Hindi Vidya Prachar Samiti's

RAMNIRANJAN JHUNJHUNWALA COLLEGE

Ghatkopar (W), Mumbai - 400086, Maharashtra, India

in assosiation with

Vanashakti & ATBS

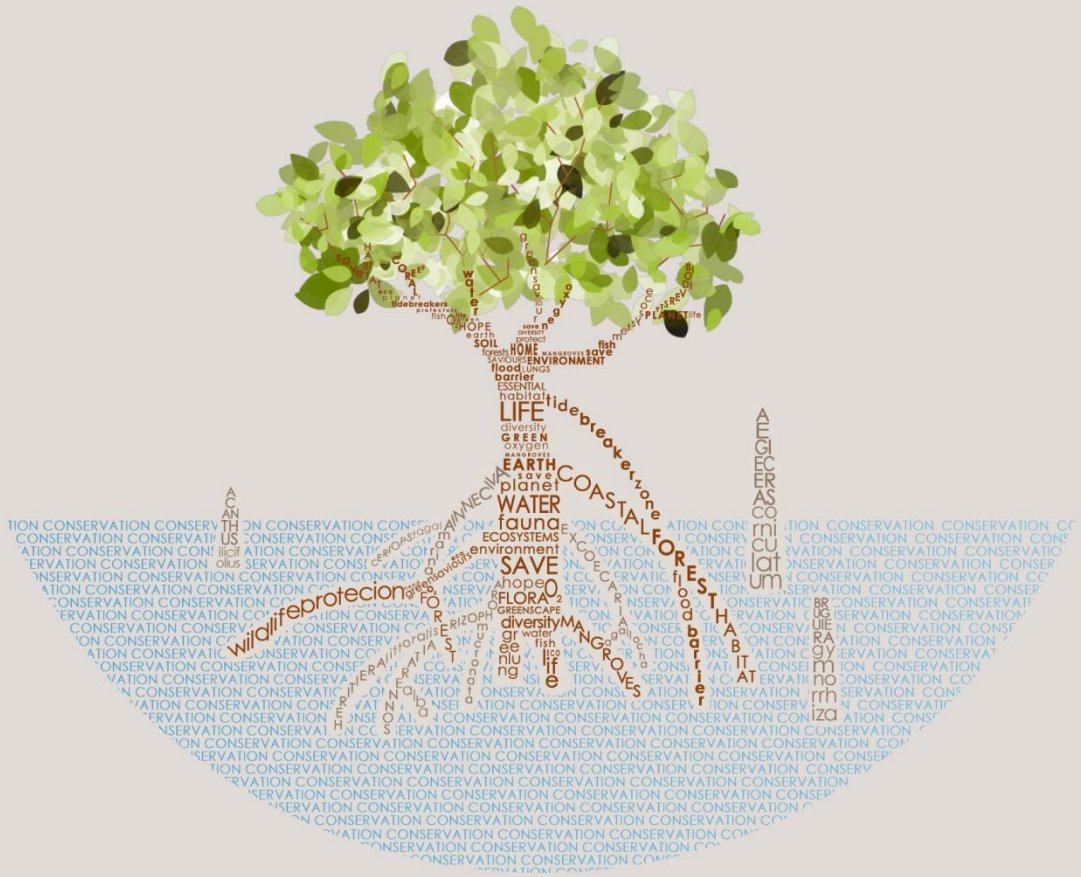
PROCEEDINGS OF

UGC SPONSORED

NATIONAL-LEVEL SEMINAR

ON

Dynamics of Mangrove Ecosystem



29-30 November, 2013

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organized by
Hindi Vidya Prachar Samiti's
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Vanashakti and ATBS

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Foreword.

Dr. Usha Mukundan
Principal, Ramniranjan Jhunjhunwala College

In the early 1970s a local train journey for an enthusiastic botanist from Kalyan to Ghatkopar would mean enjoying the lush green mangroves along the creeks. These mangroves often go unappreciated by the casual observer and may be this is the reason that we have failed to notice their rapid disappearance from the 1990s.

The mangroves represent an extremely important part of the equation of life in all of the world's tropical ecosystems. Mangroves are plants living in the tidal coastal areas between sea and land. The term has been applied to any and all species of trees which occupy this zone of life. All plants share the trait of being able to tolerate partial submersion in high salinity water and deficiency of oxygen in the soil surrounding their roots. Mangroves have evolved ways to deal with these two limitations. These adaptations to survive in conditions of physiological drought could be in the form of pneumatophores for combating oxygen limitations in the saline clayey soil, vivipary ensuring seedling survival in a hostile environment, presence of salt glands in the leaves to remove excess of salts, proton pumps in the cells to ensure salt regulation etc.

Mangrove forests are intertidal wetlands which cover more than 100,000 Km^2 of tropical coastline worldwide. These ecosystems are unique in their structure and are characterized by a variety of plants, animals, microorganisms, which have adapted to the dynamic environmental conditions. The amount of litter produced by a mangrove plant is on an average about 1 kg/square meter/year. Some of this is consumed by small animals like crabs and fishes and majority of it remains in the soil to be broken down by the microbial flora of the soil. The tidal water carries degraded waste to the open sea which feeds the planktons, thus making the mangroves an important source of food and nutrient for the flora and fauna on the reefs and oceans. As mentioned earlier the mangroves have been vanishing rapidly. There are several reasons but all of them narrow down to one component of this planet - *Homo sapienis*; and their activities like urbanization, reclamation, increased resource extraction etc. This has resulted in irreparable loss of mangroves worldwide. Mangroves serve as a buffer zone between the ocean and the shore. Their roots hold the shoreline preventing erosion and attenuating the waves. Clearing the mangrove forests makes the shoreline vulnerable to the erosive effects of the sea and significantly hinders the lifecycles of all the associated flora and fauna which need the mangroves for their survival.

This seminar has been organized by the Department of Biosciences of Ramniranjan Jhunjhunwala College in collaboration with VANSHAKTI and ATBS for two reasons: i) to revisit and rejuvenate the work which has been done in our department for more than five decades on various aspects of eco-physiology of mangroves and ii) to ignite the interest among the student community and educators to understand the intricacies of the mangrove ecosystem and to take care of the fragile mangrove ecosystem.

Contents.

Keynote address.

Dr. Deiva Oswin Stanley
Mangrove Ecologist and Ecosystems Management Specialist,
Eco Balance Consultancy, Vadodara, Gujarat

In the current scenario, the ecosystem services provided by wetlands especially mangroves are better understood, but on the other hand, degradation and conversion of wetlands continues at an astonishing quantum. This is largely due to the fact that the 'full value' of ecosystem functions is less understood or often ignored in policy-making, plans and corporate evaluations of development projects.

I want this seminar to enlighten the issues which perhaps delineate the real mangrove conservation strategies with of the formal ones which never worked on ground as drafted and also elicit the minds of all who were involved in the management of mangroves for better protection.

Mangrove and tidal wetlands provide essential and highly beneficial ecosystem services including habitat/fishery nursery function, shoreline stabilization, carbon capture and storage, and more. In general, mangroves provide the resources several communities in multiple ways i.e, products such as food, raw materials, genetic resources, medicinal resources, ornamental resources; maintain the essential ecological processes and life support systems, like gas and climate regulation, water supply and regulation, waste treatment, pollination, etc.; source of inspiration to human culture and education throughout recreation, cultural, artistic, spiritual and historic information, science and education; provide habitat for flora and fauna in order to maintain biological and genetic diversity.

Despite these cultural, economic, social values, mangroves are among the most threatened of all natural resources and over the past 50 years, approximately one-third of the world's mangrove forests have been lost through conversion to industrial, agricultural and residential developments, but most data show very variable loss rates and often neglecting or confusing the people who were involved in their management and hence, the real impact is less realized.

For years the world's leading environmental scientists have been cautioning us that a gradual warming of global climate was underway, but we paid no heed. The growing scientific consensus is that this warming is largely the result of emissions of carbon dioxide and other greenhouse gases from human activities including industrial processes, fossil fuel combustion, and changes in land use, such as deforestation including mangroves. The scientific community has adduced evidence requiring us to decrease green house gases emissions, mainly CO_2 and methane, by 60-70% from the present, for several decades. This will revolutionize the supply and demand of energy. In India, there is little doubt that climate will become more unstable in the years ahead. We will witness more unpredictable and extreme weather patterns and may be even vanishing of some low lying coastal areas. This is so as sufficient greenhouse gas emissions have already entered the hemisphere to bring about such change. However, little research has been

conducted on how climate change will affect different parts of India, our wetlands and what should be our response to it locally and nationally.

Outcomes of global climate change besides global sea-level rise, such as changes in precipitation, increases in air and sea-surface temperatures, changes in frequency and intensity of storms, changes in prevailing ocean wave heights and direction, and changes in tidal regimes may affect coastal systems, including mangroves. However, projected changes in these parameters are less certain than global change in sea-level, and the response of mangroves and other coastal systems to changes in these parameters are not well understood. Thus concerted effort is the need of the hour and at least for India, this conference may bring some guidance and suggestion.

A 20-cm rise in sea level by 2030 is expected to result from glacial melting and from the thermal expansion of the oceans as water temperatures rise. This may inundate some coastal regions and increase the risk of flooding in many areas. In case of Indian subcontinent population, density often exceeds 1,000 persons per km 2 within low elevation coastal zone (LECZ), which has placed India as one of the most vulnerable nations to climate change- induced sea-level rise.

Acquiring and analyzing remotely sensed imagery to observe historical trends in changes in position of mangrove margins is a challenge. There has also been no coordination between the limited mangroves monitoring work that has been done. The countries and territories with a mangrove monitoring program do not employ standardized techniques to enable a meaningful comparison of results from the different programs. In contrast, several countries do not possess adequate monitoring.

Mangrove boundary position will also be variable where other natural and anthropogenic forces exert a larger influence on mangrove margin position than changing sea-level. Mangrove species have specific tolerance levels for period, frequency, and depth of inundation; salinity regime; wave energy; soil and water pH; sediment composition and stability; nutrient concentrations; and degree of faunal predation; resulting in zonal distribution of mangrove species and determining if a mangrove wetland can become established and survive in a specific location.

Mangroves in India account for about three per cent of the world's mangrove vegetation. Mangrove cover in India is 4,662 sq. km, which is 0.14 per cent of the country's total geographical area. The very dense mangrove comprises 1,403 sq. km (30.10 per cent of the total mangrove cover), moderately dense mangrove is 1,658.12 sq. km (35.57 per cent) while open mangroves cover an area of 1,600.44 sq. km (33 per cent). The rate of carbon sequestered in mangrove mud is estimated to be around 1.5 ton C/ha/year. The upper layers of mangrove sediments have high carbon content (a conservative estimate is 10%). Each hectare of mangrove sediment would then contain some 700 tons of carbon per meter depth. The monetary value of the carbon sequestered by the forest is calculated by using an international price per unit amount of carbon reduced (e.g. $150 per ton of carbon in Norway). Thus mangrove sediment

has a significant role in carbon sequestration. Because the mangroves fix and store significant amounts of carbon, their loss may have impact on global carbon budget. Estimates shows that a loss of about 35% of the world's mangroves has resulted in a net loss of 3.8×1014 g carbon stored as mangrove biomass.

Over the next 25 years, unrestricted clear felling, alteration of hydrology, pollution, aquaculture, and overexploitation of fisheries will be the greatest threats. In addition to that mangroves are even more endangered due to "MASSIVE MANGROVE PLANTING DRIVES" which is interfering with the natural regeneration of a particular mangrove stand where seeds are stripped continuously for planting programs.

Planting mangroves on a large scale itself is a major threat to the naturally prevailing mangroves. The reasons are as below:

a. Fixing larger target areas
b. Seed exploitation for continuous consecutive years from same stand
c. Interference in natural forest regeneration and recruitment of the natural stand
d. High percentage failures in artificial regeneration (seed wastage)
e. Incorrect performance indicators (area coverage and density being indicators of mangrove development projects leaving behind the survival and recruitment capability)
f. Benefit extraction (compulsory fodder collection from undeveloped establishments)
g. Inadequate knowledge about the autecology of mangroves and the ecological functions of mangroves as an ecosystem (drives in planting in wrong location)

The planting targets or area of plantation that has been fixed by the organizations nowadays are extremely inconceivable and several attempts are doomed due to the lack of understanding about the autecology of mangrove species. Example: In general species like *Avicennia* and *Rhizophora* are the target species developed in nurseries planted at sites with varying elevations, tidal inundations, amplitudes, water temperature, pH, salinity, natural and human pressures and more. Mangroves are not comprised of few selected species of plants, it is an ecosystem. However restoration of an ecosystem is not justified by planting selected species of mangroves. Unless the ecosystem in a holistic form is restored, the ecosystem functions and services may not be achieved as estimated or targeted.

To supply seeds for the proposed extensive planting areas by the projects/implementing agencies, seeds from the adjacent natural forests are striped off and depleted in plenty. Therefore, the process of natural regeneration in that particular area, as well as the area up to the seed reaches naturally is interrupted. Continuous process of razing off seeds from natural patches would in long term affect the existing mangroves from regenerating future forests. Neither the mangrove plantation would exist due to the improper management nor do the natural forests exist in future due to severe stress of seed extraction.

Performance Indicator: Currently the indicator of a successful project is accounted to be the area covered by planting selected mangrove species in a stipulated time span and not the survival or

growth performance of the forest or restored ecosystem; hence funding agencies, NGOs or Industries have to take a real stand and fix up the indicator of mangrove regeneration, so as to avoid degradation of the existing and the future fragile ecosystems.

The most important aspects to be taken into account for any mangrove planting or restoration project by the funding agencies are: i) Agency's capacity, ii) Target area - considerably factual, iii) Project design modification with rational approach and technology as per on site feasibility, iv) Compensation policy, v) Realistic long term protection and conservation module.

The area of mangrove plantation given to any organization should not exceed 20 to 50 ha per season. The available potential area for restoration/regeneration or the proportion of coastline available and related details needs to be checked before project allocation to avoid compulsory ecosystem conversion and unsuitable lands being tried and failed just to implement the project and utilize funds. Assessment of fodder/fuel requirement of the target coastal village and Alternative fodder or fuel projects to be implemented before suggesting any mangrove project. The implementing organization should have the schemes to counter /reduce the pressures on mangroves at least to 30-50%. The direct pressures are to be solved or addressed before planting mangroves for the success of community based mangrove planting projects.

- A perfect balanced system of project implementation monitoring to be ensured
- Compulsory continuous monitoring scheme for forest performance assessment to be implemented periodically with stakeholders, local Forest Department and mangrove ecologists
- The members of the Monitoring Committee or the Advisory Board must be on the ground zero to advice the site specific facts than speculate or hypothesize with their experiences
- The monitoring reports need to be made public for scrutiny from any level
- Unless the community is 100 percent mangrove dependent the usage of mangrove area may be reduced by providing alternative schemes
- The protection right of any community developed mangrove forests needs to be shared with the Forests and Environment Department for long term security

Unless the development authorities and managers rationally and consciously treat the ecosystem, mangroves of the y(o)ur Nation may be increasing in statistical papers not in real grounds. Industries commitment of developing mangroves should be strictly altered towards ecological protection and not for the sake of environmental clearance.

Re-establishment of mangroves is always site specific and the strategies of restoration also vary with site. Apart from the regular nursery development and transplantation methods, dredging canals is also in practice which has failed in almost majority of the sites tried in India. Considering this, why can't we think? and go for the replicable long term sustainable hydrological restoration models.

We recommend and promulgate EMR technique for restoring the degraded mangrove areas in India. Ecological Mangrove Restoration, EMR Technique originated by Roy R. "Robin" Lewis III, Certified Professional Wetland Scientist, President, Lewis Environmental Services, Inc., Tampa, Florida, SA, has more than three decades of experience in marine wetland research. The six step approach of EMR on mangrove restoration projects are successfully evidenced in eleven countries, including Nigeria, Vietnam, Hong Kong, Thailand, Cuba, Mexico, Costa Rica and Myanmar and the technology is applicable all over the world. Combining the experience of this and the minds of Indian mangrove managers, we can reach greater heights and protect Mother India and the interdependent livelihoods.

Hydrological Restoration: Day to day mending of the restoration model is not necessary or minimal when the model merges with basic principles of ecology with ecological engineering approach. Important feature in designing a successful mangrove restoration project is determining the normal hydrology (depth, duration and frequency and of tidal flooding) of the existing natural mangrove plant communities (a reference site) in the proposed restoration area. Contrary to popular belief mangroves require some freshwater to grow well, and they are submerged only around 33% of the time. Planting mangroves along an exposed coastline or in too deep water without fresh water input is a recipe for failure. It is been suggested to follow nature and the natural hydrological pattern while trying to restore mangrove areas. Manipulation of the hydrology over the natural pattern would be long lasting and successful. This type of hydrological restoration has been successfully carried out in the developed countries like America in vast areas more than 500 to 600ha in single plots. We too have all the facilities and in house expertise to understand the hydrology of the mangrove forests and let us try to explore the perpetual success with genuine increase in the mangrove area coverage of our country.

While trying to create an identical mangrove ecosystem, efforts at least should be made sincerely with the motive of providing support to the ecosystems and to the community dependent thereof. There should be an authoritative strict regulation for the protection of mangroves exclusive of whether it is community made or natural which are present in the CRZ-I. Department of Forests should play a major role in lobbying for implementation of the Coastal Regulation Zone requirement of a 200 to 500 meter "STRICT NO DEVELOPMENT ZONE". A code for mangrove restoration/regeneration should be made as a mandate for anybody who wishes to serve the ecosystem.

To conclude, I strongly believe that the University of Mumbai has a critical role to play in to increase the mangrove cover through its incredible research capacities, in the basic key fields. I wish to say that given the unprecedented support that we have had from the Government, University Grants Commission, we in the scientific community would resolve to contribute our might to position India in the committee of nations as a formidable player as a leader in scientific and technological innovation.

Bacteria from mangrove sediments of the Indian coast: A review.

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INTRODUCTION

Mangrove ecosystem is found in the intertidal region between marine and terrestrial environment. It comprises of trees and shrubs adapted to grow in waterlogged soil which is constantly subjected to tides. Variations in the structure and function of mangrove ecosystems have generally been described solely on the basis of a hierarchical classification of the physical characteristics of the intertidal environment, including climate, geomorphology, topography, and hydrology (Feller, 2010).

Mangrove habitats contribute to complex food webs and energy transfers. The degradation of mangrove vegetative material produces detritus, which can be defined as organic matter obtained from dead and decaying bodies of organisms. It is rich in energy and contains a large active microbial population both attached and living free. Microscopic examination of decomposing mangrove leaves reveals a complex community composed of fungi, bacteria, protozoa, and micro-algae (Odum and Heald, 1975). Mangrove sediments exhibit peculiar characteristics of high temperatures, high levels of salinity, high pH, high levels of organic matter, low aeration and moisture which provide interesting substrate conditions conducive to the development of diverse microbial communities (Holguin, 2001). Pristine mangrove sediments show a transition gradient from aerobic to anaerobic conditions. They are aerobic on the surface and become anaerobic at shallow depths. This is a result of their fine texture and organic matter. Detritus supported bacterial biomass channels essential elements through the food web by providing nitrogen and phosphorus to protozoa and metazoa, and eventually to commercially important higher-tropic-level organisms such as fish and shrimp (Bano *et al.* 1997). Thus these microbial systems participate in bio-mineralization of organic matter and bio-transformation of minerals (Gupta, 2009).

Bacteria create mutualistic relationships with the mangrove trees. They provide services such as N-fixation while the mangroves trees provide root exudates, stimulating microbial growth activity. Fungi show similar relationships with the mangrove trees. Plants also supply oxygen to these organisms (www.microbewiki.kenyon.edu). There is also competition among the microorganisms because of the limited amount of nutrients available in mangroves. These competitive relationships can even be between a mangrove tree and a colony of bacteria. For example: Tannins from the mangroves control the bacterial counts in the mangrove sediments (Kathiresan *et al.*, 1998). In tropical mangroves, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2% (Alongi 1988). All of these interactions make mangroves highly efficient nutrient cyclers.

The bacteria found in the mangrove sediments can be broadly grouped as nitrogen-fixing bacteria, phosphate solubilising bacteria, sulphate reducing bacteria, photosynthetic anoxygenic bacteria and methanogenic bacteria (Sahoo and Dahl, 2009). Nitrifying bacteria are considered to be a significant group of bacteria that facilitates in mineralization of molecular nitrogen or nitrous oxide from ammonia which is derived from protein of organic debris. The reliable balance between population of nitrifying bacteria and nitrogen fixing bacteria in mangrove sediment is quiet effective for maintaining the fertility of soil sediment with respect to nitrate-nitrogen (Das, 2013). Phosphate-solubilising bacteria are potential suppliers of soluble forms of phosphorus (Vazquez, 2000). The degradation of organic matter in the anaerobic layers occurs mainly through sulfate reduction. In the anoxic sediment layers of salt marshes, 70–90% of the total respiration is by sulfate reduction (Howarth 1984). Methanogenic bacteria regulate the flux of methane in the mangrove ecosystem (Knittel and Boetius, 2009). Sulphate reducing bacteria and methanogenic bacteria are known to co-exist in mangrove sediments and play important role in nutrient cycling within this ecosystem (Taketani, 2010). Photosynthetic anoxygenic bacteria usually belong to purple sulphur bacteria and green and purple non-sulphur bacteria. They use hydrogen sulfide or other reduced inorganic sulphur as an electron donor during photosynthesis (Bryant and Frigaad, 2006). However, it is challenging to determine different components of microbial communities, their function and their interactions. Several studies have been carried out to study the microbes and their role in mangrove sediments. In the present review we report such studies carried out with respect to mangrove sediments of Indian region.

Distribution of Mangroves in India

Mangroves in India account for about 3% of the global mangroves and 8% of Asian mangroves (SFR, 2009; FAO, 2007). These mangrove habitats (69°E-89.5°E longitude and 7°N-23°N latitude) comprise of three distinct zones of East coast habitats, West coast habitats and Island Territories (Singh, 2012). According to Forest Survey of India (FSI), mangrove wetland is 3,48,710 ha out of which nearly 56.7% is present along the East Coast, 23.5% along the West Coast and the remaining 19.8% in Andaman Nicobar islands (Singh, 2012).

East Coast

The east coast of India is bestowed with world's largest mangrove forest, the Gangatic Sunderbans in West Bengal (9,600 km^2). Untawale (1986) suggested that around 80% of India's mangrove area is found on the east coast which can be attributed to the terrain, slope and deltas of rivers like river deltas of Ganges, Brahmaputra, Mahanadi, Godavari, Krishna and Cauvery which have nutrient rich alluvial soil. Several studies on bacteria from mangrove sediments have been reported from the east coast.

Sunderbans

Bhaumic and Barman (1986) reported 24 bacterial strains from mangrove swamps of Sunderbans. In the same year Roy *et al.* reported cellulolytic bacteria from the litters of Sunderban mangrove swamps among which *Kurthia bessonii*, *K. zopfii* and *Micrococcus* varians were most active. Biswas in the same year isolated chitin breaking bacteria from the decomposed litter of mangrove swamps of Sunderbans. *Brevibacterium lypolyticum* was found

as the most active strain. *Bacillus alvei*, *B. stationis*, *Kurthia zopfii* were moderate and *Brevibacterium sociovivium*, *K. bessonii* were very weak for breaking chitin of the decomposed litter complex. Ramanathan *et al.* (2008) carried out a study on microbial diversity at three sampling location viz. Canning, Jharkhali and Pakhiralay, Sunderbans. They evaluated the total microbial load, along with phosphorus solubilising, nitrogen fixing and nitrifying bacteria found in the sediments. They found that sediments associated with dense mangroves (Pakhiralay) showed highest count of cellulose degrading bacteria. Further, these authors concluded that environmental conditions played a significant role in the determination of microbial diversity as well as nutrient behaviour in the sediments. Ghosh and his collaborators (2010) studied the microbial diversity of mangrove sediments of Sunderbans using molecular approach (16S rRNA gene libraries), 8 different bacterial phyla were detected. The major divisions of detected bacterial phyla were *Proteobacteria* (alpha, beta, gamma, and delta), *Flexibacteria* (CFB group), *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Planctomycetes* and *Gammatimonadates*. They have reported for the first time the presence of *Gemmatimonades* in the mangrove sediments. Recently, Das and colleagues (2013) explored the depth profile of nitrifying bacteria, nitrogen fixing bacteria along with total microbial load and other physiochemical parameters like soil temperature, pH and salinity of sediments during pre- monsoon, monsoon and post-monsoon periods at three different sampling stations in Sunderbans. They also found that the microbial population was highest in the deep forest sediment. NO-N concentration increased from surface to 40 cm of depth but decreased from 40 cm to 60 cm suggesting that increasing depth caused unfavorable condition for microorganisms to carry out bio-mineralization processes in sediment.

Bhitarkanika, Odisha

There are few reports (Routray *et al.*, 1995; Mishra *et al.*, 1995; Gupta *et al.*, 2007; Mishra *et al.*, 2009) on bacterial population from Bhitarkanika mangroves. In 2012, Mishra *et al.* and Thatoi *et al.* have reported that nitrogen fixing bacteria (six species, namely, *Klebsiella* sp., *Azotobacter* sp., *Bacillus* sp., *B. alcalophylus, Pseudomonas* sp. and *P. putida*), Denitrifing bacteria, Phosphate solubilizing bacteria (*Pseudomonas* sp., *P. cepacia* and *P. stutzeri, Bacillus* sp., *B. lichiniformis, B. schlegelii* and *B. megaterium*), Sulphate oxidising bacteria (*Desulfotomaculum* sp., *Desulfomonas* sp., *Desulfovibrio* sp., *D. salexigens, Pseudomonas* sp.), cellulose degrading bacteria such as *Pseudomonas* sp., *Bacillus polymyxa, B. mycoides, B. brevis* were reported. Thus bacterial diversity in Bhitarkanika mangrove soil by culture method showed the predominance of bacterial genera such as *Bacillus*, *Pseudomonas*, *Desulfotomaculum*, *Desulfovibrio*, *Desulfomonas*, *Methylococcus*, *Vibrio*, *Micrococcus*, *Klebsiella* and *Azotobacter*. These authors have concluded that microbial dynamics and nutrition balance in the sediment of the Bhitarkanika mangrove forest were interdependent and salinity does not affect microbial functionalities. There was a prominent seasonal variation among the microbial population and the nutrient content. Sediment of Bhitarkanika mangrove forest harbours higher bacterial communities in comparison to fungi and actinomycetes, exhibiting great genetic diversity. Mangrove soil supports higher population of free-living nitrogen fixers, nitrifiers, de-nitrifiers, phosphate solubilizer, cellulose degraders, and sulphur oxidizers, responsible for major biogeo-chemical cycles.

Gaderu River mangroves

Raghavendrudu and Kondalarao (2007) reported occurrence of twenty two strains of actinobacteria from mangrove sediments of the Gaderu River of Gautami-Godavari estuarine system, east coast.

Chollangi

Audipudi *et al.* (2012) isolated 23 bacterial strains from Chollangi mangrove sediments. They have reported occurrence of phosphate solubilising bacteria species, fluorescent *Pseudomonas* sp., *Bacillus* sp. and *Azotobacter* sp.

Pichavaram mangroves

It extends over an area of 1100 km^2 (Venkataraman, 2007). It is the largest mangrove ecosystem in Cuddalore district, Tamil Nadu. Lakhmanaperumalsamy (1987) and later in Ravikumar (1995) reported isolation of nitrogen fixing bacteria from mangrove sediments. Three species of *Azotobacter* viz., *A. vinelandi, A. beijerinckii* and *A. chroococcum* have been identified.

Krishnamurthy *et al.* (1986) isolated Purple bacteria (*Chromatium*) and green bacteria *Chloroflexus* from sediments of Pichavaram mangroves near Porto Novo, Tamil Nadu. Ramamurthy *et al.* (1990) studied the distribution and ecology of methanogenic bacteria from Pichavaram mangrove sediments. Saravanan (1995) found magnetobacteria (*Pseudomonas mesophilica, P. caryophylls* and *Bacillus cereus)* in sediments of Pichavaram. Common genera like *Vibrio, Bacillus, Micrococcus, Pseudomonas, Aeromonas, Flavobacterium* etc. were also reported from Pichavaram mangrove sediments (Sathiyamurthy *et al.*, 1990).

Vethanayagam (1991) isolated purple photosynthetic bacterial strains from mud samples collected from four sites, Madai, Periyakkadavu, Karithurai and Chinnavaikkal, mudflats of Pichavaram mangroves (Tamil Nadu, India) in 1989 and 1990. He reported presence of two major groups of photosynthetic purple bacteria Group 1: purple sulphur bacteria (family *Chromatiaceae*, strains belonging to *Chromatium* sp.); and Group 2: purple non-sulphur bacteria (family *Rhodospirillaceae*, strains apparently belonging to *Rhodopseudomonas* sp.). Isolation of cyanobacteria from Pichavaram was accomplished by Ramchandran and Venugopal (1987) and Ramchandra Rao in 1992. Palaniselvam and Kathiresan (1998) reported that cyanobacterial species, of *Phormidium*, were well-adapted to saline stress and can be used as bio-fertilizer and in preparation of shrimp feed formulations. Nedumaran *et al.* (2008) carried out study on seasonal variations at two stations in Pichavaram. Many cyanobacterial species were found to be associated with the aerial roots of the mangrove plants, 23 cyanobacterial species were recorded in association with the mangrove vegetation of which *Lyngybya major*, *Oscillatoria agardhii* and *Phormedium tenue* were predominant. They found that *Avicennia marina* harboured maximum cyanobacterial species than the other mangrove plants.

The work in microbial diversity has been excellently summarized in the review on Pichavarm mangroves by Kathiresan, 2000. Ganesan *et al.* (2011) have isolated halophillic bacteria *Vibrio harveyi, Halomonas* sp., *Vibrio fluvialis* and *Halobacterium* sp., strains from soil samples collected

from Pichavaram Mangrove forest. These were chemoorganotrophic, Gram negative rods, which synthesized biopolymers. Lakshmipriya and Sivakumar (2012) screened Pichavarm sediments for heterotropic bacteria which produced exopolysaccharides. Abirami *et al*. (2013) have isolated photosynthetic purple non sulphur bacterium (*Rhodopseudomonas* sp.) from mangrove sediments of Pichavarm. This bacterium showed antibacterial activity against 5 clinical bacterial pathogens. Pichavaram mangroves have been exhaustively studies for their microbial communities, further these bacteria were also explored for their potential application in various fields.

Muthupet mangroves

Sudha *et al*. (2007) recorded 63 species of cyanobacteria, belonging to 21 genera and 9 families, out of which members of family *Oscillatoriaceae* were predominant and Chroococcaceae were co-dominant. This study was carried out in 2002-2003 at Muthupet mangroves, Tamilnadu. Ashokkumar *et al*. (2011) recorded 9 coliforms, *Vibrio cholerae*, *V*. *parahaemolyticus, E*. *coli, Klebsiella pneumonae, Shigella dysenteri, Streptococcus faecalis* and *Pseudomonas aeruginosa* from Muthupet mangroves, Tamil Nadu.

Kodiakkarai

Mohanraju and Natarajan (1992) studied the occurrence of methanogenic bacteria in the mangrove sediments of Kodiakkarai, Tamil Nadu, over a period of one year. They established the correlation between the environmental factors and number of methanogenic bacteria.

Vellar mangroves

Nabeel and collaborators (2008) published data on mangrove system of the Vellar estuary, South East Coast of India, which revealed that mangrove-associated microbes significantly contribute to the food web of detritus in a mangrove ecosystem. The prominent species of microbes in decomposing mangrove leaves were *Aeromonas hydrophila, A*. *punctata, Azotobacter beijerinckii, A*. *vinelandii, A*. *chroococcum, Bacillus cereus, Corynebacterium xerosis, Escherichia coli, Lactobacillus* sp.*,* and *Pseudomonas aeruginosa*. They have used stable isotopes along with the fatty acid biomarkers as tools for identifying the trophic interactions among dominant producers and consumers in the mangrove ecosystem.

Muthukuda mangroves

Muthukuda mangrove sediments (Latitude 9°54'10.20"N; Longitude 79°09'07.13"E), are located 20 km north of Thondi, South east coast of India. Govindasamy and his colleagues (2011) screened for the first time the heterotrophic bacteria from this area. *Bacillus subtilis*, *Streptococcus* sp., *Staphylococcus* sp.*, Carnybacterium* sp., *Photobacterium sp*. *Enterobacteriaceae* sp., *Escherichia coli* and *Actinobacteria* sp. were reported.

Andaman and Nicobar Islands

The Andaman and Nicobar Islands carry about 13% (Singh, 2012) of Indian mangrove cover. Shome *et al*. (1995) studied bacterial flora from mangrove sediments from South Andaman. They have reported presence of *Bacillus sp*. (50%), *Aeromonas, Vibrio, Eschrichia, Enterobacter,*

Corynaebaterium, Kurthia, Staphillococcus, Micrococcus and *Listeria*. In another study conducted in 2001, Shome and Shome have reported L-asparginase producing bacteria from the mangrove sediments of Andaman Islands.

Geeta *et al*. (2007) reported that around 50% of the total bacteria isolated from mangrove sediments of Andaman and Nicobar islands, belonged to Bacillus species. They have also reported larvicidal activity of some strains against mosquito larvae. This work has resulted in development of a novel mosquitocidal biosurfactant produced by *Bacillus subtilis* ssp. *subtilis* (VCRC B471) known as Surfactin (2010).

Gobalakrishnan *et al*. (2013) enumerated total heterotopic bacteria from Havelock Island. They isolated around 13 genera from the mangrove sediments, such as, *Escherichia, Pseudomonas, Flavobacterium, Salmonella, Vibrio, Shigella, Klebsiella, Micrococcus, Cornybacterium, Enterococcus, Aeromonas, Streptococcus* and *Staphylococcus*. There are very few reports available on the bacteria of mangrove sediments of Andaman and Nicobar Islands. This ecosystem remains largely unexplored.

West Coast

West coast occupies only 20% of the total mangrove cover of India. Gujarat has about 23% of India's estimated mangrove cover of 4.88 lakh ha. It is considered to be the second largest area along the Indian coast after Sunderbans (Singh, 2012).

Kachchh

The Gulf of Kachchh, Gujarat, India lies approximately between latitudes 22° to 23°N and between longitudes 68° to 70°30' E with an area of approximately 7300 km^2. Goutam and Ramanathan (2012) estimated population of the free living nitrogen fixing, phosphate solubilising, cellulose degrading bacteria from the mangrove sediments at 11 different locations of The Gulf of Kachchh. They observed that higher population count in the microbes synchronized with the nutrients availability in the surface water.

Goa

Sulphate reducing bacteria were reported by Saxena *et al*. (1988). Lokabharati *et al*., in 1991 isolated and studied Sulphate reducing bacteria from three mangrove stations along the Zuari Estuary, Goa. *Desulfovibrio desulfuricans, Desulfovibrio desulfovibrio aestuarii, Desulfovibrio salexigens, Desulfovibrio sapovorans, Desulfotomaculum acetoxidans, Desulfotomaculum orientis, Desulfosarcina variabilis* and *Desulfococcus multivorans* were isolated and classified into 4 genera. They were found to be nutritionally versatile.

Iron oxidising and iron reducing bacteria has been reported from mangroves of Goa and Konkan (Panchanandikar, 1993) as reported in the review by Sunil Kumar (2011). Sardesai and Bhosale (2002) isolated a unique strain of Bacillus, which was solvent toleant and hydrocarbon degrading from the mangrove ecosystem of Mandovi estuary, Goa. Marbaniang and Nazareth (2007)

isolated halotolerant *Penicillium* species from mangroves of Goa. These species were found to be resistant to heavy metals such as lead, copper and cadmium.

Dastager and Damare (2013) reported occurrence and distribution of actinobacteria group of bacteria capable of dissolving insoluble phosphates in sediments of Chorao Island, Goa, located between the Mandovi and the Mapusa rivers and the western side of the island is occupied by a thick mangrove forest of about 1.78 km^2. The geographical location of the station is 15°32'34" latitude and 73°55'15" longitude. A total of 200 bacterial isolates of actinobacteria were separated. Thirteen different isolates exhibited Phosphate solubilizing bacteria. All the isolated belonged to genera like *Streptomyces, Microbacterium*, *Angustibacter*, *Kocuria*, *Isoptericola* and *Agromyces*.

Cochin

Chandrika and Kala (1993) have reported phototropic thionic bacteria and actimycetes (fungus like bacteria) from anaerobic and micro aerophilic strata of Cochin mangroves. Joseph and Raj (2007) isolated five aerobic endospore forming bacilli from Mangrove soil at Cochin, Kerala. These bacilli were found to be highly thermotolerant (55°C), pH tolerant (upto 11) and were halotolerant.

Mumbai

Bhat and Shewade (2013) isolated bacteria from mangrove sediments from 5 different stations of CBD Belapur, Navi Mumbai. All the isolates were gram positive and belonged to genera Bacillus. These isolates exhibited L-asparginase activity and few isolates showed Protease and amylase activity. Most of them were thermotolerant (55°C) and could tolerate heavy metal stress.

CONCLUSIONS

Mangroves provide shelter to a variety of flora and fauna. They serve as breeding ground, nurseries for diverse group of animals. The living vegetation is a valuable food resource for insects, crustaceans and some vertebrates. Mangrove ecosystems have very high productivity. Most of the mangrove production is transferred to other trophic levels through litterfall and detrital pathways (Kathiresan, 2012).It is a unique ecosystem which not only influences the ecological and environmental aspect but also contributes significantly to the socio-economic perspective. The well-being of mangroves is dependent on the diverse, and largely unexplored, microbial and faunal activities that transform and recycle nutrients in the ecosystem. It is vital that the health of the benthic microbial communities be maintained because these organisms are responsible for conserving the scarce nutrients within the ecosystem. Little is known about the activities of microbes in mangrove waters and sediments and effort must be made to further elucidate the intricacies and complexities of microbial activities in mangrove ecosystems and their impact on the productivity of the ecosystem.

The bacterial community of mangrove sediments is an invaluable resource for bioprospecting. It can be used as region for discovery of new thermotolerant, halotolerant bacteria which can secrete new antimicrobials, larvicidals, enzymes etc. these bacteria can also be used in

bioremediation as many of them are found to be resistant to contamination of heavy metals. They can be used as biofertilizers. Thus mangrove sediments due to their unique environment can serve as a renewable commercial resource for sustained human activity.

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Associate algal flora in the mangrove habitat of Achara creek.

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ABSTRACT

The algal flora in mangrove habitats is very much fascinating, somewhat distinct from the fresh water habitat algae. It has been observed that this habitat supports luxurious growth of marine algae and sea grasses. It is the safest environment in which the flora present is attached with the soil, mangrove roots and stem or trunk bases throughout the year. The algae, sea grasses and phytoplankton along with the dense fauna, form a complex food chain and web for the mangrove ecosystem. The mangrove environment from all the corners is secured environment in which daily inflow of various resource organisms enter and settle every moment along with the tidal flow. The thick population of organisms every day and moment recruit such habitat for various purposes in which feeding, grazing and spawning take place quit often. This ecosystem directly provides appetizing nutritious organic food materials to the organisms. The primary production of such areas is always high except during monsoon. The algae can withstand saline water and survive well in these habitats. Their physiological functions are set up in such a way that they can cultivate and perform the physiological activities in the saline water ranging between 10-32 ppm salinity, whereas the freshwater algae do not grow or survive under such circumstances. The percentage composition of the algae is limited in terms of species composition and distribution.

KEYWORDS: Mangrove, algae, Achara

INTRODUCTION

Mangrove forest is dynamic and changes quite frequently. The study conducted highlighted the amazing fact the way these algal flora support the mangrove ecosystem. During this period it was found that in the intertidal mangrove habitats the salt tolerant algae are from three distinct habitats i.e. free floating planktonic form, benthic form which grow on mature soil substratum rich with organic matter and further enrich the soil and the third form is the periphytic algae attached on the periphery of aerial roots like pneumatophores, stilt roots, prop roots, root buttresses, flank roots and also on the trunk base of regular tidal inundated branches of these mangroves. Frequently and during tidal inundation phases, these algae receive the moisture and nutrients from the tidal waters and tidal water carrying silt. They also get the opportunity to take part in the process of photosynthesis during exposure of these algal habitats at low tides. Besides these, the periphytic algal bloom is largely grazed by the mollusc, shell fish as well as fishes.

The algal bloom shows seasonal variation in which the pre-monsoon and post monsoon seasons favour luxurious growth. The algal growth in an aquatic medium is directly correlated with the density of the fauna and high fishery yield potential. The Achara mangrove habitat supports variety of marine algae like chlorophyta, phaeophyta, rhodophyta, cyanophyta and sea grasses. The algal growth serves as the protective covering during adverse conditions preventing heavy predation of fry as well larvae of prawns fishes and molluscs. The rate of predation was inversely proportional with the density of resource organisms in the mangrove environment. The standing crops of phytoplankton in terms of biomass, chlorophyll a and total cells were recorded at high level in mangrove ecosystem. It was also noticed that the biomass of zooplankton was high during the algal bloom. The assessment of the fishery in an aquatic ecosystem is fully dependent on the total primary organic production. Estimation of primary production is usually done with phytoplankton community though other autotrophic groups such as periphyton and macrophytes that are present in varying quantities.

Seaweed industry is an economically flourishing industry in India. Nearby 700 species of seaweeds have been reported in East and West coasts of India. They can be used for the production of various photochemical purposes as well as their extracts are used in cosmetic industries. Traditionally even now the coastal living people utilise the seaweeds as fertilisers over many decades. The crushed powder of these algae is an excellent nitrogenous nutrient for the paddy fields. It acts as biofertilizer that accelerates the vegetative as well as reproductive growth of paddy fields and yields more production. The coastal people are actively engaged in the collection of the algal species like *Gracilaria* sp., *Sargassum* sp., *Turbinaria* sp., *Ulva* sp. etc. in the month of December to May. *Gelidiella acerosa* is economically important for the extraction agar. Many species of *Caulerpa* sp., *Undaria* sp., *Laminuria* sp., *Porphyra* sp., *Gracilaria* sp., *Hypnea* sp. etc. of chlorophyta, phaeophyta and rhodophyta are cultivated in different countries on the basis of their demands and various techniques are adopted for their development. In India algae are mainly used for the extraction of agar and algin.

MATERIALS AND METHODS

The mangrove habitat of Achara, was studied monthly for marine algae and sea grass distribution at A_1, A_2 and A_3 stations. The plant samples along with thallus and rhizoides were collected and washed thoroughly to remove epiphytes, accumulated mud, sand, debris and then preserved in 4% formalin for further identification. The point method was adapted for percentage contribution of individual algae.

RESULTS

The mangrove ecosystem is the main stock of varieties of organic nutrients. These nutrients are continuously distributed to the surrounding media. This is the main attraction to the economically important resource organisms. The primary production in this habitat is the main feature of the environment. Algae play an important role in the food chain of such ecosystem. In the present investigation species of seaweeds were recorded in 24 months period. The algal growth was either epiterranean or epiphytic on mangrove trunks, pnuematophores, stilt roots, prop roots and plant debris.

The percentage compositions of individual algae in relation to monsoon, post-monsoon and pre-monsoon were recorded as in Table 1,2,3 and 4. It was clearly noticed that post monsoon was the most favourable period for lavish growth of algae both qualitatively and quantitatively. During rainy season due to heavy current of fresh water and interference, they were washed off along with the flow of water. During monsoon most of the algae decompose and increase the organic load which later on provides excellent food to the juveniles of various organisms. The lagoons are the best habitat for the protection of algae in the month of June-July compared to open regions of the creek. The percentage contribution of algae in post and pre-monsoon were quite high compared to monsoon because of heavy rainfall. The cholorophyta, rhodophyta and cyanophyta were dominant groups amongst all.

Table 1:Percentage composition of chlorophyta in Achara habitat.

No.	Name of the species	Monsoon	Post-Monsoon	Pre-Monsoon
1	*Ulva lactuca*	3.12	11.15	12.20
2	*Ulva reticulate*	-	6.19	7.15
3	*Ulva fasciata*	11.13	2.18	3.17
4	*Ulva patengensis*	8.18	9.12	10.13
5	*Enteromorpha intestinallis*	16.14	11.16	12.17
6	*Enteromorpha compressa*	2.21	4.12	5.12
7	*Rhizoclorium hookeri*	4.15	1.22	2.18
8	*Chaetomorpha gracillaris*	6.17	6.15	7.15
9	*Caulerpa sertularioides*	3.45	4.18	5.12
10	*Caulerpa racemosa*	1.2	2.17	1.19

Table 2: Percentage composition of phaephyta in Achara habitat.

No.	Name of the species	Monsoon	Post-Monsoon	Pre-Monsoon
1	*Sargassum wightii*	19.13	13.15	17.18
2	*Sargassum duplicatum*	-	6.11	13.12
3	*Sargassum myriocystum*	12.12	6.19	7.12
4	*Sargassum tenerrimum*	14.16	5.12	8.01
5	*Cystoseira trinodis*	-	5.15	5.11
6	*Hormophysa triquetra*	-	8.09	6.07
7	*Dictyota indica*	12.1	11.17	5.11
8	*Giffordia mitchellae*	8.17	10.12	3.12
9	*Colpomenia sinuosa*	7.15	8.21	9.12
10	*Padina tetrastromatica*	27.17	26.69	25.17

Table 3: Percentage composition of rhodophyta in Achara habitat.

No.	Name of the species	Monsoon	Post-Monsoon	Pre-Monsoon
1	*Gelidiella indica*	-	5.12	7.12
2	*Gelidiella pusillum*	5.12	7.06	8.25
3	*Gracilaria arcuta*	21.17	23.01	18.16
4	*Gracilaria corticata*	8.12	9.04	8.02
5	*Gracilaria folifera*	14.18	12.11	10.04
6	*Gracilaria edulis*	8.12	9.02	11.12
7	*Hypnea musciformis*	-	2.11	3.13
8	*Hypnea valentiae*	-	4.12	5.07
9	*Acanthophora spicifera*	13.11	8.12	6.08
10	*Porphyra indica*	8.21	4.14	4.03

Table 4: Percentage composition of cyanophyta in Achara habitat.

Sr.No.	Name of the species	Monsoon	Post-Monsoon	Pre-Monsoon
1	*Phormidium fragile*	-	3.05	3.12
2	*Microcoleus chthonoplastes*	8.14	5.12	4.03
3	*Xenococcus chaetomorphae*	6.04	7.17	6.15
4	*Xenococcus cladophorae*	5.12	5.43	4.49
5	*Oscillatoria princeps*	2.15	4.12	2.12
6	*Oscillatoria martini*	9.17	8.12	5.11
7	*Anaebaena variabilis*	12.18	9.19	10.08
8	*Anaebaena oryzea*	7.12	8.93	9.25
9	*Calothrix crustacea*	-	6.19	1.16
10	*Spirulina* sp.	12.03	-	9.13

DISCUSSION

The lush growth of seaweeds and sea grass fully depend on the hydrobiological parameters of the aquatic environment. Amongst all the parameters, salinity, temperature and light are crucial that accelerate the bloom of algae. The salinity is directly correlated with the growth of the algae therefore monsoon is almost an unfavourable season for most of the species of seaweeds. Mangrove water is always turbid which does not allow much light to penetrate upon them during high tide. The dense forest of mangrove swamps does not allow the light to easily fall on the water surface and hence there is continuous disturbance of wavelength in mangrove habitat. This may be the main reason along with high turbidity of water to decrease the algal growth in monsoon. The grass carries maximum photosynthesis during low tide. The sea grass beds in mangrove ecosystems promote sedimentation by trapping water borne particles and by the retention of organic material derived from sea grass. This period is not that stable for most of the physical as well as chemical factors so, its natural in adverse conditions, retarded growth of many species takes place. The post monsoon then stabilises the environmental condition by which it favours the growth. Thus it is clearly understood that the luxurious growth of algae in mangrove habitat is directly related with the environmental parameters.

Thus it was noticed that phytoplankton seaweeds and sea grass beds in the mangroves of Achara contribute towards food chain in the mangrove ecosystem. Achara creek is associated

with large number of lagoons containing thick belt of mangroves, which look promising for cultivation of economically important seaweeds. The marine algae of Maharashtra coast was reported by Chauhan (1978). The seaweed resources survey along the Goa coast was conducted by Untawale *et al.* (1975). In South Konkan, the coastal living people collect the seaweeds in post-monsoon and pre-monsoon period after their normal fishing activities. During peak period species like *Sargassum*, *Padina*, *Turbinaria* are collected by many fishermen who leave fishing activity for sometime. Species like *Sargassum*, *Padina* are major constituents of the seaweeds that had been harvested for commercial purpose.

The quality of iodine, mineral, vitamins present in mainly green, brown and red algae of Gujarat coast was determined by Pillai (1956), Kappanna *et al.* (1962). The protein content in the seaweeds was estimated by many scientists like Pillai (1957), Neela (1956) in species like *Gracilaria, Turbinaria* and *Sargassum*. The seaweeds are a very rich source for agar and algin. They are also used as livestock feed and biofertilisers. Agar and algin are also used in food, dairy industries and confectionary as gelling stabilising and thickening agent mainly in the manufacture of sweets, jellies, ice-creams, also used in laboratories for experimental use. After the valuable contribution of Boergesen not much of work has been done on the morphology and taxonomy of Indian marine algae during the past four decades. A general review of the marine algae of the West coast was published by Biswas (1945), Srinivasan (1946; 1960) has given a detailed account of marine algae on the East and West coast of India and recorded 162 genera and more than 413 species of marine algae.

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Electrophoretic studies of isoenzymes of glutamate dehydrogenase (EC 1.4.1.3) (GDH) in *Cressa cretica* L.

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ABSTRACT

Cressa cretica L. is an associate halophyte which generally grows near marshy land which is less frequently washed by marine water and the soil is generally deficient in nitrogen content. The plant has adapted to nitrogen stress by evolving efficient nitrogen assimilating isozymes. In present investigation 3 isoenzymes of glutamate dehydrogenase (EC 1.4.1.3) (GDH) are separated electrophoretically from the leaves of *Cressa cretica* L.

KEYWORDS: *Cressa cretica*, glumate dehydrogenase, isoenzymes

INTRODUCTION

Markert and Moller (1959) described the term 'isozyme' for the first time for the multiple forms of an enzyme. These enzymes are with similar or identical substrate specificity, occur in the same organism but differ in certain physico-chemical properties. According to Vessel and Bearn (1962), the duplication of gene with subsequent mutations at both the parent and the daughter loci could be the biological mechanism for producing multiple molecular forms of an enzyme. However, Rider and Taylor (1980) suggested that the multiplicity of the enzyme might be due to genetic and primary causes, where organism carries multiple genes, each one coding a different type of enzyme subunit. It may also be due to posttranslational causes, as homogenous enzyme subunits are modified differently resulting in a range of subunits from a single gene. Multiple forms of an enzyme may also result due to the influence of environment on the molecules of proteins. Lumry and Erying (1954) termed these forms as 'conformational forms'.

Isozymes commonly occur in microorganisms (Brown *et al.,* 1975) plants (Liu, 1975) and animals (Georgiev, 1975) and have been extensively studied by several workers (Vezina *et al.,* 1987; Cai and Wong, 1989; Chen and Cullimore, 1989). Isozymes are known to function in the biochemical modulation of intracellular reactions (Ting *et al.,* 1975). Even though isozymes exhibit tissue specificity, which implies a significant physiological role for them, they are essentially alike in enzyme activity (Markert and Apella, 1961). According to Kay *et al.* (1967), isozymes differ from one another in several catalytic properties, as affinity for the substrate, behaviour towards coenzyme, pH optima, thermal stability and sensitivity to inhibitors.

Environmental conditions are known to have a great influence on the isozyme pattern of different enzymes. Pahlich (1972) observed changes in the isozyme pattern and electrophoretic

mobility of GDH due to environmental changes. Similarly, Srivastava and Singh (1987) also reported variation in the number of GDH isozymes due to variation in nutritional and environmental conditions. Modified activity of certain enzymes under saline environment is either due to conformational changes (Kalir and Poljakoff-Mayber, 1975) of the enzyme molecule or due to the changes in isozyme pattern (Sanglikar, 1982). Hasson-Porath and Poljakoff-Mayber (1969) based on their isozyme pattern of MDH reported that Na_2SO_4 did not affect the isozyme pattern. However, NaCl caused the appearance of new isoenzymes in pea root tips. According to Somero (1975), isoenzymes serve an important mechanism for broadening the environmental tolerance range of the organisms.

MATERIAL AND METHODS

The plant material of *Cressa cretica* L. were collected from the natural habitat and brought to the laboratory in polythene bags and used for the study of protein profile and isozymes of glutamate dehydrogenase (GDH).

Enzyme Extraction

Plants were collected from natural habitats and brought to the laboratory in polythene bags. The plants were washed with deionized water and blotted dry. One g fresh leaf material was ground vigorously in 10 ml chilled extraction buffer (0.2 M Tris-HCl pH 8, 3.5 mM $MgCl_2$ and 2.5% Polyethylene glycol) using a mortar and pestle. The homogenate was then passed through 4 layers of muslin and the filtrate was centrifuged at 10,000 rpm for 20 minutes. The debris was removed and the supernatant was used as the enzyme source. Throughout the extraction, procedure the temperature was maintained around 0°C±2°C. Minimum quantity of buffer was used to get concentrated extract.

Polyacryamide Gel Electrophoresis

PAGE was carried out at low temperature (4°C±1°C) according to the method of Zweig and Whitaker (1967). The gels used for the separation of anionic samples in the present experiment were 7.5% running gels; stacking pH 8.3. The gels were cast in neutral glass plates (7 cm long, 8 cm wide and 0.75 mm thickness with 10 wells) of vertical electrophoresis unit (BIO-RAD, MINI PROTEAN-II). The gel was polymerized with polymerizing catalysts like ammonium persulphate and TEMED (accelerator of polymerization of gel). For the electrophoresis, the buffer was prepared using 6 g Tris-HCl, 28.8 g glycine and volume was made to one liter. The buffer was diluted 10 times and pH was adjusted to 8.3 before use.

The enzyme extract was mixed with bromophenol blue as a front marker and loaded in a polymerized gel wells. The entire gel plate was fitted in the electrophoretic unit, it was flooded with running buffer and the anodal, and cathodal ends were connected to the power system. The electrophoretic run was carried out at a current of about 2 mA /well, at a constant voltage of 150 volts. The run was carried out for about 90 minutes until the bromophenol marker front migrated to the other end of the plate. Subsequently, the gel was loosened with a jet of water and removed from the glass plate.

Detection of GDH

Isoenzymes of GDH were detected on the gels by the method of Brewer and Singh (1970).The staining mixture was prepared by dissolving 0.25 M L-glutamic acid, 1.5 mM NAD, 0.163 mM phenazine metho sulphate (PMS) and 0.43 mM nitroblue tetrazolium (NBT) in 0.125 M phosphate buffer (pH 9.0). The staining mixture was prepared shortly before use as the reactants are less stable in solution.

For detecting GDH isoenzymes, the gels were incubated in the staining mixture at 37˚C. GDH activity generates NADH, which reduces phenazine, which in turn reduces NBT. The reduced NBT is an insoluble formazan, which is coloured.

RESULTS AND DISCUSSION

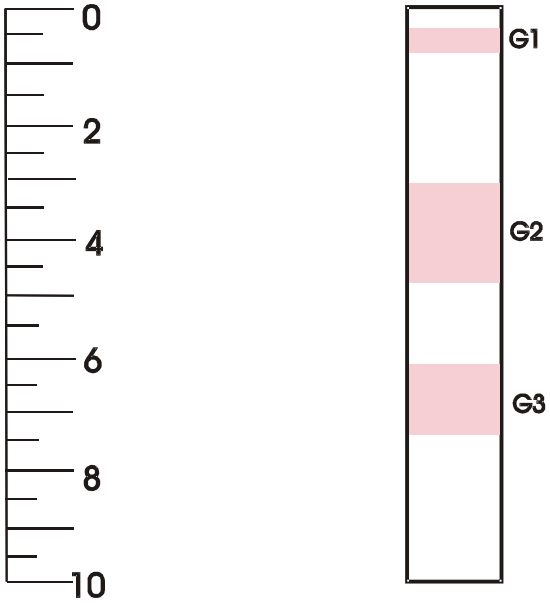

Fig. 1: Zymogram depicting isoenzymes of glutamate dehydrogenase (GDH) from the leaves of *Cressa cretica* L.

Three bands G1, G3 and G5 of Rf values of 0.062, 0.416 and 0.656 were observed representing GDH activity in the leaves of *C. cretica*. (Figure 1). Isoenzymic nature of GDH in higher plants is well established (Thurman *et al.,* 1965; Yue, 1969). Number of GDH isoenzymes varied amongst different plants studied and upto 7 isozymes have been reported in *Pisum sativum* (Hartman, 1973), *Medicago sativa* (Hartman *et al.,* 1973), *Arabidopsis thaliana* (Cammaerts and Jacob, 1985) and *Vitis vinifera* (Loulakakis and Angelakis, 1990). Ratajczak *et al.* (1986) observed 8 isozymes of GDH in lupin root nodules. According to Srivastava and Singh (1987) isozymic number of GDH enzyme varies with plant species as well as with nutritional and environmental conditions.

Kanamori*et al.* (1972) detected new isozymes of GDH on the zymogram of PAGE due to ammonia treatment. Similar observation of synthesis of new isozymes of GDH under high levels of ammonia in the cellular environment was made by several workers (Ratajczak*et al.,* 1977, Givan, 1979). According to Loulakakis and Angelakis (1991), ammonia induces expression of isozyme. Plants growing under high salt concentration are known to accumulate high

concentration of ammonia (Strogonov, 1964), which may lead to synthesis of new isozyme of GDH. Isozymic studies of GDH in leguminous plants have suggested that the GDH isozymic pattern is the result of an adaptation of the cell to nitrogen metabolism (Mazurowa *et al.,* 1980) suggesting a physiological role of GDH isoenzymes in the regulation of nitrogen metabolism. However, in the present study three isozymes of GDH were detected in the leaves. This may be in response to halophytic nature of the plant.

CONCLUSION

Electrophoretic studies of Glutamate dehydrogenase (GDH)enzyme extracted from the leaves of *C. cretica,* revealed 3 isoenzymes of Rf values of 0.062, 0416 and 0.656. From the present investigation it can be concluded that even though the plants of *C. cretica* an associate halophyte generally grow in soil which is poor in nitrogen content, it has high nitrogen content in the plant which is due to efficient enzyme machinery responsible for assimilating the nitrate and ammonia available to the plant.

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Studies of enzyme nitrate reductase in *Sesuvium portulacastrum* L., an associate halophyte.

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ABSTRACT

Sesuvium portulacastrum L. (Aizoaceae) is a pioneer, psammophytic associate halophyte of subtropical, Mediterranean regions. It dominates in coastal and warmer zones of the world. Apart from being utilized as a vegetable by local people and forage for domestic animals in the coastal area, environmentally too it is utilized for the bio-reclamation of saline soil in the arid and semiarid regions. Coastal soils as well as sea water, which permeate the soil characteristically, have a poor content of available nitrogen. In contrast, halophytes which inhabit these areas have high protein content. This is because halophytes have the ability to conserve nitrogen and recycle it through their body metabolism. Efficient enzyme mechanism for Nitrogen metabolism in halophytes has been thoroughly studied and communicated.

In present investigation, S*esuvium portulacastrum* L. is used as a model system representing an associate halophyte with efficacy in Nitrogen utilization in saline conditions. To begin with kinetics of enzyme nitrate reductase (NR- E.C.1.6.6.1) is studied in terms of effect of varying temperature, pH and concentration of enzyme and substrate. The same study would be extended to other important enzymes of Nitrogen metabolism to get an insight in efficacy of such halophytes to conserve available Nitrogen from saline soils and help in phytoremediation of saline soils.

KEYWORDS*:* Associate halophyte, nitrogen metabolism

INTRODUCTION

Associate halophytes grow in the fringe area of mangrove swamps, get inundated 1-5 times per fortnight during spring tide and are also found growing in mesophytic habitat. *Sesuvium portulacastrum* L. (Sea purslane) is one such fast growing, herbaceous, dichotomous, perennial, pioneer, psammophytic halophyte naturally growing in the subtropical, Mediterranean, coastal and warmer zones of the world. *Sesuvium portulacastrum* is found occurring on the coastlines of five continents and widely distributed as a pioneer strand species on tropical and subtropical shores (Lonard and Judd, 1997). It grows naturally in the subtropical, Mediterranean coastal and warmer areas around the world (Balasubramanian *et al*., 2006).

Sesuvium frequently grows in the backshore topographic zone on sandy beaches as the initial pioneer species just above the high tide line on barrier islands. It is also a common species on the margins of hurricane wash-over channels, disturbed roadsides, and tidal flats (Lonard and Judd, 1997). In the tropics, the species occurs on estuarine mudflats adjacent to mangrove swamps (Joshi and Bhosale, 1982), in salt marshes and on calcareous shorelines, on the margins of lagoons, on coral sand and rubble shorelines (Lonard and Judd, 1997). It is also found along coasts and river mouths and in lower mountains (Hammer, 2001).

In India, it grows among the eastern and western coastal regions as inland or seashore species including areas where mangrove plants are found. This includes coastal regions of Gujarat, Maharashtra, Goa, Kerala, Tamilnadu, Andhra Pradesh and Orissa

MATERIALS AND METHODS

Healthy plants of *Sesuvium portulacastrum* were collected from natural habitat and brought to the laboratory in a polythene bags. The plants were washed with distilled water and air-dried. These plants were used for *in vivo* assay of NR, according to the method of Klepper *et al*. (1971).

In vivo assay of NR

Leaves were cut with a pair of scissor into very small pieces. The assay mixture consisted of 2 ml of Tris-HCl buffer of pH 7.5, 2 ml of 0.1 N KNO_3 and chopped leaves (100 mg). The tubes were incubated at 37˚C for one hour. The reaction was terminated by adding 1ml of 1% sulphanilamide, followed by the addition of 0.02% NEDD. The pink colour developed was estimated spectrophotometrically using Equiptronics digital spectrophotometer (EQ-820) at 540 nm. The specific activity of the enzyme NR was expressed as mM NO_2/g fresh weight/60 min.

NR of the leaves was assayed at different pH ranging from 4 to 9. The enzyme activity was recorded as function of time from 5 to 90 minutes. Substrate variation for NR was carried out for different concentrations of KNO_3 from 100 mM and 500 mM. Temperature variation was performed at all different temperatures *viz*. 20°C, room temperature (RT), 37°C and 50°C.

RESULTS

Effect of pH on NR activity

Figure 3.6 depicts effect of varying pH on NR activity of *Sesuvium portulacastrum*. Optimum enzyme activity is observed at pH 7.5. Although the enzyme showed a two peak response to changes in hydrogen ion concentration, a pH of 7.5 was apparently more favourable than pH 5.5, since at later pH the enzyme activity was more by 10%. When pH was increased beyond 7.5, there was a rapid decline in the activity of the enzyme. In view of this, in all further experiments the pH for the enzyme assay was maintained at 7.5.

Effect of temperature variation on NR activity

Figure 3.7 depicts the results of the activity of NR enzyme when studied at 20°C, 27°C (RT), 37°C and 50°C. It was observed that at temperature of 37°C, the NR activity was maximum and was

minimum at 20°C. At 37°C, the activity was more than double than at 20°C. Hence, in further studies on NR, the incubation was carried out at 37°C.

Effect of substrate concentration on NR activity

The effect of different concentrations of substrate KNO_3 on NR activity is illustrated in figure 3.8. The rate of NR activity was linear upto 300 mM KNO_3. At close to 350 mM KNO_3 the enzyme activity reached its maximum, thereafter there was gradual decline in enzyme activity. The rate of the reaction is calculated in terms of ΔOD/g leaves/hr. V_{max} value was found to be 0.18 and Km value as 150 mM.

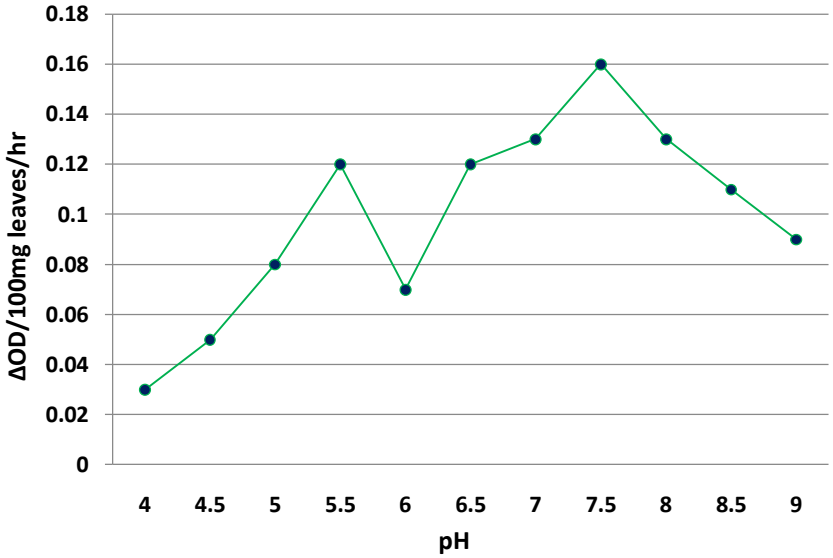

Fig. 3.6: Effect of pH variation on the *in vivo* NR activity in *Sesuvium portulacastrum*.

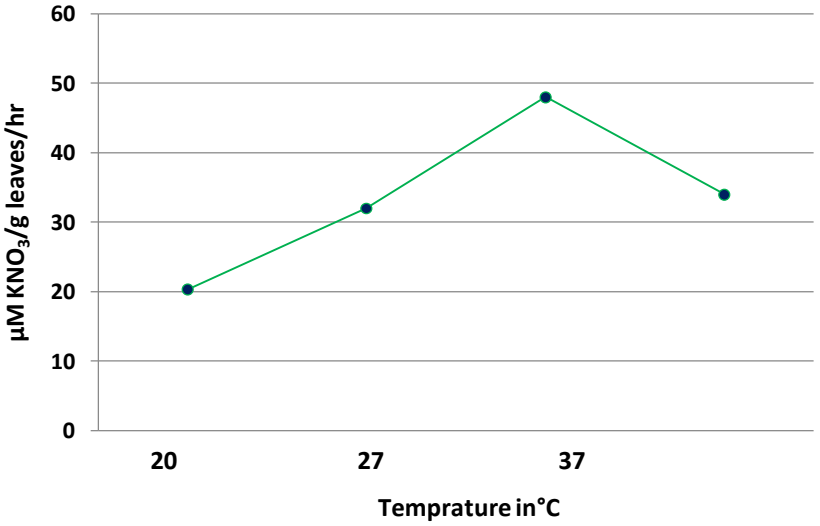

Fig. 3.7: Effect of temperature variation on the *in vivo* NR activity in *Sesuvium portulacastrum* leaves.

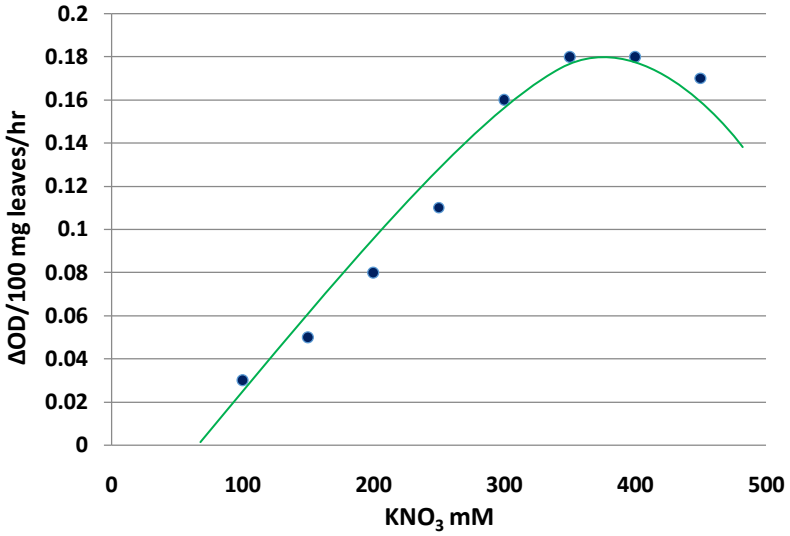

Fig. 3.8: Effect of substrate variation on the *in vivo* NR activity in *Sesuvium portulacastrum* leaves.

DISCUSSION

Stogonov (1973) has reported disturbances in the primary stages of nitrogen assimilation in plants due to salinity. This justifies sensitivity of NR system to external stress as reported by Heuer *et al.*, (1979). Soil salinity along with soil humidity and bacterial nitrification having pronounced effect on NR activity has been thoroughly documented (Mahasneh *et al.*, 1984; Doddama *et al.*, 1985).

Halophytes have been shown to contain high leaf proteins (Goodin and Mckell, 1971; Dwyer and Wolde-Yohannis, 1972). Lokhande (1983) and Ansari (2001) have reported on high nitrogen as well as protein content in plants even under low soil nitrogen condition in *Pentatropis cynanchoides* and *Cressa cretica* respectively. Such a situation can arise only through an efficient utilization of the poor nitrogen resources. It is well known that plants prefer to absorb nitrate nitrogen and therefore within the plant it is this form that nitrogen is made available for metabolic consumption and its ultimate conversion in proteins. Nitrate reductase (NR) is therefore, the key enzyme of nitrogen metabolism of plants.

NR has been widely studied in crop plants (Goodman and Caldwell, 1971; Stewart *et al.*, 1972; Bhosale, 1978; Sarangdhar, 1986) and the requirement of NO_3^- as substrate for the induction of NR is well documented (Beevers and Hageman, 1969; Filnes *et al.*, 1969; Hewitt, 1975; Rajshekar *et al.,* 1988). NR level *in vivo* is usually maintained through a balance between synthesis and turnover of enzyme (Lee and Steward, 1978). The enzyme NR is substrate inducible (Sanderson and Cooking, 1964; Oaks *et al.*, 1980; Somers *et al.*, 1983) and undergoes a rapid turnover on receiving regulatory amount of substrate (Oaks *et al.*, 1972; Asla and Oaks, 1976).

Apart from substrate regulation of NR, the enzyme activity is also light dependent. Nitrate reduction by NR depends upon the availability of electron donors produced by light dependent processes. Nitrate can either be reduced in roots or in shoots. Relative contribution of roots and shoots in the assimilation of nitrates varies with species (Oghoghorie and Pate, 1972; Pate, 1973).

Leaves are also capable of considerable participation in nitrate reductase (Beevers and Hageman. 1969; 1972). Lewis *et al.* (1982) based on their studies on nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase in roots and leaves of barley plants suggested that the leaves are the main sites of nitrate assimilation. In green tissues assimilation of nitrate is intimately linked with photosynthetic reactions, not only for the reduction of nitrate to ammonia but also for the synthesis of carbon compounds which are required for incorporation of ammonia into amino acids (Naik *et al.,* 1982)

At optimum pH and temperature concentration experimentally determined, varying concentration of KNO_3 on NR activity is quite significant. Saturation of reaction, only at higher concentration of KNO_3 is indicative of appreciable NR activity in *Sesuvium portulacastrum,* growing in natural saline environment.

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Biodiversity of microalgal species identified from Western Ghats of Maharashtra as a potential source for development of bioproducts.

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ABSTRACT
Coastline of India is over 7,500 km, which harbors a variety of specialized marine ecosystems like coral reef, seagrass beds, mangroves, algal communities, mud flats and lagoons; thus supporting wealth of marine resources. Algae can be grown in open-culture systems such as lakes or highly controlled close-culture systems; have higher productivity than the traditional crops and can be grown in adverse climatic conditions. The edible microalgae are the green algae (chlorophyta) and cyanobacteria. Microalgae contain substances of high biological value, such as polyunsaturated fatty acids, proteins, amino acids, pigments, antioxidants, vitamins and minerals. They are promising source for carbon sequestration. In the present study, the algal samples were collected from freshwaters and mangrove sites near Achara and Dhamapur forests, district Sindhudurg across the Western Coast of Maharashtra. Collections were carried out during the month of February, 2013. Microalgae *Oscillatoria* sp., *Lyngbya* sp., *Scenedesmus* sp., *Chlorella* sp., *Anabaena* sp., *Arthrospira* sp. *Selenastrum* sp. and *Synechocystis* sp. were identified purely on the basis of the morphological and microscopical observation. Five nutrient media namely BG11 media, Bold's Basal media, Jaworski's media, f/2 media and *Spirulina* media were used for growing cultures for optimum growth. Various physicochemical parameters like pH, temperature, light intensity, nutrient composition were maintained. Total lipids and phycobiliproteins were extracted from 2 unknown blue green algal species J.1 and J.2 respectively. Further we plan to cultivate algal feedstock, especially under conditions of flooded paddy, saline wastelands and mass cultivation in fishery deficient coastline. This study was the first step towards a new approach for sustainable livelihood in coastal areas.

KEYWORDS: Microalgae, freshwater, blue green algae, mangroves

INTRODUCTION

Mangroves create unique ecological environments that host rich assemblage of species. They occupy the intertidal zone, and interact strongly with aquatic, inshore, upstream, terrestrial ecosystems and offer suitable habitat to wide variety of organisms including species of vertebrates and invertebrates (Odum and Heald, 1972). Being surrounded by loose sediments, the submerged roots, trunks and leaves are islands of habitat that attract epibenthos bacteria,

fungi, microalgae and macroalgae. Large numbers of algae occur in association with mangroves, some on the above ground roots and some free living on the mud. Microalgae are of great significance to coastal processes including nutrient and oxygen cycling and form an important component of mangrove food web. Physicochemical factors like salinity, temperature, desiccation, tidal inundation, nutrient levels and light intensity produce patterns of horizontal and vertical distribution seen in mangrove algae. Sindhudurg has a semi-tropical climate; warm and humid in most of the year with three seasons: Rainy (June - October), winter (November-mid February) and summer (mid February–May). Temperatures vary between max. 32°C and monsoon winds bring heavy rains. Blue green algae are thought to have arisen approximately 3.5 billion years ago (Schopf, 1993) and have been the dominant form of life for about 1.5 billion years. As a result of this long evolutionary history, they have adapted to all types of freshwater environment – including extreme conditions (thermal springs, desiccating conditions), brackish (semi saline) conditions, high and low nutrient environments. The main objective of this study was to identify the indigenous microalgae especially blue green algae and analyze its contribution towards value added products.

MATERIALS AND METHODS

Sampling locations

Various mangrove sites in Achara and Dhamapur forest, lake situated in Sindhudurg district, State of Maharashtra, India were well studied for sampling of microalgae. Random sampling method has been applied in the algal collection procedure.

Isolation, purification and identification of microalgae

Water samples for isolation of microalgae were collected in sterile screw cap bottles. The method for isolation and purification of cyanobacteria was adapted from (Ferris and Hirsch, 1991). Algal mats were washed properly to remove the mud, suspended in liquid medium. The algal samples were preserved in 4% formalin (aqueous solution of formaldehyde). Glycerine was used for mounting the material on slide. The centric organisms were photographed using a Leica microscope with attached camera. Samples were further inoculated in BG-11 medium, Bold's Basal media (BBM), Jaworski (JM) medium, f/2 medium and *Spirulina* medium. Sample was separated teased and placed on solidified agar plate of above 5 media. The plates were incubated for 15 days, microscopically examined for the growth of cultures. Individual species were picked aseptically, sub-cultured in 250 ml Erlenmeyer flasks and incubated under continuous illumination (six bulbs, 15 watt each) at 22˚C with 16 hr light regime. The BG-11 medium was used for the isolation and maintenance of blue green algal cultures. Filamentous axenic strains were maintained on agar slants. The algae culture was identified through the manual, "Microalgae Identification for Aquaculture", by Barry H. Rosen (1990). Cultures were maintained at the 32˚C and in 15 watt bulb light intensity.

Standardization of culture conditions

Growth at different temperatures

Strains were inoculated in 250 ml Erlenmeyer flask containing 100 ml of BG-11 medium and the flask were placed in incubator shaker at various temperatures viz. 22˚C, 32˚C and 42˚C under illumination (2600 lux) with 16 hr light period for 15 days.

Cultivation of microalgae in 5 different media of varying pH

Samples from different sites were cultured in 5 different media: - Blue Green medium (BG-11) pH 7.5 (Stanier, 1971), Bold's Basal Medium modified (BBM) pH 6.8 (Stein, J.), Jaworski's medium (JM) pH 7.0 to 8.0 and f/2 medium pH 8.0 (Guillard RRL, 1962), *Spirulina* medium pH 9.0 (Chojnacka K, Noworyta, 2004).

Scaling up of microalgae using assembly of 6-L open tank

Experiment was carried out in open tanks of capacity 6 L (17cm width and 23cm height) made of Pyrex glass. White gravels were layered at the bottom of tank which acted as substratum for microalgae. Aeration was achieved by oxygen pump connected with 2 m pipe to air stone for mixing. 10% inoculum was added to 3 L medium. The tank was operated in the semicontinuous mode. The culture system was maintained at 30-35ºC. Tank was externally illuminated using two daylight tube lights with total light intensity of 2600 lux.

Biomass harvesting

The biomass production in above semicontinuous experiment was interpreted in terms of dry biomass per 100 ml suspension. For dry weight, 100 ml sample was filtered through pre weighed Whatman No. 1 filter paper. The biomass was washed twice with distilled water in order to remove salts adhered to the algal cells. The resulting biomass was dried at 70ºC in a hot air oven for 24 hr and dry weight was calculated. Weight of the dried biomass was taken until the constant weight was achieved.

Extraction of total lipids

Total lipids were extracted from fresh microalgal biomass strain J.1 unknown using slightly modified method of Bligh and Dyer. Lipids were extracted with chloroform – methanol (2:1; v/v) and separated. Chloroform layer was washed and evaporated to dryness. Thereafter weight of crude lipid obtained from sample was measured gravimetrically. Experiment was carried out in triplicates.

Extraction and purification of phycobiliproteins

Culture was harvested after 10 days of incubation under controlled laboratory conditions (temperature, pH and light) by centrifugation at 5000 g for 20 min. Harvested cell mass was frozen at 0˚C. 0.68 g of freeze dried cell mass was suspended in 5 ml sodium phosphate buffer (0.01 M, pH 7.0). Suspended cell mass disrupted by thawing at 4˚C overnight. Mixture was centrifuged at 6000 g for 15 at 8˚C, phycobiliprotein containing clear supernatant was collected. 2 ml Crude extract was added to 9 ml distilled water. 10% w/v ammonium sulphate was added, vortex, kept for overnight at 4˚C. Greenish brown proteins at bottom indicated contaminating

proteins. Supernatant was collected after centrifugation at 8000 rpm for 10 min at 4˚C. Ammonium sulphate 30% w/v was added to supernatant. Phycobiliproteins were precipitated and after centrifugation pellet was resuspended in distilled water. After dialysis, pigment was checked for purity. Absorbance of supernatant was measured by spectrophotometer at wavelengths 620, 652 and 562 nm for calculating C- phycocyanin, allophycocyanin and phycoerythrin.

RESULTS AND DISCUSSION

In this study, isolation and identification of variety of Cyanobacteria from freshwater was carried out. Microalgae *Oscillatoria* sp., *Lyngbya* sp., *Scenedesmus* sp., *Chlorella* sp., *Anabaena* sp., *Arthrospira* sp., *Selenastrum* sp. and *Synechocystis* sp. were studied on basis of morphology. Some diatoms like *Navicula* sp., protozoan and zooplankton were also observed. The optimum conditions were studied to improve the quantity and quality of value added products of blue green algae. The concentration of total lipid content of culture J.1 was 2.2 g%. Phycobiliproteins were extracted and purity of C-Phycocyanin (C-PC) was 1.981. Maximum biomass was harvested from BG11 media. The cyanobacteria such as *Spirulina* and *Nostoc* have been used as a source of protein and vitamin for humans and animals (Ciferri 1983, Kay 1991, Gao 1998, Takenaka *et al*. 1998).

CONCLUSION

This preliminary but strong study has opened various avenues of environment friendly, commercial and economically viable solution for sustainable future. Microalgae like *Oscillatoria* sp. are used as model organisms for lipid extraction. *Scenedesmus* sp., *Chlorella* sp. are enormously studied for capturing of carbon from steel, power and cement plants (Sahoo Dinabandhu, 2012). Rice plantations can utilize healthy populations of nitrogen-fixing cyanobacteria (*Anabaena*, as symbiotes of the aquatic fern *Azolla*) for use as rice paddy fertilizer. *Spirulina*, a cyanobacterium and especially *Arthrospira* sp. (free-floating filamentous) could be used as dietary supplements or whole food or as feed for aquaculture. At a functional and ecological level, size and shape play important role in terms of solute and gas exchange, absorption of light, rates of growth and cell division, sedimentation in the water column, cell/colony motility (Sigee, 2004). Analysis of DNA sequences should be done for biochemical assessment of both indigenous blue-green (16S rRNA genes) and eukaryote algae (18S rRNA and chloroplast DNA).

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Eco-physiological studies of *Pongamia pinnata* and *Canavalia cathartica* growing in saline and non-saline habitat from Ratnagiri district of Maharashtra.

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ABSTRACT

Pongamia pinnata and *Canavalia cathartica* are the leguminous plants growing as mangrove associates as well as in non-saline area of Ratnagiri district of Maharashtra. They were compared for the habitats and their leaves were studied for stomatal characters like density, length, breadth as an indicator of photosynthetic CO_2 fixation capacityand chlorophyll content as photosynthetic machinery. Soluble sugar and free proline content from various plant parts was analysed as osmolyte as an adaptive character in saline condition. Saline and non-saline soil differs in the EC, pH as well as minerals (specifically sodium and chloride) composition. Stimulation in stomatal density, reduction in chlorophyll content and accumulation of soluble sugars and free proline content in various parts of *P. pinnata* and *C. cathartica* were recorded to be ecological adaptive features.

KEYWORDS: *Pongamia pinnata*, *Canavalia cathartica*, mangrove associates, stomatal behavior, chlorophyll content, soluble sugar

INTRODUCTION

Ratnagiri has a coastline along with estuaries like Kalbadevi, Sakhartar, Shirgoan, Bhatye, Ranpar, Pawas, Gaokhadi, Purnangad etc. with mangrove vegetation. The mangrove vegetation is dominated by *Rhizophora mucronata, R. apiculata, Avicennia marina, A. officinalis, Sonneratia alba, Lumnitzera racemosa, Ceriops tagal, Exocaeria agallocha* and are associated with *Acanthus ilicifolius, Salvadora persica, Ipomea pes-caprae, Clerodendrum inerme, Derris heterophylla, D. trifoliata, Pongamia pinnata, Thespesia populnea* etc. *P. pinnata, C. cathartica* and *C. crista* plants grow as mangrove associates on borderline of mangrove patches. *P. pinnata* grows on sandy shores as well as mangrove associate, perennially and develops in a tree form. *C. cathartica* grows as climber and climbs on the mangrove plants in the vicinity like, *Avicennia marina, A. officinalis, Sonneratia alba, Excoecaria agallocha* along with *Clerodendrum inerme, Derris trifoliata, Caesalpinia crista* etc. Both the plants studied, grow in saline as well as non-saline habitat. Comparison between saline and non-saline habitats and the plants growing under saline and non-saline condition gives us the idea of the effects of salinity on the growth and

performance of the plants under natural condition. Stomatal characters, chlorophyll content of the leaves and soluble sugar and free proline content of the plants play an important role in determining how these plants adjust in the ecosystem.

MATERIALS AND METHODS

Soil analysis:

The soil samples were collected from the rhizosphere of each plant species and were dried first in air, then in oven at 60ºC and used for EC and pH determination. Similarly Soil extracts were prepared (USDA Book No 60, 1954)and used as a source of Na^+ and K^+ estimation, flame photometerically. Chloride from the same soil sample was determined from the extract prepared in distilled water by titrating against $AgNO_3$ (USDA Book No 60, 1954).

Stomata present on the leaves of *P. pinnata* and *C. cathartica* growing in saline and non-saline region were studied for their density. Peelings from upper and lower surfaces were separately observed under high power of the microscope and number of stomata was measured and stomatal density was calculated and expressed in number of stomata/ mm^2. Similarly stomatal length and breadth/ width were measured using ocular and stage micrometer, average was calculated by taking 10 readings. Chlorophylls from the leaves of *P. pinnata* and *C. cathartica* growing in saline and non-saline region were estimated according to the method of Arnon (1949).

P. pinnata and *C. cathartica* plants growing in saline and non-saline were analysed for their chemical constituents. Different plant parts root, stem, rachis and leaves were separated, dried and used for analysis of soluble sugar and free proline content. Soluble sugar content was estimated using dry powders by using the method of Dey (1990). The free proline content was estimated colorimetrically, according to the method of Bates *et al*. (1973).

RESULT AND DISCUSSION

Habitat analysis:

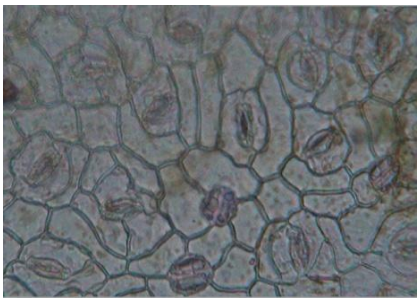
Saline and non-saline soil differs in the EC, pH as well as mineral composition. Different characters of habitat in which *P. pinnata* and *C. cathartica* grow were recorded in Table1.It indicated that the soil pH varies with the location. pH of soil in the rhizosphere of *P. pinnata* in saline region was neutral but in the non-saline region it was slightly acidic while *C. cathartica* grows in acidic soil and soil in saline region is more acidic. EC of the soil in saline region in these three places was more than that of corresponding non-saline region. Similarly sodium, potassium as well as chloride contents in the soil were always more in saline soil than non-saline soil.

Table 1. Characteristics of soil collected from root zones of plants growing in saline and non-saline habitat.

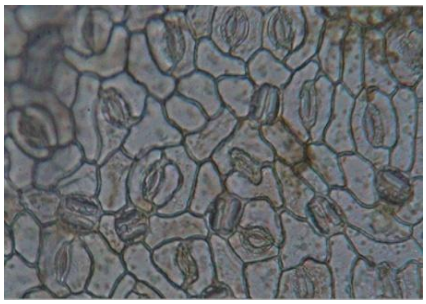
Plants	Soil	pH	EC (m mhos/cm)	Inorganic elements g/100g		
				Sodium	Potassium	Chloride
P. pinnata	Saline	7.01	1.0	1.24	0.68	1.24
	Non-saline	6.44	0.1	0.49	0.38	0.12
C. cathartics	Saline	5.08	1.332	1.46	0.78	1.48
	Non-saline	6.37	0.387	0.66	0.44	0.15

Stomatal characters:

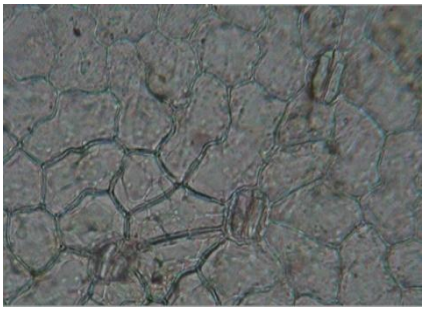
Stomata, influence transpiration rate, stomatal conductance and photosynthesis to a great extent and play an important role on growth and development of plant. Stomatal density can be taken as an indicator of transpiration potential, water use efficiency and photosynthetic CO_2 fixation capacity. Fig. 1 shows stomata present on the leaves of *P. pinnata* and *C. cathartica* growing under saline and non-saline conditions. Table 2 shows stomatal density as well as size (length and breadth) of stomata on upper as well as lower epidermis. In *P. pinnata,* stomata were restricted to lower epidermis of the leaves while in *C. cathartica* stomata exist on both the



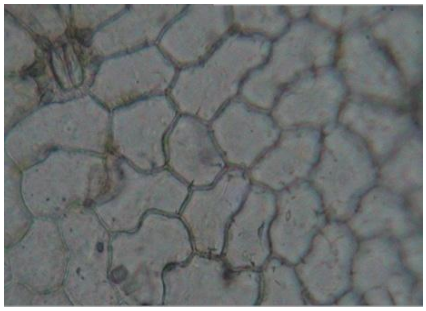
***C. cathartica* saline lower epidermis**



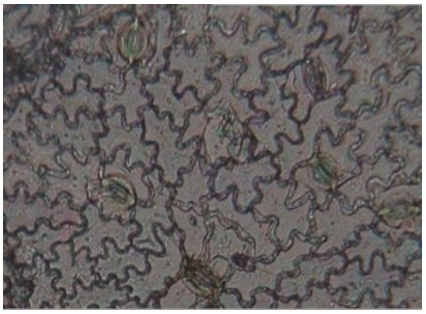
***C. cathartica* non-saline lower epidermis**



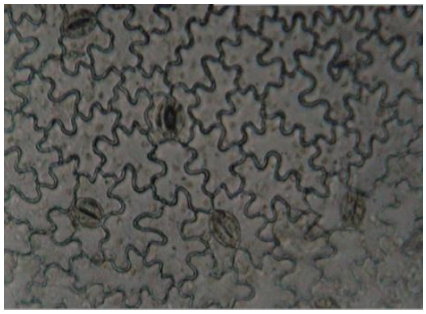
***C. cathartica* saline upper epidermis**



***C. cathartica* non-saline upper epidermis**



***P. pinnata* saline lower epidermis**



***P. pinnata* non-saline lower epidermis**

Fig. 1: Stomata on the leaves surfaces in the three legume species growing in saline and non-saline conditions.

surfaces but on upper surface they were restricted to vein regions. Variation in the presence of stomata has been recorded by Joshi *et al.* (1976) in *R. mucronata*, *B. gymnorhiza*, *A. ilicifolius*, *K. rheedei*, *A. majus*, *E. agallocha* with stomata on lower side and in *S. acida*, *L. racemosa* and *S. persica* they are present on both the surfaces. Stomata of *P. pinnata* leaves are anisocytic and are present on lower surface (The Ayurvedic Pharmacopoeia of India, 1999). The study of epidermal features of leaves of *C. cathartica* revealed two distinct types of stomata i.e. paracytic and anisocytic (Rodrigues and Torne, 1990).

Table 2: Comparison of stomatal characters of leaves of plants growing in saline and non-saline conditions.

Plant	Region	Stomatal density (No. of stomata/ mm^2)		Stomata length (µm)		Stomata width (µm)	
		Upper	Lower	Upper	Lower	Upper	Lower
P. pinnata	Saline	Nil	211 ±33	-	23.07 ±3.05	-	17.38 ±3.72
	Non-saline	Nil	90 ±14	-	24.33 ±1.52	-	14.85 ±1.53
C. cathartica	Saline	35 ±12	263 ±31	23.78 ±2.42	34.45 ±3.16	15.94 ±2.35	27.52 ±2.42
	Non-saline	28 ±9	162 ±31	24.33 ±2.13	35.71 ±3.35	17.38 ±1.64	28.12 ±2.77

The stomatal density varied with the surface when they were present on both surfaces. In *C. cathartica* stomata were present on both sides of the leaf and their density was more at lower side. It was clear from the observations (Table 2) that density of stomata on upper side was less than on lower surface. Similar observations were recorded by Joshi *et al.* (1976) in mangroves *S. acida*, *L. racemosa* and *S. persica*.

In both the plants studied, the stomatal density was more in the leaves of plants growing in saline region. Stimulative effects of salinity on stomatal density was noted in cotton (G*ossypium* sp.) genotypes RAHS-14, LRA-5166 and AK-235 by Basanagouda (2007), in *Phaseolus vulgaris* L. by Kaymakanova *et al.* (2008) and in mangrove *Bruguiera gymnorrhiza* seedlings by Xiao *et al.* (2010) under experimental conditions. Plants growing in saline conditions showed more stomatal density than growing in non-saline region in both the plants studied. Difference in the length and breadth/width of stomata was negligible. Increasing number of the stomata under saline condition can be a stress adaptation mechanism of these plants in order to increase transpiration and therefore to increase water uptake.

Chlorophylls from leaves of *P. pinnata* and *C. cathartica* growing in saline and non-saline conditions are shown in Fig 2. *P. pinnata* and *C. cathartica* plants growing under saline conditions contain less chlorophyll a and chlorophyll b and total chlorophyll than the plants growing in non-saline conditions. Under both saline as well as non-saline conditions Chl a: b ratio was more in from plants growing under saline conditions.

Since chlorophylls take part in the conversion of solar energy into chemical energy, their level in the leaf tissue was one of the important feature governing photosynthetic efficiency of plants. Chlorophyll content in the leaves was dependent on endogenous factors like rate of pigment synthesis, rate of pigment degradation, stage of leaf development etc. and some environmental factors like shade, light, temperature, drought, water-logging, soil salinity etc.

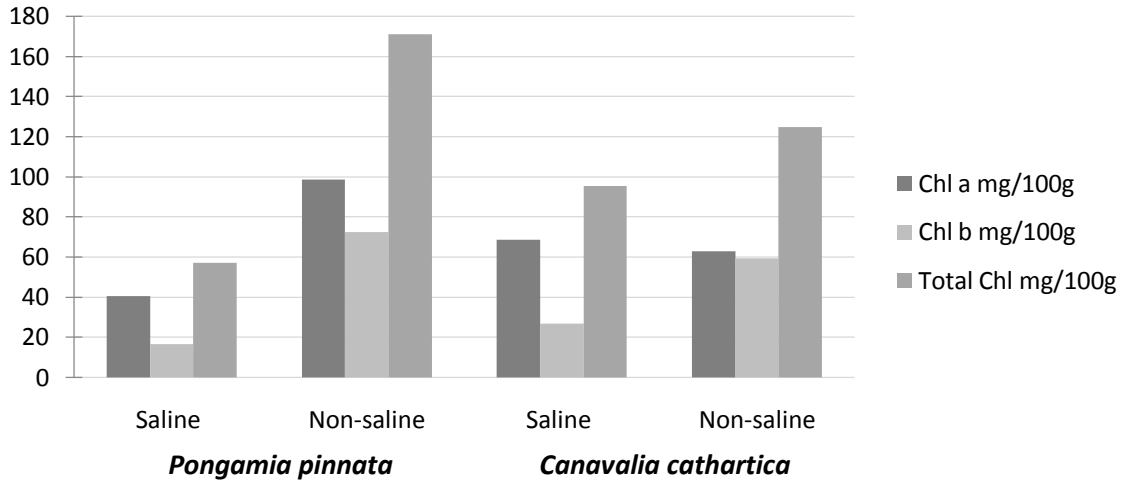
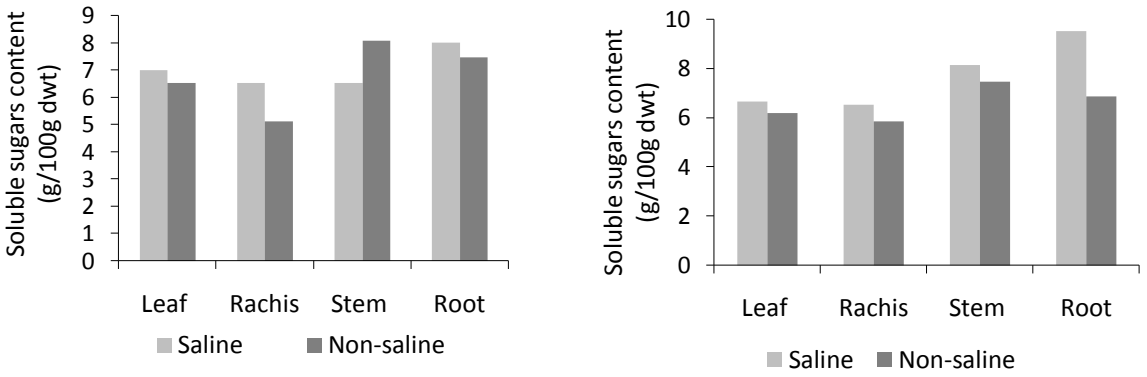


Fig. 2: Chlorophyll content from leaves of plants growing under saline and non-saline area.

In *Thespesia populnea,* a mangrove associate, Kotmire (1983) noticed higher values of chlorophyll a, b and total chlorophyll from the plant growing under non-saline condition indicating the adverse effect of salinity of chlorophyll content similar to our observation in *P. pinnata* and *C. cathartica*. Tuffers *et al*. (2001), recorded no difference in chlorophyll content in the leaves of *Avicennia marina* plants growing in two localities of high and low salinity conditions. Total chlorophyll content from two mangroves *Avicennia marina*, *Bruguiera gymnorrhiza* and mangrove associate *Hibiscus tiliaceus* growing at the two sites having low salinity and high soil salinity have been recorded by Naidoo *et al*. (2002). Leaves of *A. marina* and *B. gymnorhiza* from highly saline area and *H. tiliaceus* from less saline area showed more total chlorophyll indicating difference of trend of total chlorophyll content in mangrove and mangrove associate as regards the effect of salinity of the habitat on the pigment status. In mangrove *Laguncularia racemosa* Sobrado (2005) recorded more leaf Chl a and Chl b content in plant treated with 30% NaCl than control (0%), but there is decline in Chl a: b ratio. Assessment of chlorophylls was done by Nandy (Datta) *et al*. (2009) from leaves of five mangrove species under saline and non-saline conditions. They recorded 18%, 13% and 0.7% higher total chlorophyll content of the leaves of *Bruguiera gymnorhiza*, *Exocaeria agallocha* and *Heritiera fomes* growing in non-saline condition, while in *Phoenix paludosa* and *Xylocarpus granatum* from the plant growing in saline conditionthe total chlorophyll content was 22% and 7% higher res. However, these workers noticed that the ratio of Chlorophyll a and b was higher in plants growing in non-saline soil.

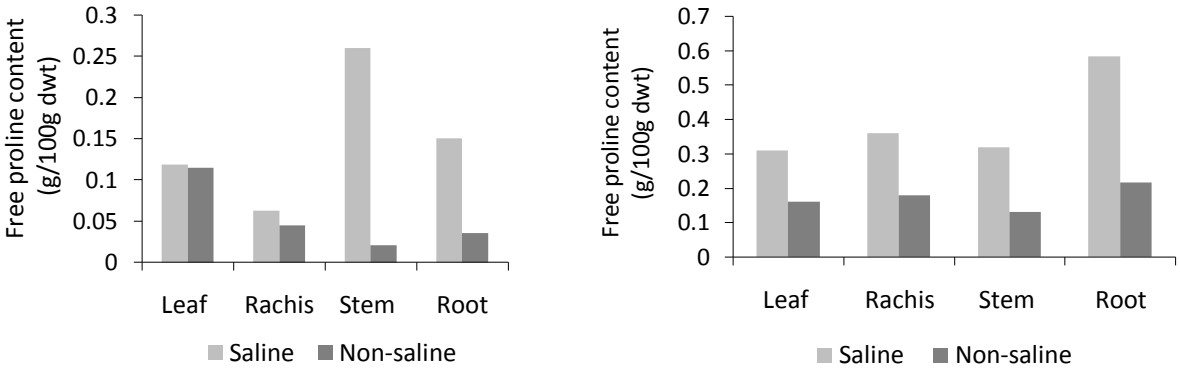
Soluble sugar content from various parts of *P. pinnata* and *C. cathartica* growing in saline and non- saline conditions is shown in Fig.3. Soluble sugar content is comparatively high in leaf, rachis and root tissue in *P. pinnata* and leaf, rachis and stem tissue of *C. cathartica* plants growing under saline conditions. It seems that soluble sugar is one of the components in maintaining osmotic potential of these plants under saline condition.



A) *Pongamia pinnata* B) *Canavalia cathartica*

Fig. 3: Soluble sugars content in parts of plants growing in saline and non-saline area.

Synthesis of soluble organic compounds like soluble sugars (Abebe *et al.*, 2003; Ashraf and Bashir 2003), allow the plant to overcome this failure and re-establish a water potential gradient which in turn gives the possibility to absorb water and restore plant turgor (Xiong and Zhu, 2002). In *Thespesia populnea,* Kotmire (1983) recorded higher amount of total sugar and starch in the leaves from saline condition plants. Free proline content in shown in Fig. 4. The level of free proline in different parts of *P. pinnata* plant, growing in non-saline habitat was in the order of stem>root>leaf rachis whereas this order in case of plants from saline habitat was leaf>rachis>root>stem. In *C. cathartica* plant growing in non-saline region showed the proline pattern as root>rachis>leaf>stem whereas in plants growing in saline habitat showed root>rachis>stem>leaf pattern. Thus in both species there was a definite effect of habitat on the accumulation of proline.



A) *Pongamia pinnata* B) *Canavalia cathartica*

Fig. 4: Free Proline content in parts of plants growing under saline and non-saline area.

Free proline content in all the plant parts of both plants under study showed higher levels in plants growing under saline condition. Similar observations were recorded in *Thespesia populnea* by Kotmire (1983),in seven halophytes (highest in leaf tissue of *Frankenia hirsuta* and lowest in *Camphorosma monspeliaca* L. ssp. *monspeliaca*) by Oncel (1988), in the leaves of *Alysicarpus vaginalis,* a wild legume plant by Chandrashekar and Sandhyarani (1994), in eight species of *Atriplex*, two species of *Sesbania* by Ismail (1998). Datta and Ghose (2003) recorded presence of high amount of free proline in mangrove associate *P. pinnata*, Slama *et al.* (2006) in a succulent halophyte *Sesuvium portulacastrum*, as an osmolyte. Nandy (Datta) *et al.*, (2009) analysed free amino acids including proline from leaves of five mangrove species *Bruguiera gymnorrhiza*, *Exocaeria agallocha, Phoenix paludosa, Heritiera fomes, Xylocarpus granatum* under saline and non-saline conditions. Their quantitative analysis showed a considerable high amount of free amino acids from the plants grown in saline environment. In both plants studied the free proline level is relatively more in different parts of plants growing under saline habitat clearly indicate active role of proline in salt tolerance process.

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Vertical distribution of mangrove related higher marine fungi in Raigad district of Maharashtra.

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ABSTRACT

Mangroves are tropical and subtropical forests comprising trees of many unrelated genera that share the common ability to grow in estuarine and coastal environments. Mangroves being detritus based ecosystem, fungal populations are involved in detritus processing. The term marine fungi, actually denotes the fungi which have the capability to grow and reproduce under aquatic conditions where the salinity of water is high. Fungal diversity may also vary from one mangrove to another.

An attempt was made to study the vertical distribution of higher marine fungi on *Sonneratia apetala* at Dharamtar creek in Raigad District of Maharashtra. *Sonneratia apetala* plant was marked at five levels ranging from 0.5 m to 2.0 m. One thousand samples were collected throughout the year. Samples collected from *Sonneratia apetala* were examined for sporulating marine fungi, which gives the record of 24 species. 17 species from ascomycota, 1 species of basidiomycota, 1 species from mucoromycotina, 4 species from hyphomycetes and 1 species from coelomycetes were recorded. Salinity and temperature are the major factors affecting the diversity of marine fungi. Species with bitunicate asci as well as immersed and carbonaceous ascocarps were recorded above mean tide level. Unitunicate asci with immersed ascospores, basidiomycota and hyphomycetes were found in both above and below mean tide level. Fungi with long neck, unitunicate asci and hyaline ascospores were distributed in the wide zone. It is interesting to note that there was no any species restricted only either below the mean tide level or beyond the mean tide level.

KEYWORDS: Mangrove, marine fungi, *Sonneratia apetala,* ascomycota, hyphomycetes

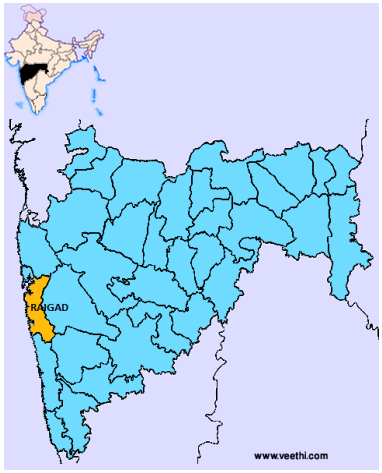
INTRODUCTION

Marine mangrove fungi usually grow on dead and decayed leaves, prop roots, stems, pneumatophores; drift wood and seedlings of mangrove. Due to the diurnal fluctuations in the tidal level substrates at low tide level get exposed for short periods and remain submerged for long periods. Substrates at high tide level remain exposed for longer periods and submerged for

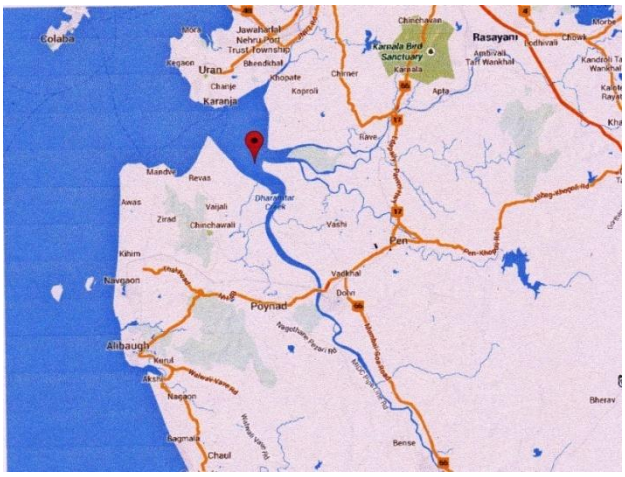
shorter periods. Substrates at very higher level get the occasional splashes of tidal water and remain exposed for very long period. This unique environment allows growing some specific fungi in varying conditions (Kohlmeyer and Kohlmeyer, 1979; Hyde and Jones, 1988). Ascomycetes are well adapted group of higher fungi to intertidal mangroves as compared to basidiomycetes and deuteromycetes (Kohlmeyer and Kohlmeyer, 1979; Hyde, 1990). Adaptation of higher marine fungi shows unique morphological features in ascocarp, ascus and ascospores. Earlier observations on the vertical distribution of marine fungi in mangroves were made Kohlmeyer and Kohlmeyer (1979) and Aleem (1980). Chinnaraj (1993) has studied vertical distribution of higher marine fungi on *Rhizophora mucronata*. An attempt has been made to study the vertical distribution of higher marine fungi on *Sonneratia apetala* at Dharamtar creek in Raigad District of Maharashtra.

MATERIALS AND METHODS

The present study was carried out in a *Sonneratia apetala* stand at Dharamtar creek in the Raigad district. The tidal amplitude varies from 0.04 to 4.68 m (Indian tide tables of Mumbai port).



Location of study area



Dharamatar Creek ends in Arabian sea

Satellite map of Dharamtar creek

***Sonneratia apetala* marked for vertical zonation**

Dharamtar creek: (Alibag Taluka; 18^{0}40'N and 73^{0}00'E) Dharamtar is one of the domestic port situated along Amba river 20 km east of Alibag. This place is on the border of Alibag and Pen Taluka. One of the Ispat Company is established along the creek. There is a good mangrove vegetation along the creek but found to be under threat of industrialization and urbanization. There is an Ispat company along the creek and port activities are also found to be increased in

last few years. Loading and unloading of the goods like fossil coal, iron ores, chemicals and drazing activities are affecting the mangrove vegetation of the area. Total number of mangrove species recorded was 13. Important mangrove species are *Avicennia officinalis, Bruguiera cylindrical, Excoecaria agallocha, Sonneratia apetala* etc.

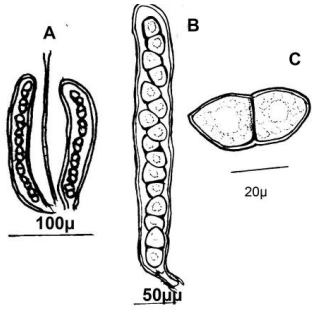
Sonneratia apetala at Dharamtar creek from highly eroded stands were selected and marked at 5 levels as below:

- Level 1: Below 0.50 meters
- Level 2: 0.50 to 1.00 meters
- Level 3: 1.50 to 2.00 meters
- Level 4: 1.50 to 2.00 meters
- Level 5: above 2.00 meters

Four collections were made from January 2009 to December 2009 and in each collection 25 samples were obtained at each tidal level (total 125 samples). The samples were collected from the respective collection sites and placed in large polythene bags for transport to the laboratory they were examined immediately as well as following the incubation in the moist chambers. Samples were examined by direct microscopic observation method (Hyde and Jones, 1988; Kohlmeyer and Kohlmeyer, 1979). Samples were also incubated for 7-10 days in the moist chamber and they observed for sporulating fungi from 7-10 days.

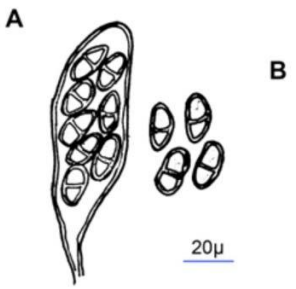
RESULT

One thousand samples collected from *Sonneratia apetala* were examined for sporulating marine fungi, which gives the record of 24 species. Out of which 17 species from ascomycota, 1 species of basidiomycota, 1 species from mucoromycotina, 4 species from hyphomycetes and 1 species from coelomycetes were recorded. *Curvularia lunata* and *Verruculina enalia*, were found to occur in all the tidal levels while *Aigialus mangrovei*, *A. parvus*, *Aniptodera* sp. are found to be restricted only at above mean tide level and *Cirrenalia tropialis* was recorded at highest tide level. The highest species diversity on was observed at above mean tide level and at lowest low tide level very poor species diversity was recorded. Fungal species at above the highest tide level were also recorded; the diversity of fungal was very low and these species were represented by *Cirrenalia tropialis, Halocyphina villosa, Verruculina enalia, Curvularia lunata, Massarina velatospora* and *Zopfiella* sp. These species may be capable to grow in adverse conditions also.



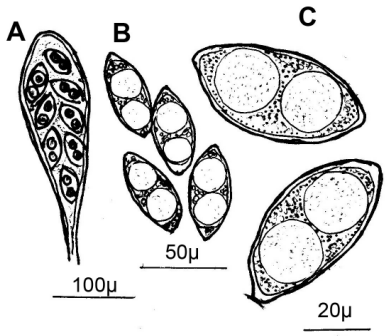
***Verruculina enalia* (Kohlm.) Kohlm. & Volkm.-Kohlm.**

A. Asci with paraphyses; B. Ascospores in asci; C. Ascospore



***Aniptodera* sp.**

A. Asci with ascospores; B. Ascospore



***Halosarpheia ratnagiriensis* S.D. Patil & Borse**

A. Ascospores in asci; B. Ascospores

Table 1: List of fungal species occurred at various levels on *Sonneratia apetala*.

No.	Name of the fungal species	L 1	L 2	L 3	L 4	L5	Total
1.	*Aigialus parvus*	-	06	07	01	-	14
2.	*Aniptodera mangrovei*	-	05	03	02	-	10
3.	*Aniptodera mangrovei*	01	08	08	02	-	19
4.	*Antenospora quadricornuta*	-	04	04	03	-	11
5.	*Aniptodera* sp.	-	-	06	-	-	06
6.	*Aspergillus niger*	05	12	06	02		25
7.	*Aspergillus ustus*	04	07	03	-	-	14
8.	*Cirrenalia basiminuta*	-	-	06	02	-	08
9.	*Cirrenalia tropialis*	-	-	04	01	01	06
10.	*Clavatospora bulbosa*	01	10	09	05	-	25
11.	*Cunninghamella elegans*	-	16	08	-	-	24
12.	*Curvularia lunata*	03	08	07	03	01	22
13.	*Halocyphina villosa*	-	02	11	04	01	18
14.	*Halosarpheia marina*	-	04	09	03	-	16
15.	*Halosarpheia minuta*	01	05	08	05	-	19
16.	*Halosarpheia ratnagiriensis*	-	07	07	04	-	18
17.	*Hysterium* sp.	01	02	08	-	-	11
18.	*Lulworthia grandispora*	-	16	14	09	-	39
19.	*Massarina velatospora*	-	09	07	02	01	19
20.	*Phoma* sp.	-	08	06	01	-	15
21.	*Savoryella lignicola*	-	05	03	03	-	11
22.	*Verruculina enalia*	01	04	10	09	01	25
23.	*Zalerion maritimum*	-	02	06	07	-	15
24.	*Zopfiella* sp.	-	07	06	03	-	16

DISCUSSION

Schhaumann (1968; 1969) was the first to consider vertical distribution of marine fungi on stationary wooden structures. Lplleuer (1969) however found no evidence for vertical zonation on the prop roots of *Rhizophora* sp. and the pneumatophore of *Avicennia* sp. Vertical zonation of fungi on salt marsh grasses and perennial herbaceous plants has been well documented: Spalina (Gessner and Kohlmeyer, 1976); *Acanthus illicifolius* (Sadaba *et al*., 1995) *Juncus roemarianus* (Series of papers by Kohlmeyer and Volkmann-Kohlmeyer 1998; 1999) and *Phragmites australis* (Poon and Hyde, 1998) with marine fungi growing at the bases of the plants

and terrestrial species on the apical part of the shoots. Petersen and Koach (1997) found evidence of vertical zonation of marine fungi over a narrow tidal range on oak and larch poles. *Marinospora calytrata, M. longissima, Lulworthia* sp.*, Halosphaeria appendiculata* and *Ondiniella torquata* were commonly recorded from the lower zone (sub tidal) while *Sphaerulima oraemaris, Marinosphaera mangrovei* and *Leptosphaeria pelagica* were recorded from the upper tidal zone (supralittoral). Study by various workers showed that fungal succession on mangrove wood exposed in seawater (Tan *et al.*, 1989; Leong *et al.*, 1991).

Studies on the vertical distribution of on mangroves from different geographical regions showed that most of the species occurring on intertidal mangrove wood have an affinity towards certain tidal levels (Hyde, 1988; 1989; 1990; Hyde and Jones, 1988). The highest species diversity was observed above mean tide level. High species diversity was observed at the same zone at Brunei but with different species composition (Hyde, 1988; 1989; 1990). Differences in the species composition have been reported in earlier studies, also on different mangrove host studied (Hyde 1990b) as well as different mangrove areas studied (Borse, 1988; Hyde 1988, 1989, 1990; Hyde *et al*., 1990; Jones and Kuthubutheen, 1989; Leong *et al*., 1991). High species diversity at above mean tide level may be due to the suitable environmental and functional situations as occurrence of water at every high tide, low temperature and light intensity due to the shade caste by canopy and better chances of transmission and inhabitation due to the tidal water.

Species with bitunicate asci as well as immersed and carbonaceous ascocarps were recorded above mean tide level (e.g. *Massarina velatospora*) species with identical morphology were recorded at the same level at Brunei (Hyde, 1990). Unitunicate asci with immersed ascospores, basidiomycota (*Halocyphina villosa*) and hyphomycetes were found in both above and below mean tide level (Hyde, 1990; Hyde and Jones 1988). Fungi with long neck, unitunicate asci and hyaline ascospores (*Halosarpheia minuta*), species from Eurotiales (*Aspergillus niger*), Hyphomycetes (*Curvularia lunata*) fungi with cleistothecial ascomata *(Verruculina enalia)* were distributed in the wide zone. It is interesting to note that there was no any species restricted only either below the mean tide level or beyond the mean tide level. *Aniptodera* sp. was found to be restricted only at level 3.

Salinity and temperature are the major factors affecting the diversity of marine fungi were well illustrated by the data of Booth and Kenkel (1986). Salinity affects the diversity of fungi colonizing the *Acanthus illicifolius* is illustrated by Sadaba (1996). During the dry season when salinities were high, marine fungi were predominant, conversely in the wet season when the salinities were low terrestrial fungi were dominant (Sadaba, 1996). Fungi with bitunicate asci and immersed ascoscarps were found both above and below mean tide level (*Verruculina enalia*) Fungi with unitunicate asci and immersed ascocarps (*Aniptodera* sp.*);* Unitunicate asci and appendaged ascospores *(Halosarpheia ratnagiriensis)* were recorded both above and below mean tide level. One of the hyphomycetes, *Curvularia lunata* is found to be distributed in all zones. Fungal species*, Aniptodera* sp. was found to be distributed in a very restricted zone.

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Critically endangered mangrove species along the coast of Maharashtra: Victims of human encroachment.

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ABSTRACT

Present paper provides an array of habitat characteristics of critically endangered mangroves like *Xylocarpus granatum*, *Cynometra iripa*, *Heritiera littoralis* and *Dolichandrone spathacea* along the coast of Maharashtra. The populations of these species are fragmented species have very narrow ecological amplitudes. A heavy anthropogenic impact as well as recalcitrant nature of seeds adds to the problems of rarity and local extinctions.

KEYWORDS: *Xylocarpus, Cynometra, Heritiera,* recalcitrant, endangered

INTRODUCTION

On the coast of Maharashtra, mangroves grow in a narrow strip of coastal habitats, estuaries and creeks. Characteristically these are sandwiched in between the sea and human settlements. Therefore, mangrove areas are reducing very fast. Reduction in area is prominent on the boundaries where some of the important mangrove species are growing. These include *X. granatum, C. iripa, H. littoralis* and *D. spathecea*. The populations of these species are completely vanished. Mangroves of Maharashtra have been evaluated for their IUCN status (Bhosale *et al.*, 2002).

MATERIAL AND METHODS

Extensive field visits were arranged in different estuaries of Maharashtra for survey and documentation of mangrove species, since last 13 years. Numbers of PRAs (PRA: Participatory Rural Appraisal) were arranged for collection and validation of information on mangrove floristic. Habitat parameters such as physicochemical properties of soil, water, tidal amplitude, species association, zonation etc. are studied from time to time. Nursery and bioassay techniques are standardized for field regeneration. Present compilation is the result of these long term attempts in the field of mangrove conservation.

RESULT AND DISCUSSION

Table 1: Occurrence of critically endangered mangroves along the coast of Maharashtra and their population characteristics.

No.	Species	No. of estuaries in which species occurs	Population characteristics
1.	*Xylocarpus granatum*	5	Mostly fragmented, consisting not more than 50 trees
2.	*Cynometra iripa*	3	Mostly fragmented, consisting not more than 10 trees, invading wet places along estuaries especially in upstream regions.
3.	*Heritiera littoralis*	1	Very small population with 35 individuals, devoidof seedlings and saplings.
4.	*Dolichandrone spathacea*	4	Very fragmented in the form of single or rarelycouple of trees.

Table 2: Present habitat status of critically endangered mangrove species along the coast of Maharashtra.

No.	Species	Location	Soils	Tides	Threats
1.	*X. granatum*	Midstream fringing forests, rarely on riverine forest habitats, some-times in the Horticultural farms on the edge of estuaries in coconut plantation, along the fencings. Mostly flourish in fringing habitats at the foot hills along the estuaries with perennial fresh water inputs in the form of small streams.	Gravelly, sandy, non-saline to moderately saline sometimes hyper saline, well drained	Moderate tidal activity, cut off of tides due to bund construction at some places.	Human inference on mangrove habitat, bund construction, encroachment, dredging of sand. Conversion of mangrove land for agricultural purpose as well as aquaculture. Cutting for fuel, indiscriminate. Collection of seeds for medicinal purpose. Seed eating by monkeys. Recalcitrant nature of seeds.

2.	*C. iripa*	Midstream to upstream. Strictly inthe fringing forest habitats with perennial fresh water inputs in the form of small streams at thefoot of hills	Gravelly, non-saline to slightly saline, well drained	Flourish in moderate tidal activity areas, cannot tolerate submergence, also found in the terrestrial habitats.	Human interference on mangrove habitat, bund construction, encroachment, cut off of fresh water inputs, erosional deposits from hills, cutting for fuel, recalcitrant nature of seeds and insect infestation
3.	*H. littoralis*	Protected shorelines, borderlines of mangroves in low tidal activity creeks at the foot of hillock in privately owned terrestrial land in glycophytic conditions, along the fencing in coconut plantation on the bank of estuaries**.**	Gravelly, sandy, non-saline to moderately saline, well drained	Require moderate tidal activity, presently it is decreased due to habitat modification**.**	Shoreline erosion, habitat modification, cutting, recalcitrant nature of seeds, unavailability of proper sites in field for regeneration
4.	*D. spathacea*	Fringing forest patches along mid-stream to upstream, rarely mudflats and riverine sites. Sometimes under glycophytic conditions.	Gravelly, sandy, muddy, non-saline to saline, some-times hyper saline, water logged	Moderate tidal activity, tidal regions are modified at some places due to human interference	Indiscriminate cutting and felling, habitat modifications, dumping of waste, seedlings are very hard to survive

Mangroves of Maharashtra comprise 27 species viz. *Rhizophora mucronata, R. apiculata*, *Kandelia candel*, *Ceriops tagal*, *Bruguiera gymnorrhiza*, *B. cylindrica*, *B. parviflora*, *Sonneratia alba*, *S. casuolaris*, *S. apetala*, *Avicinnia officinalis*, *A. marina*, *A. marina* var. *accutissima*, *Acanthus ilicifolius*, *Exocaeria agallocha*, *Lumnitzera racemosa*, *Aegiceras corniculatum*, *Baringtonia accutangula*, *B. racemosa*, *Carallia brachiata*, *Salvadora persica*, *Derris heterophylla*, *Cerbera odollam*, *Xylocarpus granatum*, *Cynometra iripa*, *Heritiera littoralis* and *Dolichandrone spathacea* (Chavan, 2013). Among these former 23 were well known to researchers and discussed in many compilations in the last century. Occurrence of *X. granatum, C. iripa, H. littoralis* and *D. spathacea* are recently recorded from the present laboratory and monitored for various aspects.

Table 1 depicts the number of estuaries along the coast of Maharashtra in which the species occur. *X. granatum* was recorded first time only from Achara estuary in Sindhudurg district. Later on, its occurrence was recorded in some other estuaries like Vijaydurga, Vetye, Nivati etc. PRA studies revealed that the species was common to those estuaries. At some places local inhabitants were able to show its habitat locations. According to them large trees of the species were existing, strikingly all the sites are along the border lines of fringing patches. In vernacular language *X. granatum* is known as 'Bhelanda'. This name was similar to all the estuaries where it grows. It clearly indicates its luxuriant presence in the past.

Population structure and size of these species are disturbed. Populations were typically fragmented. In case of *D. spathacea,* isolated individuals were observed. Population of *X. granatum* and *C. iripa* consists of very few seedlings, saplings and mature trees but the same of *H. littoralis* and *D. spathacea* consists of only trees. Seedlings were recorded very rarely. It is alarming from regeneration point of view.

Table 2 depicts location of the species in estuaries, soils, tidal status and potential threats. Strikingly all the species inhabit borderline locations; soils were mostly gravely and well drained with moderate tidal activity. Human interference was the most common threat.*C. iripa* and *X. granatum* were studied for autecology and seed recalcitrance from our laboratory. This study revealed that, seed recalcitrance was the most important threat to regeneration of these species. The same was observed in case of *H. littoralis* also. Seed biology study of *D. spathacea* is under progress in our laboratory. However, immediate conservation action is needed.

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Survey of arbuscular mycorrhizal fungi associated with *Avicennia* (Mangrove plant).

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ABSTRACT

An attempt has been made to survey arbuscular mycorrhizal fungi associated with *Avicennia* plant. Soil samples and roots of *Avicennia* were collected from two localities of Srivardhan area. Two genera with six species were reportedfrom above localities. The genus *Glomus* was most common with four species with two species of *Scutellispora* were reported. The average number of AM propagules per 100 g soil ranged from 40 to 55. The percentage root infection was ranging between 20 to 50 percent.

KEYWORDS: Arbuscular mycorrhizal fungi, *Avicennia*

INTRODUCTION

Avicennia is one of important mangrove plant. It is grown invarious types of saline soils. The arbuscular mycorrhizal (AM) fungi play important role as a biofertilizer for promoting plant growth. They also play an important role for uptake and accumulation of phosphorus and other ions like copper, zinc etc.

MATERIALS AND METHODS

Avicennia plantis cultivated on two different localities of Srivardhan area of District Raigad at an interval of 30 days. These samples were analyzed for presents of mycorrhizal fungi. The isolation of AM propagules was done by wet sieving and decanting method (Gerdemann and Nicolson 1963). The percentage root infection was measured by Phillips and Hayman's (1970) method. Isolated spores were identified by using the Manual of Schencknk and Percz (1990). The number of propagules were counted under trinocular research microscope.

RESULTS AND DISCUSSION

The survey of AM fungi was carried out from two localities of Sriwardhan area. The first soil samples were collected at the interval of 30 days from rhizosphere of *Avicennia* plant. The genera *Glomus* and *Scutellispora* were found associated with *Avicennia*. The genus *Glomus* was most abundant with four species two species of *Scutellispora*. The rhizosphere soil from locality 2 had maximum number of AM propagules 40 per 100 g of soil in the month of June. Locality 2 to maximum percentage of root infection was 50% and minimum was 20%.

Table No. 1 Arbuscular mycorrhizal fungi reported from soil.

No.	AM Fungi	Locality1	Locality2
1	*Glomus fasciculatum*	+	+
2	*Glomus geosporum*	-	-
3	*Glomus hoi*	+	-
4	*Glomus macropora*	-	+
5	*Scutellispora calospora*	+	-
6	*Scutellispora minuta*	-	+

+Present; - Absent

Table No. 2 Number of propagules per 100 gm of soil and percentage root infection.

No.	Month	Locality1 No. of Prpa.	Locality1 % root infect.	Locality2 No. of Prpa.	Locality2 % root infect.
1	June-13	40	20%	40	20%
2	July-13	42	30%	43	20%
3	Aug-13	48	40%	45	30%
4	Sept-13	50	40%	48	40%
5	Oct-13	52	50%	50	40%
6	Nov-13	55	50%	55	50%

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Ecology and population dynamics of *Avicennia marina* in Navi Mumbai.

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ABSTRACT

Mangroves, inhabiting the ecotone between terrestrial and marine habitats, face a great variety of stresses. To overcome these, the mangrove plants have evolved a host of adaptations. They are physical, physiological as well as biological adaptations. *Avicennia marina* (Forssk.) Vierh, with two varieties, *A. marina* var. *marina* and *A. marina* var. *acutissima,* commonly known as grey or white mangrove are the most abundant mangroves along the estuaries and creeks of Mumbai and Navi Mumbai. The importance of mangroves in protecting the coast line, ensuring the fisheries production and conserving the biodiversity has been time-honored. The current study is an extension of study of comprehensive ecological status of the proposed site of International Airport coming up at the geographical center of New Mumbai having longitude 73°04'18" and latitude 18°59'33". Though the major part of the work was done in three years 2008, 09 and 10, visits to the site continued and the data presented here has been collected during the year 2011-12. *Avicennia marina* was found to hold a key position in the estuarine swamp forests as evidenced by the ecological indices. Reproduction and propagation potential of the plant was found to be high explaining why it is the most abundant plant in this swamp habitat.

KEYWORDS: *Avicennia marina,* population dynamics

INTRODUCTION

The climatic diversity has resulted in establishment of diverse forest communities in India. Among them are the Mangrove forests along the coast line of the country. In 1968, Champion and Seth revised the classification of coastal vegetation by segregating littoral (strand) and estuarine (swamp) forests. The tidal swamps were characterized further by Rao and Sastry (1974). Since then a number of reports on mangrove forests and communities from different geographical regions of India have been published (Kothari and Rao, 1995; Blasko and Aizpura, 1997; Naskar and Mandal, 1999 and 2008; Kathiresan, 2000; Shindikar *et al.,* 2009; Vijay Kumar and Vijaya Kumar, 2012; Ram and Shaji, 2013).

Avicennia marina (Forssk.) Vierh. is a very common, widely spread mangrove species of paleotropics. It is found in South Asia, Australia, East Africa and Middle East. Since all mangrove ecosystems occur within mean sea level and high tidal elevations, increase in sea level may cause

increased mortality of some and establishment of other species (Duke *et al*., 1998). Shidikar *et al. (*2009) have described the diversity of mangroves along Thane Creek, in accordance with the varying habitats. It may appear as a shrub growing to a height of 2-4 meters or may be a short tree upto 7 meters tall. It has pencil like pneumatophores and trunk with yellowish or brownish gray bark. The leaves are opposite, stipulate and petiolate with ovate-lanceolate lamina. They are glabrous above and tomentose sivery white beneath.

MATERIALS AND METHODS

The area of about 475 Ha bordering Panvel Creek and Ulve as well as Gadhi River Estuaries was surveyed over a span of 5 years from 2007 to 2012 for comprehensive ecological status (between 2007 and 2012 as a part of EIA study of the proposed International Airport) and later as an individual venture.

The mangrove stands along banks of the Ulve and Gadhi River Estuaries and Panvel Creek have been the focus of the current study. The vegetation was analysed by the linear point intercept LPI transect method as per the method described by Obura (1995), modified by Beenaerts and Berghe (2005) and later by Lam *et al.* (2006). The data was analysed and the ecological indices of diversity were calculated using the methods described by Heip *et al.* (1998).The sediment samples collected at the sites of transects was analyzed by the standard methods described by Murdoch *et al.* (1997).

RESULTS AND DISCUSSION

The stands of mangroves were found growing along the shores of Ulve-Gadhi-Panvel Creek system in a belt mostly 3-5 meters broad. Though the vegetation was essentially mixed in nature, it was predominated by *Avicennia marina* (Forssk.) Vierh.

The plants encountered in the habitat with their relative importance in the community (n/N X 100) and relative dominance $(n/N)^2$ X 100 is given in table 1. These indices have been based on their average number encountered along the triplicate transects taken at 12 locations in three seasons (pre-monsoon, monsoon and post-monsoon) over the span of 2 years. It can be easily deduced from the data that *Avicennia marina* var. *marina* is the commonest and most dominant species in the locality. The associate mangroves like *Acanthus ilicifolius* and *Clerodendron inerme* are successively dominant plant species in the habitat. The grasses *Scirpus littoralis,* and *Urochondra setulosa* are more abundant during monsoon but their number dwindles after November so that they become less conspicuous in other seasons. The grass *Cyperus arenarius*, though less abundant is significance since it is listed as a threatened species (Anitha, 2011). *Derris trifoliata* is a climber that has invaded the habitat only recently (Borkar *et al.,* 2002).

Since *Avicennia marina* was most abundant in the region, and in IUCN Red Data Book it is listed as 'least concern' species (Duke *et al.,* 2010) it was decided to ascertain reasons for the same. The central idea for floral biology was taken from Clarke and Meyerscough (1991). The *Avicennia marina* plants were found to flower mostly between March and July. On average 2-4 flower clusters with 1-8 flowers were found growing in the axil of each bract, though Clarke and

Meyerscough (1991) had reported 3-4 flower clusters with 1-14 flowers in each. The flower buds in each cluster were found to open in acropetal succession. Each flower was noticed to remain open for 2-4 days. There is protandry in *Avicennia marina* and this as proposed by Primack *et al.* (1981) promotes outcrossing in mangroves.

Fruits were found to develop in about 7-9 days, had persistent calyx and contained a single seed covered in a relatively thin pericarp. The fruit fall was noticed to coincide with commencement of rains and was observed all through June and most of the July. The tidal activities were found to gather fruits in huge masses where germination would set in. About 75-85% fruits germinated every monsoon, which was in agreement with the observations of Patwardhan and Pejaver (2002) at Thane. Despite the high germination rate most propagules of *Avicennia marina* do not take to root or otherwise perish over a span of few months and hardly 5-7% can grow into a sapling.

Table 1: The plant species, their families and Relative Importance values (R. Imp. %) as well as Relative Dominance values (R. Do. %).

Name of the plant	Family	R. Imp. %	R. Do. %
Avicennia marina var. *marina*	Avicenniaceae	32.65	10.66
Avicennia marina var. *acutissima*	Avicenniaceae	05.02	00.25
Avicennia officinalis	Avicenniaceae	02.87	00.08
Aegiceras corniculatum	Myrsinaceae	02.51	00.06
Bruguiera gymnorhiza	Rhizophoraceae	03.11	00.09
Rhizophora mucronata	Rhizophoraceae	02.15	00.04
Sonneratia apetala	Sonneratiaceae	02.27	00.05
Exoecaria agallocha	Euphorbiaceae	02.39	00.05
Salvadora persica	Salvadoraceae	06.58	00.04
Derris trifoliata	Fabaceae	04.54	00.21
Acanthus ilicifolius	Acanthaceae	11.12	01.24
Clerodendron inerme	Verbenaceae	03.46	01.20
Thespecia populne	Malvaceae	00.84	00.007
Urochondra setulosa	Poaceae	06.82	00.46
Scirpus littoralis	Cyperaceae	07.66	00.59
Cyperus arenarius	Cyperaceae	02.63	00.07
Prosopis chilensis	Mimoseae	01,91	00.04
Cassuarina equisetifolia	Casuarinaceae	01.43	00.02

The soil samples were taken from various locations where there was accumulation of propagules of *Avicennia marina* and the results of their analysis are displayed in Table 2. It was noticed that the survival of propagules was better in location numbered 3, 4, 5, 7, 8 and 10, indicating that the texture of soil best suited for germination and sustenance of saplings is fine or loam.

Table 2: Results of analysis of soil samples from 10 different locations along mangrove stands.

Parameter	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Texture	Coarse	Coarse	Fine	Loamy	Fine	Coarse	Loamy	Loamy	Coarse	Fine
pH	7.4± 0.1	7.6± 01	6.9± 0.08	6.8± 0.1	6.6± 0.14	7.2± 0.11	7.0± 0.1	6.8± 0.1	7.4± 0.13	6.7± 0.1
NO_x-N (mg/100g)	0.28± 0.04	0.19± 0.02	3.07± 0.06	2.54± 0.1	2.88± 0.11	0.72± 0.05	2.19± 0.1	2.36± 0.13	1.03± 0.07	3.52± 0.12
PO_4-P (mg/100g)	0.035± 0.006	0.047± 0.011	0.08± 0.02	0.11± 0.008	0.24± 0.015	0.092± 0.009	0.21± 0.04	0.09± 0.03	0.04± 0.008	0.19± 0.04
Org. Carbon (g%)	0.14± 0.02	0.21± 0.06	1.29± 0.14	1.06± 0.09	2.17± 0.32	0.23± 0.05	0.88± 0.06	1.15± 0.1	0.34± 0.05	2.2± 0.41

The maximum survival rate was noticed at locations 3, 5 and 10, suggesting that finer the texture of soil better the survival. With finer texture of soil, the organic carbon contents as well as the availability of biogenic nutrients was better. In monsoon, the pH of soil appears to be slightly acidic in fine textured soils, probably because of organic acids and amino acids produced in the decomposition of organic matter and due to heavier leaching of salts. Similar observations have been reported by Patel *et al.* (2010) along Gujarat coast and by Alleman and Hester (2011) in Lousiana.

CONCLUSION

It can be inferred from the study that *Avicennia marina* is predominant mangrove species along the coasts of estuaries and creeks of Navi Mumbai, probably because of abundant seed production, high percentage of germination and whenever and wherever there is fine soil the saplings establish successfully.

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Hydrological status of mangroves from selected areas around saltpans in Mumbai.

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ABSTRACT

Mangroves are salt-tolerant (halophytes) plants found around the saltpans in Mumbai. Mangroves occur in the intertidal areas of creeks, lagoons and high coastal mudflats. They can withstand low oxygen levels and high salinity conditions. In view of the ecological and economic importance of the mangroves, in addition to their increasing loss due to deforestation, the present investigation was carried out to study the hydrological status of salt pans around the mangroves from Mulund and Bhandup areas in Mumbai. The water samples were collected during the period May-June as pre monsoon samples and July-August as monsoon samples, to assess the hydrological status and compare them seasonally. Various parameters like BOD, COD, DO, CO_2, salinity, hardness, and alkalinity were estimated using standard methods of water analysis (APHA). It was found that dissolved oxygen, COD, salinity, hardness and alkalinity, decreased during the monsoon period while the BOD and CO_2 levels increased indicating, more degradation of organic matter in the water. The water samples were also observed for the planktonic existence. Some zooplanktons like copepods, lucifer, zoea larvae, mysis, fish eggs, fish larvae and phytoplanktons like diatoms, dinoflagellates - *Noctiluca* were observed during the monsoon period indicating the availability of nutrients.

KEYWORDS: Mangroves, salinity, hardness, alkalinity, saltpans, planktons

INTRODUCTION

Water forms an essential component of all living organisms. The various el elements present in any water body provide nutrients for the growth and development of aquatic organisms. Most of the aquatic organisms rely on dissolved oxygen present in water. The oxygen present also degrades the organic matter, thus making life sustainable for aquatic organisms. The various inorganic and organic substances determine the health of the ecosystem. The present investigation was carried out to assess the hydrological status of water present around saltpans in Mumbai. Mangroves are salt-tolerant plants found in Mulund and Bhandup areas. They can withstand lowoxygen levels and high salinity contents. The present study was carried out from saltpans around Mulund and Bhandup areas in Mumbai to compare the physico chemical characteristics of water during pre-monsoon and Monsoon periods. The various parameters like salinity, dissolved oxygen, free CO_2, total alkalinity, hardness were analysed. The presence of

phytoplankton and zooplankton isindicative of the physico-chemical factors affecting them (Sarwade, 2013). The ecological state of the ecosystem is not only reflected by the chemical component of water but also by other factors (Karr *et al.,* 2000).

MATERIALS AND METHODS

The water samples were collected seasonally in June and August at 1-5 m depth in dried plastic cans of 5 litres capacity around the salt pans in Mulund and Bhandup areas in Mumbai. The various parameters like salinity, dissolved oxygen, hardness, free CO_2 and total alkalinity were analysed by standard methods of water analysis (APHA).

RESULTS AND DISCUSSIONS

The various hydrological parameters affect the life of various aquatic organisms in different ways. Quality of water plays an important role in the chemical and organic status of water reservoir, therefore it is necessary to check and maintain water quality standards through proper management strategies (Goswami, 2013). The seasonal variations in physico-chemical parameters of Mulund and Bhandup areas are represented below in Table 1 and Table 2 respectively and in Figure 1 and Figure 2

Table 1.

Parameters	Pre-monsoon	Monsoon
DO (mg/lt)	1.46	1.20
Salinity (g/kg)	13.93	0.75
CO_2 (mg/lt)	Absent	Absent
Hardness(mg/lt)	286	44
Total Alkalinity (mg/lt)	210	225

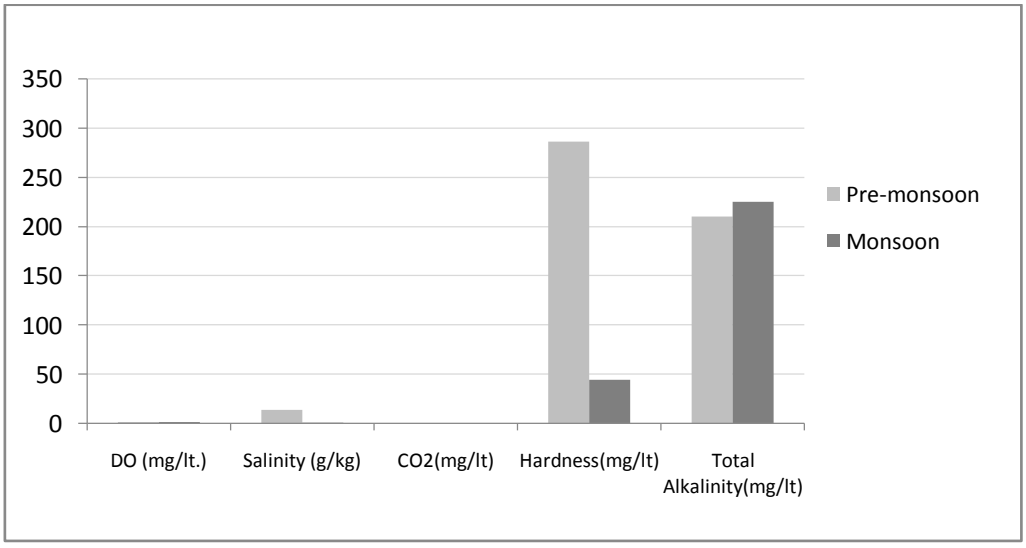

Figure 1.

Table 2.

Parameters	Pre-monsoon	Monsoon
DO (mg/lt.)	2.01	1.81
Salinity (g/kg)	7.64	1.43
CO_2(mg/lt)	113.65	209
Hardness(mg/lt)	212	42
Total Alkalinity(mg/lt)	100	200

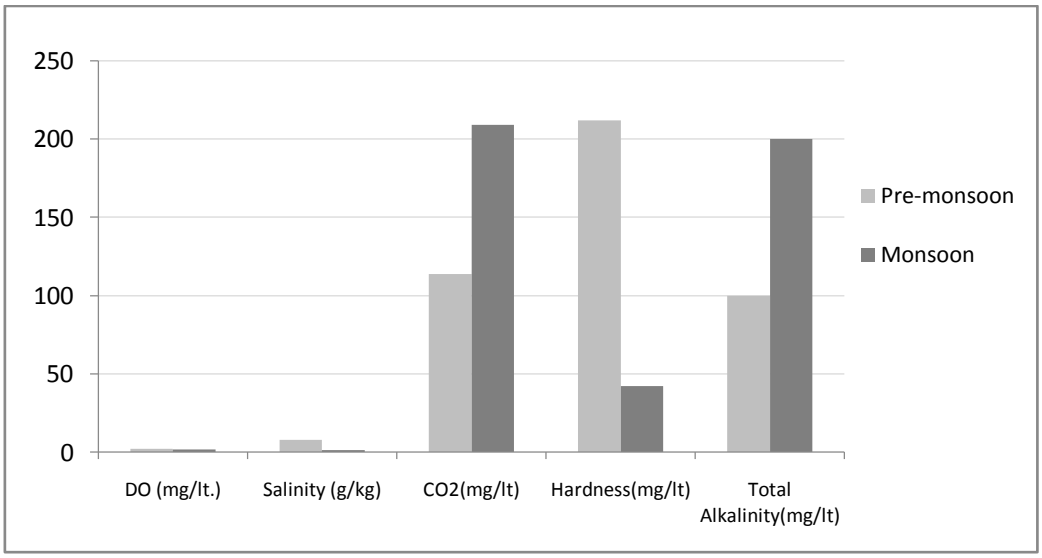

Figure 2.

The present investigation showed that the parameters like dissolved oxygen, salinity and hardness decreased during the rainy season which may be due to dilution by run-off and drainage caused by heavy precipitation. Alkalinity is a measure of the amount of ions present that can react with or neutralize H^+ alkalinity is generally due to salts of carbonates, bicarbonates, phosphates, nitrates, hydroxyl ions, etc. High values of alkalinity were recorded during the rainy season which may be due to introduction of HCO_3 into the water. CO_2 values increased during the monsoon. Carbon dioxide input may exceed the buffering capacityof salt-water. The pH of water decreases, but carbonate sediments may also act as buffer. The carbonate sediments will dissolve in the reaction as:

$$H^+ + CaCO3 = Ca^{2+} + HCO_3^-$$

This reaction introduces additional HCO_3^- into the water, increasing the alkalinity and the buffering capacity of salt-water (Thurman and Burton).

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Quality evaluation of bioactive markers from medicinally important mangroves *Avicennia marina* and *Sonneratia apetala* using validated HPTLC method.

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Abstract

Despite several publications concerning mangrove forests, a clear understanding of the dynamics in mangrove ecosystems is just beginning to emerge. Mangroves have been a source of several bioactive compounds, they have been used in folklore medicines and the extracts have proven activity against human, animal and plant pathogens too. *Avicennia marina* Forssk. (Avicenniaceae) and *Sonneratia apetala* Buch.-Ham. (Lythraceae) are two shrubby, medium-sized mangrove trees found in intermediate estuarine zones in all intertidal regions. Leaves of *A. marina* have been reported to possess antimicrobial, antioxidant, anti-candidal and cytotoxic activities whereas leaves of *S. apetala* are reported to possess antimicrobial and antifungal activities. The present work, quality of the leaves of these two mangroves has been evaluated using HPTLC technique. Simultaneous estimation of three triterpenoids, namely β-sitosterol, lupeol and ursolic acid has been carried out using a single mobile phase from the leaves of *A. marina* and *S. apetala*.

Chromatographic separation was achieved on TLC plates pre-coated with silica gel $60F_{254}$ using toluene: methanol (8:1, v/v) as the mobile phase. A compact spot of ursolic acid at Rf value of 0.30 ±0.02, β-sitosterol at R_f value of 0.46 ±0.02 and lupeol at an Rf value of 0.59 ±0.02 was observed in the ethyl acetate extracts of the plant samples at 366 nm. Methanolic sulphuric acid reagent (10%) was used as the derivatizing reagent. The method has been validated as per ICH guidelines and can be a useful as an analytical tool for quality evaluation of these two mangroves and other plants rich in these three triterpenoids.

KEYWORDS: Mangroves, HPTLC, simultaneous estimation, β-sitosterol, lupeol, ursolic acid

INTRODUCTION

Mangrove forests have been utilized for many functions including wood production, firewood and charcoal (Tomlinson, 1994). However, wood-related activities or industries are very destructive and the rates of mangrove renewal do not match this at all (Kairo *et al*., 2001). Recently, it has been strongly recommended that mangroves should be considered as a valuable

source for chemical constituents with potential medicinal use. Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents (Khafagi *et al*., 2003).

Avicennia marina Forssk. (Avicenniaceae) commonly called as *Tivar* is a small mangrove tree growing upto 3-14 m in height with small yellow flowers. The leaves are thick, ovate-lanceolate shaped and glossy with silvery-white lower surface. It is found in the intermediate estuarine zones of all intertidal regions along the coasts of East Africa, Asia up to Australia. *Sonneratia apetala* Buch.-Ham (Lythraceae) commonly called as *Kandal* is another small mangrove tree reaching 20 m in height. It has simple, opposite and leathery leaves whereas the flowers are apetalous, green in color and fleshy. *S. apetala* is specifically found along the coasts of India, Bangladesh, Myanmar and China. Leaves of *A. marina* have been reported to possess antimicrobial, antioxidant, anticandidal and cytotoxic activities whereas leaves of *S. apetala* are reported to possess antimicrobial and antifungal activities (Teja and Ravishankar, 2013). Though both these plants have been reported to possess Flavones, flavanoids, terpenoids and phytochemicals like betulin, β- sitosterol (Zhu *et al*., 2009) (Ji *et al*., 2005) etc., no chromatographic method exists till date for their quality evaluation. Also, no monograph for these plants is available in any of the pharmacopoeias.

Thus, in the present work, quality parameter limits have been established along with the microscopic analysis of the leaves. Further, the quality of the leaves of these two mangroves has been evaluated using HPTLC technique. Simultaneous estimation of three triterpenoids namely β-sitosterol, lupeol and ursolic acid has been carried out using a single mobile phase from the leaves of *A. marina* and *S. apetala*.

MATERIALS AND METHODS

Plant materials

Avicennia marina and *Sonneratia apetala* was collected from Airoli, Mumbai and the herbarium of the sample was authenticated from Agharkar Reasearch Institute, Pune. Sample was carefully segregated, cleaned and oven dried at 37°C to constant weight, powdered, sieved (BSS 85) and stored in airtight containers.

Drugs and chemical

Standards were procured from Sigma Aldrich, Germany and working standards were prepared as per requirement. All other chemicals used were of analytical grade.

Microscopy

Thin transverse sections of the leaf across the lamina and crossing the midrib were taken, stained with dilute safranin and observed under 45X magnification using light microscope equipped with a camera. Further, the powder of the dried leaves was also evaluated microscopically and distinctive characters were noted.

Physicochemical evaluation

The quality of the leaves was assessed by determining the proximate parameters like foreign organic matter, ash content, acid insoluble and water soluble ash content and loss on drying using standard pharmacopoeial methods (The Indian Pharmacopoiea, 2010).

Phytochemical analysis

The powder of dried leaves was then subjected to a phytochemical evaluation by successive soxhlet extraction with various organic solvents in order to analyze the percent extract of major class of compounds present in the raw materials using the method reported by Harborne (Harborne, 2007).

HPTLC Conditions

Chromatographic separation was achieved on TLC plates pre-coated with silica gel 60 F254. Samples were spotted using the CAMAG Linomat 5 sample spotter (CAMAG Muttenz, Switzerland) equipped with syringe (Hamilton, 100 µL). Plates were developed in a glass twin trough chamber (CAMAG) saturated with mobile phase toluene: methanol (8:1, v/v). Densitometric scanning was carried out using CAMAG TLC Scanner 4 equipped with winCATS software and CAMAG -Reprostar 3 was used for photo-documentation.

Method Validation

The developed HPTLC method for estimation of β-sitosterol, lupeol and ursolic acid was validated as per ICH guidelines for the parameters like sensitivity, linearity, precision, recovery, specificity and ruggedness.

Estimation of the markers

The quantity of the markers was calculated using the regression equation obtained from the regression analysis of the calibration curve.

Statistical analysis

The statistical analysis of the results obtained was done using Microsoft Excel 2007.

Safety evaluation

Safety study of the hydroalcoholic extract of the leaves of *A. marina* and *S. apetala* was conducted in mice as per OECD guidelines (No. 420, fixed dose procedure). The mice were fasted overnight for 10-14 hours and administered with the extract (2.0 g/kg) orally. The animals were observed individually during the first 30 min for all reflexes, periodically during the first 48 hours with special attention given during the first 4 hours (short-term toxicity) and daily thereafter for a total of 14 days (long-term toxicity) for alteration from general behavior and clinical symptoms like alteration of skin and fur texture, ptosis, excessive salivation, breathing problems, diarrhea etc. Daily body weight, food and water intake record was also maintained. The results were compared with control group (orally administered with DW).

RESULT AND DISCUSSION

The transverse section of the leaf of *Avicennia marina* shows single upper and lower epidermis followed by a multilayered palisade and 2-3 layered spongy tissue in the mesophyll region. The distinct feature is the presence of T-shaped salt excretory glands on the surface of the leaf. The T. S. also shows the presence of sunken stomata symbolizing reduced transpiration rates found in the plant. Sclerenchyma cells were found present on the lower surface in the section crossing the midrib. The leaf section of *S. apetala* also shows similar vascular bundle structure as in *A. marina*. The leaf T. S shows single epidermal layers on both the surfaces of the leaf. In the mesophyll region, the plant shows the presence of palisade tissue on both surfaces followed inwards by the spongy tissue layers. The leaf shows presence of air pockets on the surface. Powder microscopic evaluation supported light microscopy by the presence of glandular cells in the powder of *A. marina* along with spiral xylem vessel, fibres and a mass of sclerenchymatous cells. This data correlation emphasizes on powder microscopy as a potent quality evaluation tool. Leaf powder of *S. apelata* showed the presence of spiral xylem, fibre and sclerenchyamatous cells. Treatment with Lugol's reagent also confirmed the presence of starch grain in the sample.

The values obtained for foreign organic matter, ash content, acid insoluble and water soluble ash content, loss on drying and the extractive values in various solvents have been summarized in the table 1 and 2. As no monograph is available, limits have been prescribed. For both the samples, percent extractive value in 50: 50 hydroalcohol was found to be maximum; among which *S. apetala* showed higher results. Leaves of both the plants were found to be rich in alkaloids. On the contrary, the fraction of fats and waxes i.e. the neutral extract was least (Table 3). In HPTLC, mobile phase composition of toluene: methanol (8:1, v/v) showed good resolution of ursolic acid, β-sitosterol and lupeol from other phytoconstituents simultaneously. The Rf values of ursolic acid, β-sitosterol and lupeol were found to be 0.31, 0.48 and 0.59 respectively. The method developed was validated as per ICH guidelines. The method was further applied in the detection and quantitation of the biomarkers simultaneously from the hydroalcoholic extract of the leaves of *A. marina* and *S. apetala*. The content of ursolic acid, β-sitosterol and lupeol in *A. marina* was found to be 1.4286 ±0.0064, 0.0934 ±0.0010 and 0.7575 ±0.0180 mg/g of the sample respectively. In *S. apetala,* the content was found to be 2.1582 ±0.0268 and 0.0832 ±0.0032 mg/g of ursolic acid and β-sitosterol respectively. Lupeol was not detected in the hydroalcoholic extract of the leaves of *S. apetala*.

The safety of both the drug was established by acute oral toxicity study carried out on mice at 2.0 g/kg body weight. The plants were found to be safe as it showed no abnormal fluctuation in body weights and food and water intake of the animals. Clinical symptoms of toxicity were also found to be absent during the period of the study and no mortality was recorded. The safety study of the mangroves revealed that the in form of hydroalcoholic extract of the leaves, they can be considered safe with a wide margin for oral use.

Table 1: Preliminary analysis.

Parameters	Observed values (%)		Suggested limits (%)	
	A. marina	***S. apetala***	***A. marina***	***S. apetala***
Foreign organic matter	0.41 ±0.0112	0.43 ±0.0152	0.378-0.444	0.393-0.485
Total ash	17.27 ±0.2001	11.78 ±0.0660	16.669-17.871	11.589-11.985
Acid insoluble ash	0.90 ±0.0013	0.65 ±0.0596	0.896-0.904	0.478-0.836
Water soluble ash	4.30 ±0.1373	4.88 ±0.5672	3.889-4.711	3.180-6.583
Loss on drying	9.67 ±0.4588	8.89 ±0.1800	8.297-11.050	8.356-9.437

Table 2: Extraction in different solvents.

Solvents	Extraction (%)	
	A. marina	***S. apetala***
Ethanol	8.96	10.52
Methanol	10.24	11.96
Toluene	6.16	5.00
DW	16.32	17.64
Ethyl acetate	3.04	3.70
Acetonitrile	0.26	0.64
Cyclohexane	0.10	0.04

Table 3: Phytochemical analysis.

Parameters	(%)	
	A. marina	***S. apetala***
Fats and waxes	0.472	0.66
Fibres	72.69	71.58
Terpenoids and Phenolics	3.511	2.957
Quaternary Alkaloids and N-Oxides	2.961	3.05
Alkaloids	14.57	17.68
Undetected	5.796	4.073
Total	94.204	95.927

Table 4: Optimized chromatographic conditions.

Parameters	Specifications
Stationary Phase	Merck silica gel 60 F_{254} HPTLC pre-coated plates
Sample Applicator	Camag Linomat 5
Development distance	85 mm
Derivatization	10% Methanolic sulphuric acid reagent
Densitometric scanner	Camag scanner 4
Software	winCATS planar chromatography manager software version 1.4.7
Lamp, wavelength	Mercury, 366 nm
Photodocumentation	Camag Reprostar 3

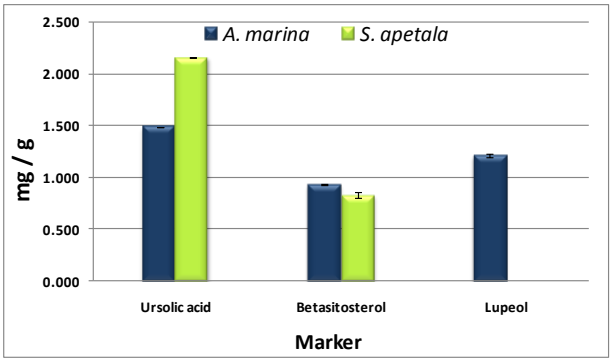

Figure 1: Content of bioactive markers from *A. marina* and *S. apetala*

CONCLUSION

The present research work involves the pharmacognostic and phytochemical evaluation of two mangroves; *A. marina* and *S.* apetala along with simultaneous estimation of three important biomarkers form the hydroalcoholic extract of the leaves of these plants. As no monographs are available for these plants, the present research work can be used as baseline database for the compilation of a monograph. The developed HPTLC method can be used as a quality control tool for these as well as, plants reported to contain these markers.

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