without causing any symptoms of disease to the host (De Bary, 1866). Generally endophytes are microorganisms such as fungi, bacteria and actinobacteria which have the ability to colonise within the plant tissue both inter or intracellularly. Endophytes are ubiquitous and found in all the plant species. The host and endophyte show a mutual relationship. The host provides suitable conditions and abundant nutrition promoting the growth of endophytes, whereas the endophytes in return provide protection and thereby increasing the survival rate of the host (Strobel *et al.,* 2004).

Fungal endophytes are the most commonly reported organisms (Elsebai *et al.,* 2014). Marine derived endophytic fungi have been obtained from marine plants (algae, seagrasses, mangroves, and driftwood), marine invertebrates (sponges, corals, ascidians, and holothurians) and vertebrates (Rateb and Ebel, 2011). Marine derived endophytic fungi are excellent source of unique natural products (Teuscher *et al.,* 2006; Suryanarayanan *et al.,* 2009; Mishra *et al.,* 2014; Hong *et al.,* 2015). Those associated with macroalgae have recently gained attention as an untrapped biological resource with potential to yield novel bioactive metabolites (Debbab *et al.,* 2012; Flewelling *et al.,* 2015). Reports on biodiversity of endophytic fungi associated with seaweeds of southern India is quite meagre, especially from south west coast of India (Suryanarayanan *et al.,* 2010). Hence, it is necessary to understand the distribution and diversity of endophytic fungi associated with seaweeds to make prudent bioprospecting decisions.

Materials and Methods

Sargassum wightii was collected from Kovalam (Kerala) and Kanyakumari (Tamil Nadu) during the period from May 2013 to September 2016. The alga with no visible signs or symptoms of disease were collected and transfered to sterilized conical flask and taken to the laboratory immediately. The samples were processed within 12 hours in the laboratory. It was washed thoroughly in running tap water in order to remove nonspecific fungal propagules adhered to the alga . Then the alga was surface sterilized with 70% ethanol for 1 min and shaken in 1.2% Sodium hypochlorite solution for 5 min. Samples were then washed three times with sterile distilled water by shaking. Various segments of the surface sterilized alga was cut and plated on to PDA medium which was supplemented with Chloramphenicol (150 mg/L) to prevent the

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growth of bacteria. Control of algal thalli before and after sterilization was also plated to check the contamination.

All plates were incubated at 28ºC for 3 days or until fungal growth was observed. Using aseptic technique, emergent hyphae were transfered and purified on sterile PDA plates. The pure culture was stored in slants under 4ºC for further studies. The fungal cultures obtained were identified by their spore morphology and colony characters using standard taxonomic key (Domsch *et al.,* 1980; Sutton, 1980; Ellis, 1971; Ellis, 1976) and also with the help of taxonomic experts.

Results

A total of 8 species of endophytic fungi belonging to 7 genera were isolated from the host alga *Sargassum wightii* (Table-1). Among these, five endophytes belong to Hyphomycete (*Aspergillus pencillioides*, *Aspergillus* sp., *Eurotium amstelodami, Curvularia* sp. and *Cladosporium* sp.), three fungi belong to Ascomycete (*Pestalotiopsis* sp., *Cheatomium globosum*, *Fusarium* sp.).

Cheatomium globosum was isolated from *Sargassum wightii* collected from Kovalam and Kanyakumari. From both the sites endophytic fungi was isolated from the blade. The algae collected from Kovalam harboured two *Aspergillus* spp. i.e., *Aspergillus penicillioides* and *Aspergillus* sp. A teleomorph of *Aspergillus amstelodami* i.e., *Eurotium amstelodami* was also isolated from the stype of the host alga collected from Kovalam. *Pestalotiopsis* sp., is a true endophyte found associated with the blade of *Sargassum wightii* which was collected from Kovalam, whereas *Fusarium* sp. was derived from the alga collected from Kanyakumari. The Sac fungi, *Curvularia sp*. was isolated as an endophyte from the alga collected from Kovalam. It was isolated from the blade of the host.

Discussion

In recent years there are several reports on biodiversity of endophytic fungi associated with macroalgae and the chemical diversity of bioactive natural products isolated from these diverse endophytes. The metabolites isolated from endophytes showed wide ranging biological activities such as, antimicrobial, antialgal, anticancer, antimalarial, antioxidant, insecticidal, AChE modulator, Tyrosine kinase inhibitor etc. (Flewelling *et al.,* 2015; Hong, *et al.,* 2015; Bugni and Ireland, 2004) which prove the value of this unique biological resource.

Table-1. Fungi associated with *Sargassum wightii*

Host Alga	Place of Collection	Fungi
Sargassum wightii	Kovalam	*Aspergillus* sp.
	Kovalam	*Aspergillus pencillioides*
	Kovalam	*Cheatomium globosum*
	Kanyakumari	*Cheatomium globosum*
	Kovalam	*Cladosporium* sp.
	Kovalam	*Eurotium amstelodami*
	Kanyakumari	*Fusarium* sp.
	Kovalam	*Pestalotiopsis* sp.
	Kovalam	*Curvularia* sp.

Several previous studies reported the presence of endophytes in marine algae especially *Sargassum wightii*. Presence of *Aspergillus* spp. and *Cladosporium* sp. as an endophyte of *Sargassum wightii* was reported earlier from Mandapam coast, Tamil Nadu by Suryanarayanan *et al.* (2010). Venkatachalam *et al*. (2015) also reported the presence of *Aspergillus* spp. and *Cladosporium* sp. from *Sargassum* sp. collected from the eastern coast of Tamil Nadu. *Fusarium* sp. was isolated from the host alga from Kanyakumari in the present study. Similarly Venkatachalam *et al*. (2015) and Thirunavukkarasu *et al*. (2015) also isolated *Fusarium oxysporum* from *Sargassum wightii* collected from south east coast.

Kaushik *et al*. (2014) reported the presence of *Pestalotiopsis* sp. in *Sargassum wightii* collected from Rameswaram. In this investigation, *Pestalotiopsis* sp. was isolated from the alga collected from Kovalam coast, Kerala. *Eurotium amstelodami* was isolated from the algal sample of Kovalam. But Du *et al*. (2017) reported *Eurotium cristatum* from *Sargassum thunbergii*. In earlier investigations *Cheatomium* sp. was found to be associated with various green and brown algae and not from *Sargassum wightii* (Suryanarayanan *et al*., 2012; Cui *et al*., 2010). But in this study *Cheatomium globosum* was derived from *Sargassum wightii*.

Altogether 8 fungal endophytes were isolated from *Sargassum wightii* alone of which *Cheatomium globosum* and *Eurotium amstelodami* were isolated for the first time from *Sargassum wightii*. The diversity of endophytic fungi associated with marine algae is known to a very lesser extent. Proper identification of fungi is critical to understand the diversity, host specificity, if any, ecological implications and also to derive unique natural products. Hence, a thorough investigation is necessary to record the diversity of algicolous fungi from various species of marine algae.

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Isolation and characterization of nitrogen fixing endophytic bacteria from *Cladophora vagabunda* (Linnaeus) van den Hoek

S. EMEEMAL SHARON AND G. RANI*
Department of Plant Biology and Plant Biotechnology
S.D.N.B. Vaishnav College for Women, Chromepet, Chennai - 600 044, Tamil Nadu, India
**Corresponding author : ranvenshre@gmail.com*

ABSTRACT

A diazotrophic bacterial strain was isolated from the green marine alga *Cladophora vagabunda* (Linnaeus) van den Hoek using Nitrogen Free basal medium (NFb) in which this isolate formed a characteristic pellicle indicating the ability of this bacterium to fix atmospheric nitrogen. This isolate was further purified using NFb solid medium supplemented with yeast extract (100 mg l^{-1}) as N source. The identity of this bacterium was determined based on the morphological, physiological, biochemical and molecular (16S rDNA) analyses. Cells are positive for oxidase, catalase and ammonia production. Utilization of different sugars and organic acids as carbon substrates and growth on different amino acids were also performed on this isolate. Resistance to different antibiotics was also studied. The 16S rDNA sequence analysis indicated that this isolate is closely related to *Bacillus cereus* with 100% sequence similarity. The other closely related organisms with this isolate are *Bacillus anthracis* (97.45 %), *B. thuringiensis* (97.81%) and *B. toyonensis* (97.81%). This is the first report on the occurrence of nitrogen fixing endophytic bacteria *Bacillus cereus* in marine alga *Cladophora vagabunda*.

Introduction

Endophytes are microorganisms (Bacteria, Fungi and Actinomycetes) that occur within plant tissues and having a symbiotic association. Many microbes are known to collaborate with marine algae to form mutually beneficial association. These endophytic microorganisms isolated from marine algae are recognized as potential sources of novel natural products for utilization in medicine and agriculture. (Lane *et al.*, 2000; Owen and Hundley, 2004). Recent molecular studies on endophytic bacterial diversity have revealed a large number of species. Endophytes promote plant growth and yield, suppress pathogen, solublize insoluable phosphate or contribute nitrogen to plants (Monica and Esperanza, 2006). Kaaria *et al.*(2015) reported that large number of endophytic microbes associated with marine algae collected from Kenyan coastal region showed antimicrobial activity. Reports indicated that more numbers of *Escherichia coli, Enterococcalbacteria, Staphylococcus aurens, Candida albicans* are associated with fresh and marine *Cladophora* species (Ola *et al.,* 2016).

Many marine algal species provide shelter to micro organisms as one of the protective mechanisms against heavy biofouling. These endophytic microorganisms get plenty of organic substances secreted by the algal cells. Endophytic interaction between marine algae and microorganisms has also been observed in some seaweeds which provide an interesting biotic environment for these bacterial communities (Se-Kwon Kim, 2013). The seaweed surface provide a suitable substratum for the settlement of microorganisms and also secretes various organic substances that function as nutrients for multiplication of bacteria and the formation of microbial biofilms (Steinberg *et al*., 2002; Staufenberger *et al*., 2008; Singh *et al.*, 2013). Some water-soluble monosaccharides such as rhamnose, xylose, glucose, mannose and galactose are part of algal polysaccharides that constitute part of the cell wall (Popper *et al*., 2011) and storage material (Lahaye and Axelos, 1993; Michel *et al*., 2010a,b). These algal polysaccharides are potential source of carbon and energy for numerous marine bacteria (Hehemann *et al*., 2012) that helps to facilitate seaweed–bacterial associations (Steinberg *et al.,* 2002; Lachnit *et al*., 2013). Therefore, interactions between seaweeds and bacteria have attracted the attention of many researchers worldwide.

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There are many reports on the isolation, screening, diversity and activity of nitrogen fixing endophytic bacteria (Florence *et al.,* 2016). Most of the nitrogen fixing bacteria are isolated from the plants, soil and roots. However, there are very few reports on endophytic bacteria associated with marine algae. Endophytic bacteria in *Spirogyra* and *Oedogonium* have been reported by Ganesh Iyer (2012). The present work is on isolation, characterization and identification of nitrogen fixing bacteria from *Cladophora vagabunda*. The biochemical characterization of nitrogen fixing bacteria was determined based on the utilization of different carbon substrates with different amino acids(Muthukumaraswamy*et al*., 2002).The molecular identification of this isolate was performed by 16S rDNA gene sequence analysis.

Materials and Methods

The seaweed *Cladophora vagabunda* (Linnaeus) van den Hoek was collected from Kovalam coast, which is 25 km from South of Chennai. The alga was identified by refering the publication 'The Marine benthic flora of Southern Australia' (Hoek and Womersley, 1984). The sample was collected in sterile plastic bags (Poly Vinyl), transported aseptically to the laboratory for further processing. The material was washed with sterile seawater followed by two minutes wash in 70% ethanol and one minute wash in 2% sodium hypochlorite. The sample was rinsed with sterile seawater for five minutes with shaking and then dried with sterile tissue papers (Denise *et al.,* 2002). The samples were cut into 2-3 cm long segments using a sterile scalpel. The cut segments were then macerated using pestle and mortar, serially diluted and it was then inoculated into NFb semisolid medium. All the tubes were incubated for 24- 48 hours at 30°C. A veil like pellicle initially formed at the base of the medium which later moved to the surface of the medium as a pellicle indicated the diazotrophic nature of this bacterium. Then this pellicle was streaked on the NFb plates supplemented with yeast extract as N source (100mg l^{-1}). After incubation for 5 days, the colonies were sub-cultured to obtain pure isolates and stored at 4°C for further study. The number of colonies per ml was calculated.

Morphological characters such as cell shape, cell size, pigmentation, edge and margin of the bacterial culture were recorded. The effect of Indole, Methyl red, Voges-Proskauer test, Citrate utilization, Lactose, Sucrose, Glucose fermentation, Triple sugar iron agar, salt tolerance, Urease test, Nitrate reduction test, Starch, Casein hydrolysis, Pectinase, Cellulose assay, Production of HCN and solubilization of inorganic calcium phosphate were performed by adopting the methods given by Challa Krishnakumari *et al*. (2013). 1% concentration of twenty two different carbon sources such as glucose, sucrose, malic acid, arabinose etc., were added separately to NFb medium to study their effect on bacteria after replacing malate. Different amino acids such as alanine, valine, arginine, etc, were added separately to the NFb medium to study their effect on growth of this bacterium (Cavalcante and Do¨bereiner, 1988). The isolates were cultured overnight in Mueller Hinton agar (Mueller and Hinton, 1941) medium prepared using seawater (Table-1) The plates were incubated by inverting for 24 hours at 37°C in different concentrations of antibiotics namely Amikacin (AK 30mcg/disc), Ciprofloxacin (CIP 5 mcg/disc), Ampicillin (A 10 mcg/disc), Chloramphenicol (C 30 mcg/disc), Gentamicin (GEN 10 mcg/disc), Tetracycline (TE 10mcg/disc), Penicillin-G (P 10 units/ disc), Streptomycin (S 10mcg /disc) and the zones of inhibition were noted and recorded by disc diffusion method (Atlas, 1993).

16S rDNA Amplification and Sequencing

Genomic DNA was isolated using the method of Ausubel *et al*. (2003).The cells from culture plate were scrapped in sterile 0.9% saline water and made pellet in 2.0mL centrifuge tube for further processing of genomic DNA isolation. Quality assessment of genomic DNA was performed by 1% agrose gel electrophoresis. Amplification and sequencing of 16S rDNA gene was done by Eurofins Scientific (Bangalore). For species level identification, sequences were compared with the Genbank database using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results and Discussion

The diluted seaweed extract inoculated in the NFb medium clearly showed the pellicle formation thus confirming the presence of nitrogen fixing bacteria. This was sub-cultured to obtain pure isolates and stored at 4^0C for further morphological, physiological and biochemical studies (Fig. 1). Bacterial numbers associated with *Cladophora vagabunda* was1.6 X 10^7 cells g^{-1}. The colony characterization showed the diameter ranging from 0.3 - 0.4 cm, yellowish pigmentation on NFb plates and microscopic observation revealed cocci shaped gram positive bacterial cells.

The biochemical reactions of the bacteria showed the presence of oxidase enzyme by the change of oxidase disc to blue colour. Addition of hydrogen peroxide on the culture drop showed the effervescence thus showing the presence of catalyse enzyme in the bacteria. The bacteria also showed the production of ammonia with Nesseler's reagent. The isolate showed the positive results for Methyl red, Citrate utilization

Table-1. Composition of Mueller Hinton Agar

Ingredients	Gms / Litre
Beef Extract	2.00gms
Acid Hydrolysate of Casein	17.50gms
Starch	1.50gms
Agar	17.00gms
Distilled water	1000ml
final pH 7.3 ± 0.1 at 25ºC	

and Triple sugar iron Test, Urease, Casein hydrolysis, Nitrate reduction. The isolated endophytic bacteria treated with various antibiotics indicated that this bacterium was susceptible to Penicillin-G and resistant to Amikacin (30mcg), Ampicillin (10mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Gentamicin (10mcg), Streptomycin (10mcg), Tetracycline (10mcg) (Fig.2). The isolates were not able to grow in the medium supplemented with aminoacids as N source such as Arginine, Valine, Leucine, Phenyl alanine but good growth was observed in the NFb medium supplemented with the aminoacids such as Alanine, Aminobutryic acid, Cysteine, Histidine, Serine, Tryptophan, Lycine, Methionine. The alga *C.vagabunda* was reported to posses the amino acids like histidine and lycine (Rani, 2007) and this may be a reason for the association of this bacterium with the alga.

The isolate did not utilize malonic, oxalic and oxoglutaric acids as carbon sources for its growth and utilized organic acids like Malic,Azelicand sugars like Fructose, Galactose, Glucose, Lactose, Mannitol, Meso-erythriolMeso-inositol, Maltose, Mannose, Rhamnose, Sorbitol, Sucrose, Xyloseas carbon substrates. Popper *et al*. (2011) demonstrated that some of the above mentioned carbon sources such as Rhamnose, Xylose, Glucose, Mannose and Galactose are the cell wall constituents of alga which may promote the association of this bacterium with *C.vagabunda*. Recent reports indicated that algal polysaccharides serve as a potential source of carbon and energy for numerous marine bacteria (Hehemann *et al*., 2012), that produce specific molecules, which in turn facilitate the seaweed–bacterial associations (Steinberg *et al*., 2002; Lachnit *et al*., 2013). 16S rDNA sequence analysis indicated that this isolate shows 100 % sequence similarity with *Bacillus cereus* (Accession No KYA47542). The other closely related organisms with this isolate are *Bacillus anthracis* (97.45%), *B.thuringiensis* (97.81%) and *B. toyonensis* (97.81%). This is the first report on the occurrence of nitrogen fixing endophytic bacteria *Bacillus cereus* in the marine alga *C.vagabunda*.

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