APPLICATION OF BIOTECHNOLOGY IN THE CONSERVATION OF ENDANGERED PLANT SPECIES FOR GENETIC ENHANCEMENT (BT/TF/19/02/91)

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M.S.SWAMINATHAN RESEARCH FOUNDATION
Centre For Research On Sustainable Agricultural
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MADRAS

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PROJECT TEAM

Dr. M.S. SWAMINATHAN	Principal Coordinator
Dr. M.S.S. MOHAN	Senior Scientific Officer
Dr. HEMAL S. KANVINDE	Senior Scientific Officer
Mr. P. BALAKRISHNA	Senior Scientific Officer
Ms. S. LAKSHMI	Research Assistant
Mr. K.M. KADIRVELU	Technical Assistant

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Dr. G. Madhava Reddy
Director
Centre for Plant Molecular Biology
Osmania University, Hyderabad

Dr. S. Ulaganathan
Lecturer
Centre for Plant Molecular Biology
Osmania University, Hyderabad

Mr. B.N. Nayak
Divisional Forest Officer
Bhitarkanika Wildlife Sanctuary
Cuttak District, Orissa

Mr. Joseph Jogindernath
District Forest Officer
Cuddalore, Tamil Nadu

Dr. Leela J. Bhosale
Department of Botany
Shivaji University
Kolhapur, Maharashtra

Prof. D.D. Awasthi
Retd. Professor of Botany
National Botanical Reserch Institute
Lucknow

Dr. G.N. Hariharann
Research Fellow
Bharatidasan University, Trichy

Dr. V. Selvam
Ms. G. Uma
Ms. H.D. Subashini
Mr. S. Madhusudhanan
Centre for Research on Sustainable
Agricultural and Rural Development, Madras.

Contents

APPLICATION OF BIOTECHNOLOGY IN THE CONSERVATION OF ENDANGERED PLANT SPECIES FOR GENETIC ENHANCEMENT

INTRODUCTION

This project was sanctioned by the Department of Biotechnology, Government of India, (BT/TF/19/02/91), with effect from February 1, 1992. The original project proposal contained provision for the construction of a molecular biology laboratory as well as for equipping it. The approved project however contained provision only for the purchase of equipment and for recruiting scientific and supporting staff to carry out the research. Therefore a building was taken on rental lease for one year to establish the laboratory. The purchase of equipment, and the recruitment of scientific and supportive staff was phased in a manner that maximum benefit can be derived from the available infrastructure.

Appointments for the positions of 3 Senior Scientific Officers Gr II, 1 Research and 1 Technical assistant were made after conducting interviews by a committee including the nominee of DBT. The appointments were made effective from March, 1992. The purchase of equipment sanctioned by the DBT was done in phases and the first phase purchases were made during the financial year 1992-93, as per DBT guidelines by calling tenders. A purchase committee chaired by Prof. M S Ananth, Head, Department of Chemical Engineering, IIT, Madras was constituted for this purpose. The eqipment already arrived is now under effective use. The scientific work began with extensive collection trips to different areas.

As part of its commitment to undertake the sanctioned project necessary infrastructural facilities were designed in the permanent site of the Foundation at Taramani, Madras. A well designed laboratory is being developed to cater to the needs of the scientists working in molecular biology, microbiology and tissue culture. The building is being dedicated on April 14, 1993 (Annexure I).

The following facilities have been provided in the laboratories which would become fully functional from June 1993.

(1) A well equipped molecular biology laboratory with all necessary facilities to carry out biotechnological work

(2) A radio-active isotope laboratory for handling radio isotopes,

(3) A specialised dark room for developing and printing photographs and autoradiographs,

(4) A centralised instrumentation facility to hold most of the analytical instruments,

(5) A microbiology laboratory with culture room facilities,

(6) A laboratory kitchen and wash facilities,

(7) A separate enclosure to house a fume hood,

(8) A cytology laboratory,

(9) Extensive tissue culture facility with clean rooms,

(10) A cold room and a growth chamber

are the facilities that are provided. Besides these, the laboratories are being supported by state of art mist propagation chambers, and an extensive green house for multiplying rare and endangered plants. All the laboratories are designed according to international standards and specifications. Also, the design favours minimal contamination, conserves energy and effectively supports reserach activities in itself. Solar energy is being used for the first time in the country, as a primary source of energy in the laboratories.

A DBT Review Committee Meeting was held, on the 18th December 1992, under the Chairmanship of Prof.T.N. Khushoo. A copy of the review committee report and its comments are appended in this report as annexure .

With full- fledged facilities being available, it was decided to fill-up the rest of the sanctioned project appointments. An advertisement in leading national newspapers was made in March, 1993. Necessary equipments sanctioned under the project will be purchased as soon as the funds for the current financial year 1993 - '94 are released.

Aim of the Project

Sustainable advances in biological productivity is indispensable for meeting the food, fuel, and other needs of our growing population. Biological diversity is essential for achieving sustainable gains in productivity per units of land, water, energy and time. We can neither sustain a national food security system nor face the challenge of climate change if we fail to conserve and utilize in a sustainable manner our genetic wealth in flora, fauna and microorganisms. Several organizations have come forward to prevent genetic erosion at the ecosystem, species and intra-specific levels. There is however, no sustained and coordinated research efforts are designed for saving the species under threat of extinction.

There is a general agreement that we are experiancing considerable genetic erosion. In our country, the Botanical Survey of India has so far published three Red Data Books (1987, 1988 and 1990). These three Books contain a list of 814 threatened taxa of Indian flora. In the lists of extinct, possibly extinct, endangered, vulnerable or rare species published by the Botanical Survey of India as many as 123 species occur in the state of Tamil Nadu alone (Table 1). The Botanical Survey has further indicated that atleast 17 can be grown in Madras (Table 2) It is such plants which are in need of conservation.

Table 1. Name of 123 species listed as extinct, possibly extinct, endangered, vulnerable or rare which occur or are known to have occured in Tamil Nadu, India.

Species	Status in India	Distribution within Tamil Nadu
Acranthera grandiflora	Endangered	Tirunelveli
Actinodaphne bourneae	Endangered	Palani hills
Actinodaphne lanata	Endangered	Nilgiris
Actinodaphne lawsonni	Rare	Nilgiris
Amomum microstephanum	Rare	Anamalais
Anoectochilus rotundifolius	Endangered/ possibly extinct	High Wavies
Antinostrophe serratifolia	Rare	Anamalais
Aponogeton appendiculatus	Indeterminate	Madras
Atuna travancorice	Indeterminate	Courtallum
Belosynapsis kewensis	Endangered	Tirunelveli/ Kanyakumari
Bentinckia codapana	Rare	Tirunelveli
Bulbophyllum albidum	Rare	Nilgiris/ Tirunelveli
Bulbophyllum acutiflorum	Rare	Nilgiris
Bulbophyllum elegantulum	Vulnerable	Nilgiris
Bulbophyllum catiense	Vulnerable	Nilgiris
Bunium nothum	Possibly extinct	Nilgiris
Campanula alphonsii	Rare	Nilgiris/ Palani hills
Capparis diversifolia	Rare	Tirunelveli
Capparis fusifera	Rare	Tirunelveli
Capparis rheedii	Rare	Tirunelveli
Capparis shevaroyensis	Vulnerable	Ramanathapuram
Carex christii	Indeterminate/ possibly extinct	Nilgiris
Carex pseudoaperta	Indeterminate	Nilgiris
Carex vicinalis	Indeterminate	Nilgiris
Cayratia pedeta	Rare	Nilgiris
Cayratia roxburghii	Vulnerable	Tirunelveli
Ceropegia barnesii	Endangered	Nilgiris
Ceropegia decaisneana	Rare	Anamalais/ Nilgiris
Ceropegia fimbriifera	Vulnerable	
Ceropegia maculata	Endangered/ possibly extinct	Anamalais
Ceropegia metziana	Rare	Western Ghats
Ceropegia thwaitesii	Vulnerable	Kodaikanal
Ceropegia omissa	Endangered	Courtallum
Ceropegia spiralis	Vulnerable	Area not furnished
Ceropegia pusilla	Rare	Nilgiris

Chrysoglossum hallbergii	Indeterminate & insufficiently known	High Wavies
Cleoxme burmanni	Indeterminate	Ramanathapuram
Coelogyne mossiae	Vulnerable	Nilgiris/ Palani hills
Commelina hirsuta	Rare	Nilgiris/ Palani hills
Commelina tricolor	Vulnerable	Karadimalais
Commelina wightii	Vulnerable	Nilgiris/ Palani hills
Corymborkis veratifolia	Rare	Nilgiris
Cotomeaster buxifolius	Vulnerable	Nilgiris/ Palani hills
Crotolaria clavata	Endangered	Coimbatore/ Madurai/ Salem
Crotolaria digitata	Rare	Kolli/ Palani hills
Crotolaria fysonii	Endangered	Palani hills
Crotolaria globosa	Rare	Nilgiris/ Courtallum/ Dindug
Crotolaria kodaiensis	Endangered	Palani hills
Crotolaria longipes	Endangered	Kolli/ Nilgiris
Crotolaria priestleyoides	Rare	Anamalais/ Nilgiris
Crotolaria peduncularis	Rare	Anamalais/ Palani hills
Crotolaria rigida	Rare	Nagapattinam/ Coimbatore/ Tirunelveli
Crotalaria scabra	Rare	Coimbatore/ Kanyakumari/ Salem/ Tirunelveli
Cyathea nilgirensis	Endangered	Nilgiris
Decaschistia rufa	Endangered	Chengalpattu
Desmos viridiflorus	Endangered	Anamalais/ Coimbatore
Dictyospermum ovalifolium	Rare	Western Ghats
Elaecarpus venustus	Vulnerable	Kanyakumari
Elaphaglossum beddomei	Rare	Nilgiris/ Anamalais
Elaphaglossum nilgiricum	Endangered	Nilgiris
Elaphaglossum stigmatolepis	Vulnerable	Nilgiris/ Palani hills
Eragrostis rottleri	Presumed extinct	Tranquebar
Eria albiflora	Rare	Nilgiris
Eriochysis rangacharii	Presumed extinct	Nilgiris
Eugenia discifera	Endangered	Sethur hills
Eugenia singampattiana	Endangered/ possibly extinct	Tirunelveli
Euonynus angulatus	Endangered	Nilgiris
Euonynus serratifolius	Endangered/ possibly extinct	Anamalais/ Nigiris
Goniothalamus rhynchantherus	Rare	Tirunelveli
Habenaria barnesii	Rare	Nilgiris
Hedyotis albonervia	Endangered	Tirunelveli
Hedyotis barberi	Vulnerable	Area not frunished
Hedyotis buxifolia	Rare	--
Hedyotis cyanantha	Rare	--
Hedyotis eualata	Rare	--

Hedyotis ramarowii	Vulnerable	--
Hedyotis swersioides	Rare	--
Hedyotis hirsutissima	Possibly extinct	Nilgiris
Helichrysum perianigerum	Rare	Coimbatore
Humboldtia decurrens	Rare	Tirunelveli
Humboldtia bourdilloni	Endangered	Courtallam
Humboldtia unijuga	Endangered	Tirunelveli
Hydrocotyl conferata	Rare	Nilgiris/ Palani hills
Impatiens neo-barnesii	Endangered	Nilgiris
Indigofera barberi	Rare	S.Arcot/ Shevroy hills
Indotristicha tirunelveliana	Rare & vulnerabl	Tirunelveli
Liparis biloba	Vulnerable	Nilgiris
Miliusa nilagirica	Vulnerable	Nilgiris/ Anamalais
Murdannia juncoides	Rare	Courtallum
Murdannia lanceolata	Vulnerable	Red hills
Nueracanthus neesianus	Endangered/ possibly extinct	North Arcot
Nothopegia aurea-fulva	Endangered	Tirunelveli
Ophiorrhiza brunosis	Presumed extinct	Nilgiris/ Palani hills
Ophiorrhiza pykarensis	Possibly extinct	Nilgiris
Orophea uniflora	Rare	Tirunelveli
Palaquium bourdillonii	Extinct/ Indeterminate	Tirunelveli
Paphiopedilum druryi	Endangered/ possibly extinct	
Peucedanum anamallayense	Indeterminate	Anamalais
Pimpinella pulneyensis	Possibly extinct	Palani hills
Piper barberi	Rare	Kanyakumari
Popowia beddomeana	Rare	Tirunelveli
Psychotri globisephala	Endangered	Courtallum
Rhynchosia velutina	Vulnerable	Tanjore/ Tirunelveli/ Kanyakumari
Salacia beddomei	Rare	Anamalais
Santapaua madurensis	Endangered	Madurai/ Pudukottai/ Tanjore
Senecio kundaices	Endangered	Nilgiris
Smilax wightii	Rare	Nilgiris
Sphaeropteris crinitu	Endangered	Nilgiris
Syzygium gambleanum	Endangered	Kanyakumari
Syzygium courtallense	Endangered	Courtallum
Teucrium plectanthoides	Vulnerable	Tirunelveli
Thottea barberi	Vulnerable	Tirunelveli

Vanasushava pedata	Rare	Anamalais/ Palani hills
Vanda wightii	Possibly extinct	Nilgiris
Vanilla wightiana	Rare	Tirunelveli/ Kanyakumari
Vernonia pulneyensis	Endangered	Palani hills
Vernonia recurva	Endangered/ possibly extinct	Anamalais
Wendlandia angustifolia	Presumed extinct	Tirunelveli
Willisia selenginoides	Rare	Anamalais
Youngia nilgiriensis	Endangered	Nilgiris

Source : Botanical Survey of India

Table 2 List of some threatened species for collection and cultivation at the Research Centre of the Foundation.

Cynometra bourdilloni Gamble.

Hamboldtia decurrens Bedd. ex Oliver.

H. unijuga Bedd.var. unijuga

Decaschistia rufa Craib.

Kingiodendron pinnatum (Roxb. ex DC) Ilarms

Calliandra cynometroides Bedd.

Dialium travancoricum Board.

Pterospermum reticulatum Wt & Arn.

Hildegardia populifolia (Roxb.) Sch.& Endi.

Iriolaena lushingtonii Dunn.

Isonanadra billosa Wt.

Bentinckia codapanna Berny ex Roxb.

B. nicobarica (Kurz.) Becc.

Mangifera andamanica King.

Calamus dilaceratus Becc.

Korthalsia rogersii Becc.

Coripha macropoda Lindel ex. Kurz.

The project aims at using both conventional and latest tools of propagation, including tissue culture to conserve such rare and endangered plants. The tools of molecular biology and recombination DNA technology have come handy today especially to understand the amount of diversity present among such plants and to identify the genes responsible for specific characters.

The second component of the project is the study of application of bioindicators. The cumulative effect of ever increasing population, unsustainable agricultural practice, various pollutions caused by anthropogenic input has become major threats to biodiversity. The level of pollution is recognized as a potential selective force, which by inflicting stress on various individual sensitive species, may upset the balance of the ecosystem and lead to the dominance of more tolerant species. Various plant and animal species which show positive sensitivity to such perturbations are called bioindicators and are of vital importance in monitoring ecosystem health.

Bioindicators are considered superior to other analytical techniques because they are low in cost and less energy consuming. Many species can be classified into sensitive, intermediate and tolerant areas. But an assessment of pollution using an indicator organism is always influenced by various factors such as its genetic make-up, metabolism, environmental condition and exposure dynamics

II EXPERIMENTAL STUDIES

1. Collection, propagation, field evaluation of endangered species listed in Red Data Book of Botanical Survey of India, RFLP studies of mangroves, generic DNA library.

The present investigation is designed to mobilize biotechnological technique to save such plant species under threat of extinction and to use latest biotechnological tools to understand the nature and extent of genetic diversity at the intra-specific level and for undertaking genetic enhancement work.

The work carried out during the present period of investigation are as follows:

(I) Collection and Propagation

a. The studies were initiated with the identification and collection of certain mangrove species. The collection was made of the following species Rhizophora mucronata, R. apiculata, Avicennia, alba, A. marina, Xylocarpus, Kandelia candel, Heretiera, Bruguiera Aegialatis Porteresia coarctata. All of them are being successfully maintained at the nursery attached to the laboratory.

b. Seed/propagules were collected for establishment of nursery.

c. Conventional propagation methods were tested

d. Physiological studies were carried out with Avicennia marina as model

e. Certain modern methods of propogation like hydroponics were carried out

f. Electron microscopic studies of Avicennia marina were carried out with a view to identify the possible changes in the structure and function of salt glands with special reference to different salt treatments

g. Different formulations like Artificial Sea Water (ASW), sodium chloride, combination of sodium chloride and calcium chloride and a mixture of different salts were used and tested against the growth to find out the rate of growth and at what particular treatment does the plants establish well.

h. Preliminary investigation on evaluation of the sample species is being carried out.

Collection of traditional cultivars (possibly extinct) are being done and two such rice cultivars "Renda Number Nellu" and "Pulidhikara Nellu" as they are locally called by the tribals of Kolli Hills, Salem District, Tamil Nadu are being grown in controlled conditions for evaluation.

RESULTS

Collection

The collection, propagation and field evaluation of plant species forms a prelude for the project whose baseline envisages

a strong hold of the collection.

The objectives of the project include the saving of plant species in Peninsular India which are to be done in a phased manner, through micropropagation and other appropriate methods also. The 'Hotspot' locations for Mangroves are being identified now. Besides, the endangered plant species, mangroves are also being identified and collected.

Collections are being done on a regular basis at the following sites

1. Pichavaram
2. Bhitarkanika
3. Ratnagiri
4. Chorao Islands

The collections are aimed at finding,

a. The presence of a particular species, if consistent or otherwise
b. The relative species richness
C. The value and relationship between different edaphic factors at the site.

The collection trips were also done at the following sites.

1. Western Coast
2. Eastern coast
3. Andamans (Prior to the initiation of the project)

The material collected was brought both as plants and as propagules, in sufficient numbers so that a nursery can be raised in the laboratory garden and the site. They were planted in mixed compost and were maintained with fresh water. We are also using ASW (artificial sea water) as a substitute to fresh water and to simulate the conditions of natural growth.

PICHAVARAM

The Pichavaram Reserve Forest is located in the South Arcot District of Tamil Nadu and occupies an area of approximately 1400 ha. The mangroves are limited to a 12 km stretch by the rivers Vellar and Coleroon, which is cris crossed by a large number of channels and creeks. The 25 mangrove and associate species found in this forest belong to 15 families and are listed in Table 3. Most of the vegetation is under very heavy biotic stress (indiscriminate felling and grazing by animals). Regular collection trips were made and the plants were collected as seeds or as propagules from Pichavaram.

BHITARKANIKA

The Mangroves of Bhitarkanika are found in the Mahanadi Delta in combination with its tributaries namely Brahmani, Baitarini, Patshale, Dhamara and Devi Rivers. These tributaries form many inlets and mudflats with numerous creeks and channels and supporting mangroves on an area of 141.44 sq.km. The coast is shallow and allows the formation of large deltas with great amount of sedimentation and subsidence caused by compaction and down wrapping. This has given rise to different topographic conditions to the delta and causing the differential distribution of mangrove species.

The Bhitarkkanika Wildlife sanctuary came into existence in 1975 and has 83 villages within its boundary. The Vanduay and

Table 3. List of mangrove and associate species of Pichavaram forest

Name of the species
Mangroves
Acanthus ilicifolius L.
Aegiceras corniculatum (L.) Bl.
Avicennia marina
Avicennia officinalis L.
Bruguiera cylindrica L.
Ceriops decandra (Griff.) Ding Hou.
Ceriops tagal (Perottet) C.B.Robinson
Excoecaria agallocha L.
Lumnitzera racemosa (Willd.)
Lumnitzera littorea (Jack) Vo. gt
Rhizophora apiculata Bl.
Rhizophora mucronata Lamk.
Rhizophora lamarckii?Montr.
Salicornia brachiata Roxb.
Sonneratia apetala B. Ham.
Xylocarpus mekongensis Pierre
Mangrove associates
Aeluropus lagopoides (L.) Trin.
Arthrocnemum indicum Moq.
Clerodendrum inerme (L.) Gaertin
Cyperus sp
Derris heterophylla (Willd.) Back
Salvadora persica
Sesuvium portulacastrum L.
Suaeda maritima (L.) Dum
Suaeda monoica Forsk.

other estuaries areas near the mouth are mainly vegetated by Avicennia Sonneratia and Aegialatis. The middle estuarine zones are dominated by Rhizophora and Sonneratia while the upper estuarine areas containing more of freshwater and are dominated by Heritiera Excoecaria and Xylocarpus. Bannerjee and Rao (1990) have extensively studied this area for five years and have reported a total of 63 species of mangrove and associate species (Table 4).

RATNAGIRI

The central position of Ratnagiri district of Maharashtra is traversed by rivers with parallel drainage. The rivers originate in the Sathyadrian scrap and drain into the Arabian Sea. Only the estuarine portions have deposition of sediments. The mangroves of Ratnagiri district are restricted to small areas.

The vegetation is dominated by mangroves capable of withstanding hydrological currents & salinity namely A. marina S. alba and R. mucronata. The mangrove species occurring along the small estuaries at Ratnagiri are listed in Table - 5.

The R. mucronata trees from this area have good growth characteristics and thereby their propagules were collected and grown in the nursery.

Table 4. List of mangrove and associate species of Bhitarkanika

Acanthus ilicifolius L.
A. volubils Wall.
Acristichum aureum L.
Aegilati rotundifolia Roxb.
Aegiceras corniculatum (L.) Blasco
Aglaia cucullata (Roxb.) Pellegrin
Avicennia alba Blume.
A. marina (Forsk.) Vierh.
A. officinalis L.
Brownlowia tersa (L.) Koster
Bruguiera cylindrica (L.) Bl.
B. gymnorhizza (L.) Savigny.
B. parviflora (Roxb.) W. & A. ex Griff.
B.sexangula (Lour.) Poir.
Caesalpinia bonduc (L.) Roxb.
C. crista L.
Cerbera manghas Linn.
Ceriops decandra (Griff.) Ding Hou
C. tagal (Per.) Rob.
Clerodendrum inerme Gaertn.
Cynometra iripa Kostl.
Dalbergia spinosa Roxb.
Derris scandens (Roxb.) Benth
D. heterophylla (Willd.) Back & Bakh.
Excoecaria agallocha L.
Fimbristylis ferruginia (L.) Vahl.
Finlaysonia obovata Wall.
Heliotropium curassavicum L.
Heritiera fomes Buch. Ham.
H. littoralis Dryand.
H. kanikensis Mj. et Ban.
Hibiscus tiliaceous L.
Instia bijuga (Colb.) Kunt.
Ipomoea tuba (Schl.) G. Don.
Kandelia candel (L.) Druce.
Lumnitzera racemosa Willd.
Merope angulata (Willd.)
Mucunga gigantea (Willd.) DC.
Myriostachia wightiana (Nees ex Steud) Hood. f.

Nypa fruticans Wurmb.
Phoenix paludosa Roxb.
Porterecia coarctata (Roxb.) Tateok
Rhizophora apiculata Bl.
R. mucronata Poir.
R. stylosa Griff.
Salacia trinoides (Willd.)
Salvadora persica Linn.
Sarcolobus carinatus Wall.
S. globosus Wall.
Salicornia brachiata Roxb.
Sesuvium portulacastrum L.
Suaeda maritima Dumort.
S. nudiflora Moq.
S. monoica (Forsk.) ex Gmel.
Sonneratia apetala Buch. Ham
S.alba J.Smith
S. caseolaris (L.) Engl.
S. giriffithii Kurz.
Tamarix troupii Hole.
T. erichoides Rottl.
T. dioica Roxb.
Thespesia populnea (L.) Sol.ex Corr
T. populneodes (Roxb.) Kostel
Tylophora tenuis Bl.

Table 5.List of mangrove and associate species of Ratnagiri

Acanthus ilicifolius Linn.
Acrostichum aureum Linn.
Aegiceras corniculatum (L.) Balsco.
Aeluropus lagopoides (L.) Trin.
Avicennia alba Blume.
A. marina var. acutissima Stapf & Mold.
A. officinalis L.
Ceriops tagal Perr.
Clerodendrum inerme Linn.
Cyperus spp.
Derris heterophylla (Willd.) Back.
Excoecaria agallocha Linn.
Halophila beccarii Aschers.
Lumnitzera racemosa Willd.
Premna integrifolia Linn.
Rhizophora apiculata Bl.
Rhizophora mucronata Lamk.
Salvadora persica Linn.
Sesuvium portulacastrum (L.)
Sonneratia alba Smith.
Stenophyllus barbata Roxb.
Thespesia populnea (L.) Soland.

CHORAO ISLAND

The mangroves of Chorao islands in the Mandovi river of Goa have been preserved as a National Park for birds and wildlife. The mangroves of Goa cover an area of 2000 ha. of which about 100 ha of mangroves area found in Chorao islands. The Chorao island has a freshwater inflow from Mandovi river. One of the most important river in the state in monsoon season whose fresh water rush off caused heavy siltation near the island. The mangrove vegetation of this island comprises of 15 species as listed in Table-6.

This island has a well developed nursery and has a seedlings of R. mucronata, A. marina, L. alba and Kandelia. These are transplanted to afforestation zones of the near by areas of the island.

PROPAGATION

Mangroves are a collection of diverse plant species with adaptations to saline conditions and to flooding. One of the adaptation is a novel mode of seed germination termed as vivipary and cryptovivipary. The Rhizophoraceae members have viviparous mode of germination and Avicenniaceae have a cryptoviviparous mode of germination. In such a germination the embryo starts developing while still attached to the mother

Fig. 1 a - Mangrove Vegetation of Bhitarkkanika

b - Mangrove Vegetation of Pichavaram

c - Mangrove Vegetation of Ratnagiri

A

B

C

Table 6. List of mangrove and associate species of Chorao island, Goa

Acanthus ilicifolius L.

Acrostichum aureum L.

Aegiceras corniculatum (L.) BL.

Avicennia alba Blume.

A. marina (Forsk.) Vierh.

A. officinalis L.

B. gymnorhizza (L.) Savigny.

B. parviflora (Roxb.) W. & A. ex Griff.

Derris heterophylla (Willd.) Back.

Excoecaria agallocha L.

Kandelia candel (L.) Druce.

Rhizophora apiculata Bl.

R. mucronata Poir.

S.alba J.Smith

S. caseolaris (L.) Engl.

mangroves species do not have such adaptations and give rise to simple fruits and seeds like Excoecaria, Acanthus and Lumnitzera.

Most of the seeds and propagules are ready/mature just prior to the onset of monsoon and are collected in that period. Propagules of Rhizophora can be stored in moist condition for a period of a month, without a change in its rooting potential (Gaykar 1991). But other species should be planted immediately. Seeds of Xylocarpus have a dormancy period of 4-8 weeks before they germinate. While Heritiera the fruit wall needs to be broken and the embryo detached before it starts germinating. The very phenomena of seed set prior to monsoon shows the physiological need of freshwater for the seeds to germinate and develop in their initial stages.

Mature propagules and seeds were collected from various regions of India (Table-7). All seeds/propagules were planted in plastic bag containing red soil and farm manure (2:1). They were watered on alternate days with fresh waters. Single seed per bags for all species except Avicennia where 3-4 seeds per bag were shown. Fig 1-5.

The problems faced in field plantation, are
a> duration of inundation
b> human disturbances
c> cattle grazing
d> algal matting covering the propagules
e> destruction by crabs, barnacles and oysters.

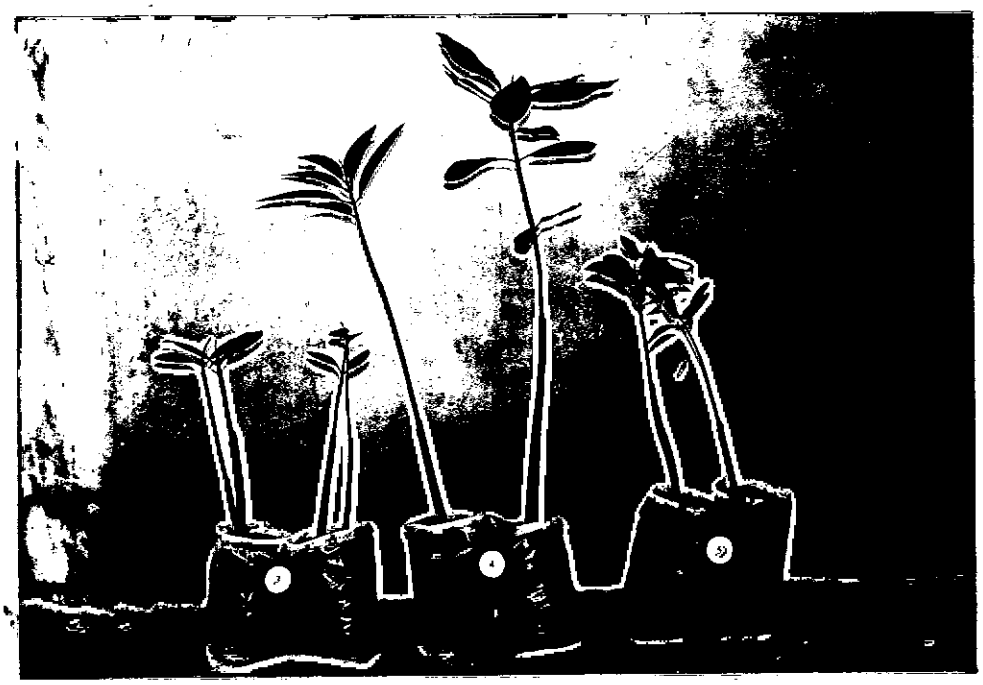
Thus it becomes important to grow rare and endangered plants in the nursery to a predetermined stage of growth and transplant them in well protected areas. Monitoring of these

Table 7. Plant species collected from some resource areas

Name of the species	Maharashtra	Goa	T.Nadu	Orissa	Andamans
Aegialatis rotundifolia Roxb.	-	-	-	+	-
Aegiceras corniculatum (L.)	+	-	-	-	-
Avicennia alba Blume.	-	-	-	+	-
A. officinalis L.	-	-	+	-	-
A. marina (Forsk.) Vierh.	-	-	+	+	-
Bruguiera cylindrica L.	-	-	+	-	-
B. gymnorrhiza (L.) Savigny.	+	-	-	-	-
B. parviflora (Roxb.) W. & A	-	-	-	+	-
Cerbera manghas Linn.	-	-	-	+	-
Ceriops decandra (Griff.) Di	-	-	+	-	-
Ceriops tagal (Perottet) C.B	+	-	-	-	-
Cynometra ramiflora	-	-	+	-	-
Heritiera fomes Buch. Ham.	-	-	-	+	+
H. littoralis Dryand.	-	-	-	+	+
Kandelia candel (L.) Druce.	+	+	-	-	-
Lumnitzera racemosa (Willd.)	-	-	-	-	+
Rhizophora apiculata Bl.	+	-	+	-	-
Rhizophora mucronata Lamk.	+	+	+	-	-
Xylocarpus granatum	-	-	-	-	+

Fig 2 a - Variation among Rhizophora mucronata collected from Pichavaram, South Maharashtra, Andamans (from left)

b - Variation among Rhizophora apiculata collected from Pichavaram and Ratnagiri (from left)



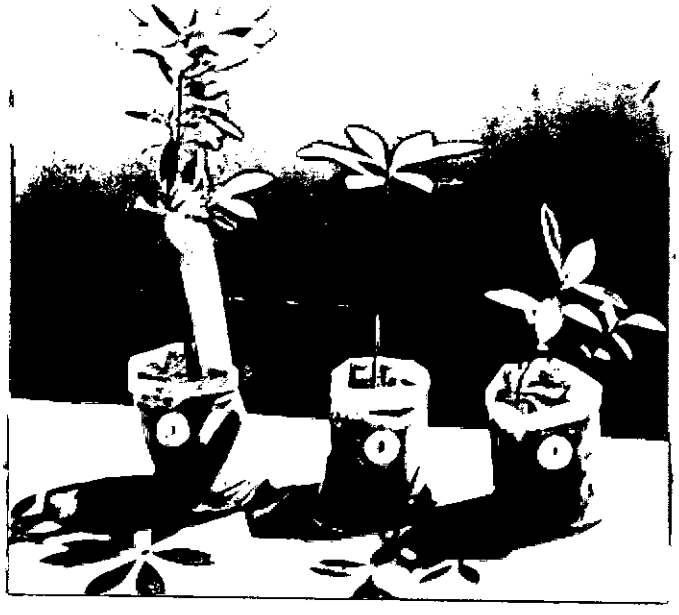
A

B

Fig 3 a - Variation in the seeds of Avicennia collected from Muthupet

b - Variation among Bruguiera collected from Ganapathipule, Chorao islands and Pichavaram (from left)

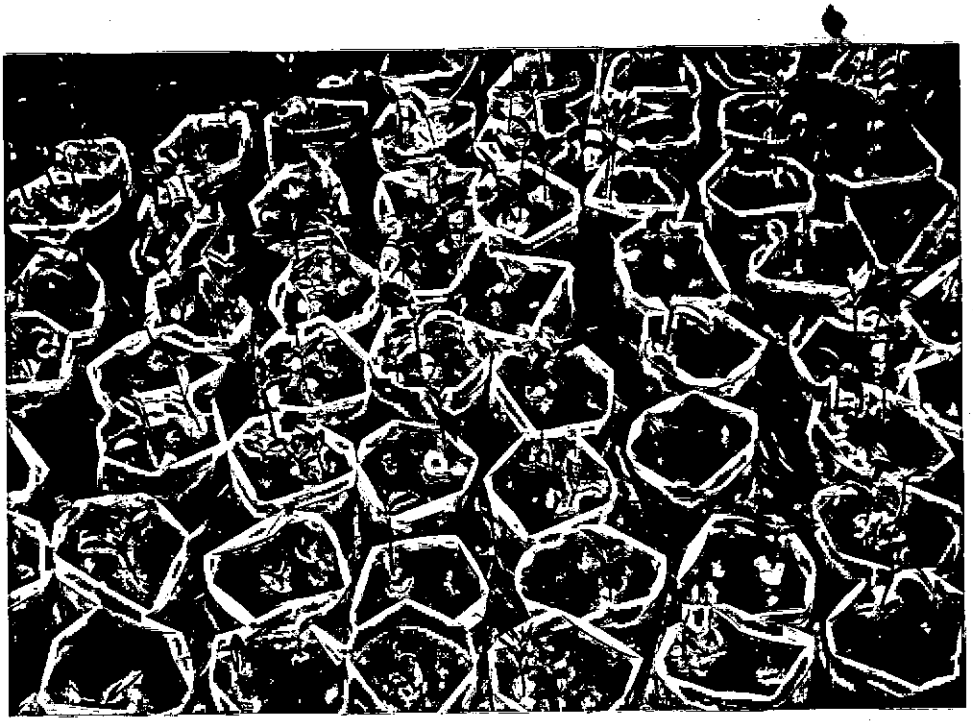
A



B

Fig 4 a - Avicennia seedlings maintained in the nursery.

b - General view of the nursery holding mangrove plants collected from different areas.

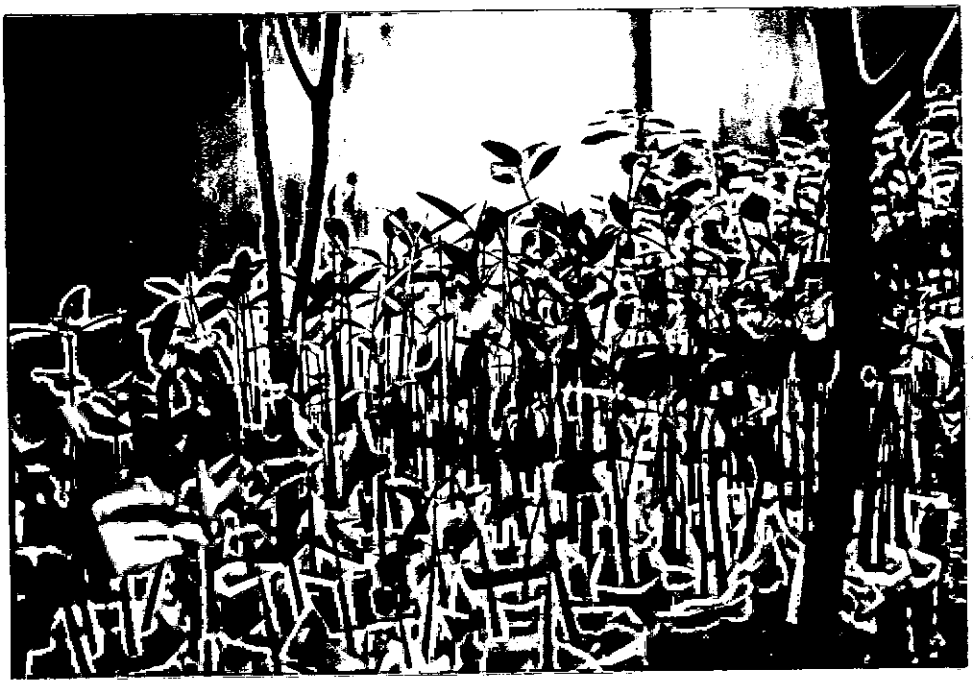


A

B

Figure 5 a - Nursery of Rhizophora plants collected from different areas

b - A general view of the nursery



A

B

transplantation is also very important.

With this in mind the following experiments were planned essentially to see how best the mangrove species can be grown under nursery condition, what are the prerequisites,effects of such environmental conditions on the growth and habit of plants and the structural morphological changes these plants adapt to. The experiment was initiated with one sample species of Avicennia. The details of work is presented hereunder.

STUDIES ON THE PHYSIOLOGY AND GROWTH PARAMETERS IN MANGROVES

Avicennia marina seeds were collected from Pichavaram during December 1992 and from Muthupet during January 1993. The seeds were brought to the laboratory, and were maintained in polythene bags containing equal amounts of soil and compost mixed thoroughly. Two bags containing 4 plants were assigned for each experiment. The collection from Muthupet was exclusively used for electron microscopic studies.

The plants were watered with respective treatments (see below) once in alternate days, (250ml) and growth parameters were recorded once in every 7 days for 36 days. In the first experiment one set of plants were treated with fresh tap water without adding any salt component and this was used as the control. In the second experiment plants were treated with Artificial Sea-Water (ASW) of 10%, 30% and 50% concentrations. The third experiment contained using of 100mM 300mM and 500mM sodium chloride alone while the fourth set up had sodium chloride and calcium chloride in the ratio 1:1 (w/w) dissolved in distilled water was used in the concentration of 100mM, 300mM and 500mM. In the last set a mixture of 4 salts (Na_2 SO_4 : NaCl : $CaCl_2$: $MgCl_2$ in the ratio 10:5:4:1) were used. The treatments were given keeping in mind to find the most effective combination of salts or fresh water that would be required for the successful growth and establishment of Avicennia plants in the nursery.

Electron Microscopy

Leaves of Avicennia from Pichavaram and Muthupet and those treated with fresh water (expt 1) and ASW (expt 2) were prepared for microscopy. Small pieces (3-5 mm) of tissue were cut and fixed in 2% glutaraldehyde (prepared in phosphate buffer pH 6.8) for 24 hours then they were washed in phosphate buffer for 2 hrs and dehydrated through ethanol series (50%, 60%, 70%, 80% 90% ethanol and two changes in absolute ethanol). The materials were dried in CPD (critical point drier) and mounted on stubs using a double adhesive tape with abaxial and adaxial surfaces facing upwards. The dried sample was coated with gold using a gold sputter and observed using a scanning electron microscope (Jeol ASID 40).

Growth parameters

Growth measurements in terms of total height, internodal length were taken using a millimeter scale once in 7 days after the treatment was given. An average of 4 plants in duplicate was taken as mean of all measurements expressed. Total height of the plant from the surface of the soil upto the growth tip was taken as total height and the internodal lengths were calculated from when they begin to appear. The data were pooled and graphs and histograms were constituted using the data of total height and internodal lengths respectively.

Response to Fresh water and ASW

Growth both in terms of total height and internodal length was significantly reduced by the treatment of fresh water than by ASW(Fig 6); there was, however, no significant difference in growth of Avicennia after 15 days of treatment in the control (fresh water). In the different ASW treatments given maximum growth was observed in 10% ASW followed by 30% and 50%. The increase in internodal length and the number of leaves formed were higher in 10% treatment, growth normally stopped in 50% ASW treated plants after 29 days. Also the total mean height of the plants grown with 10% ASW was the maximum reaching 17.65 cms while that of 50% ASW treatment stopped at 9.0 cms.

Response form NaCl and NaCl + $CaCl_2$ treatment

Growth of plants with sodium chloride (100 mM, 300 mM and 500mM) showed maximum with 100 mM treatment followed by 300 mM and 500 mM treatments (Fig7,8). Though the rate of growth was maximum in 100 mM treated plants, 300 mM treated plants showed maximal growth only during the first 7 days (12.1cm). Afterwards it maintained a slow rate of growth and reached a maximum of 14.15 cm only, while the 100 mM treatment showed the rate of growth as from 8.4 cm during the first 7 days to 12.65 cm on the 36th day. The rate of leaf formation was rather slow only with 2 leaves being formed during first 7 days in 300 mM and 500 mM treatments. Interestingly the rate of growth of internodes was

Fig.6 Variation in mean height of Avicennia with ASW treatment

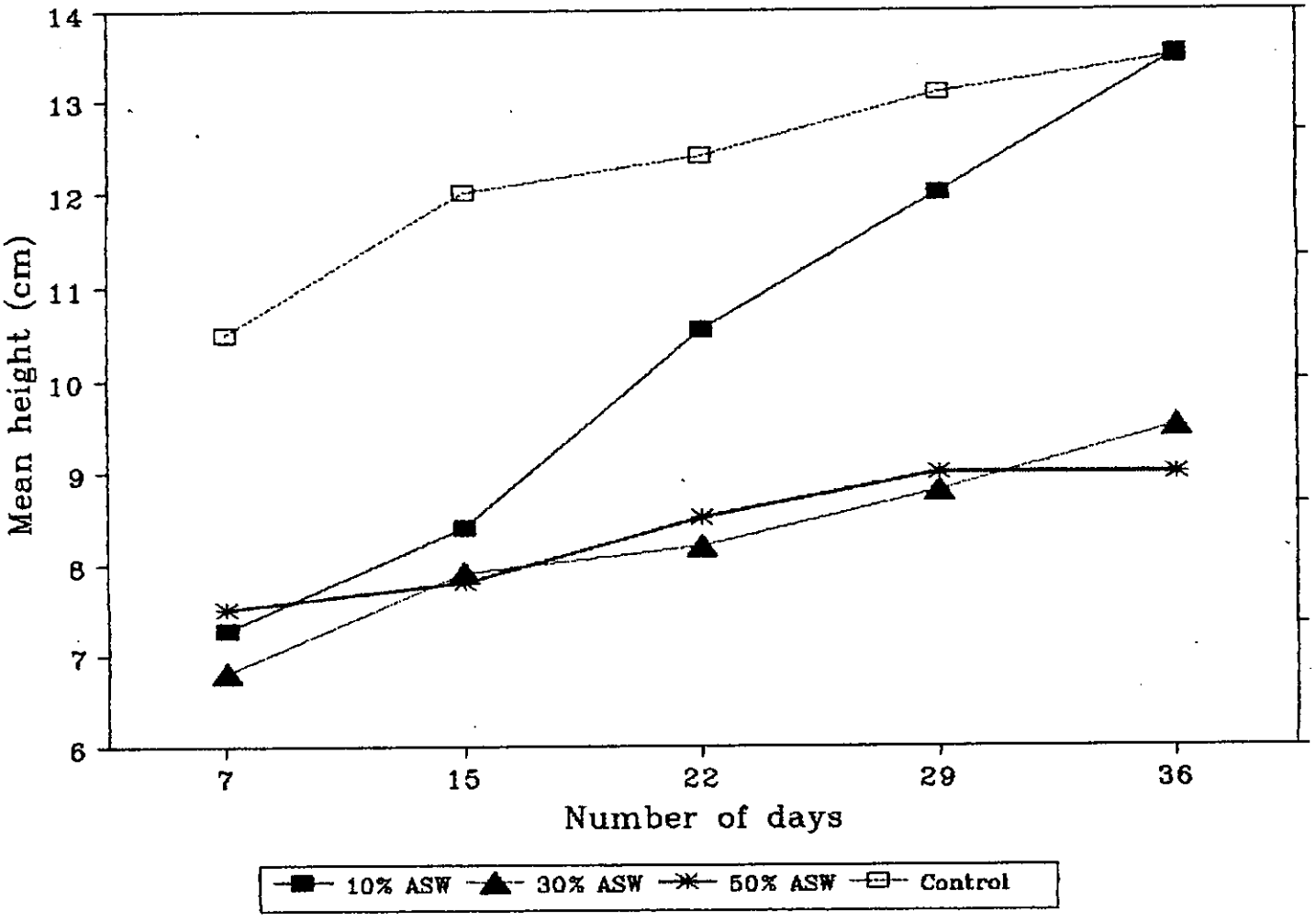


Fig.7 Variation in mean height of Avicennia with NaCl treatment

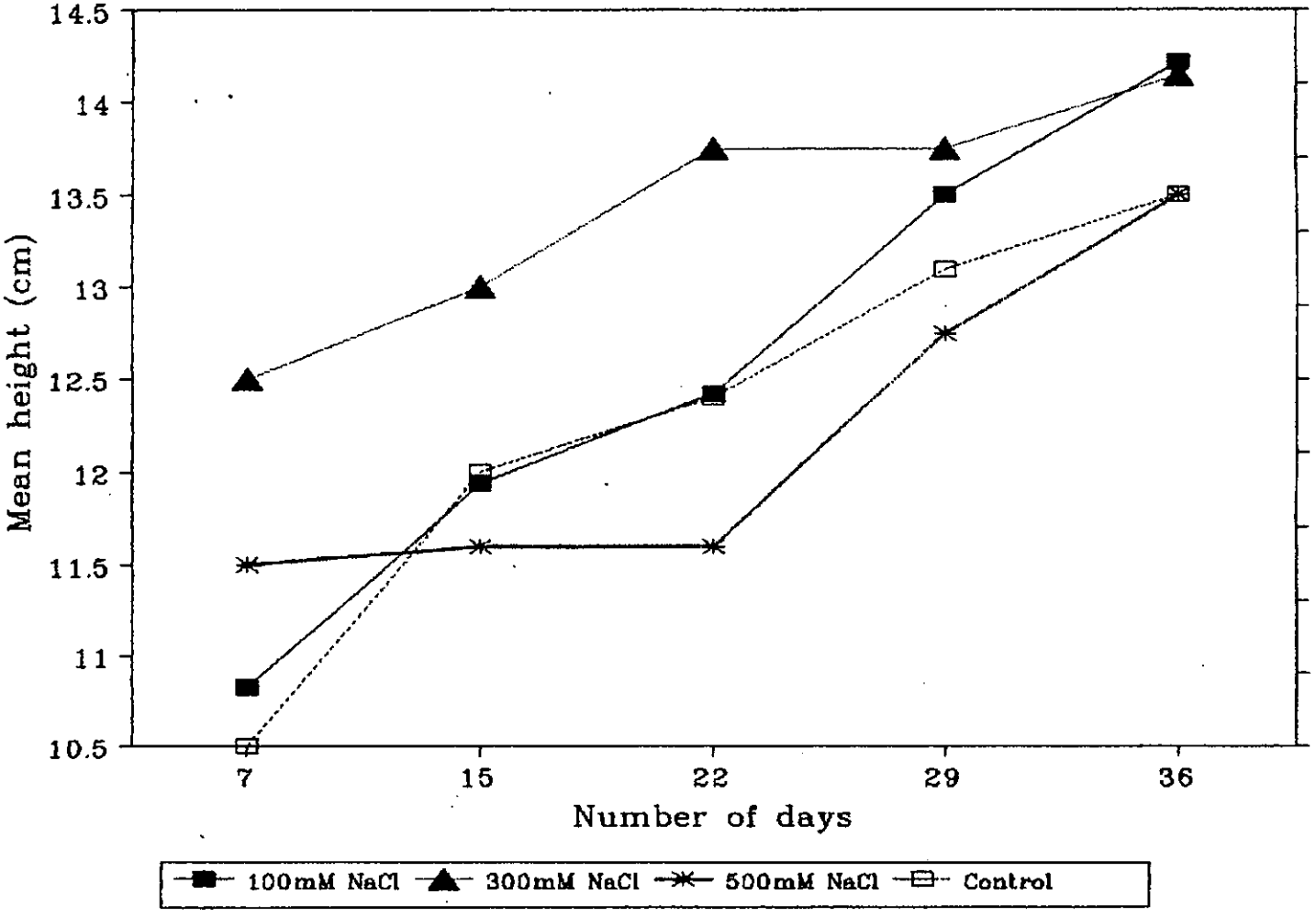
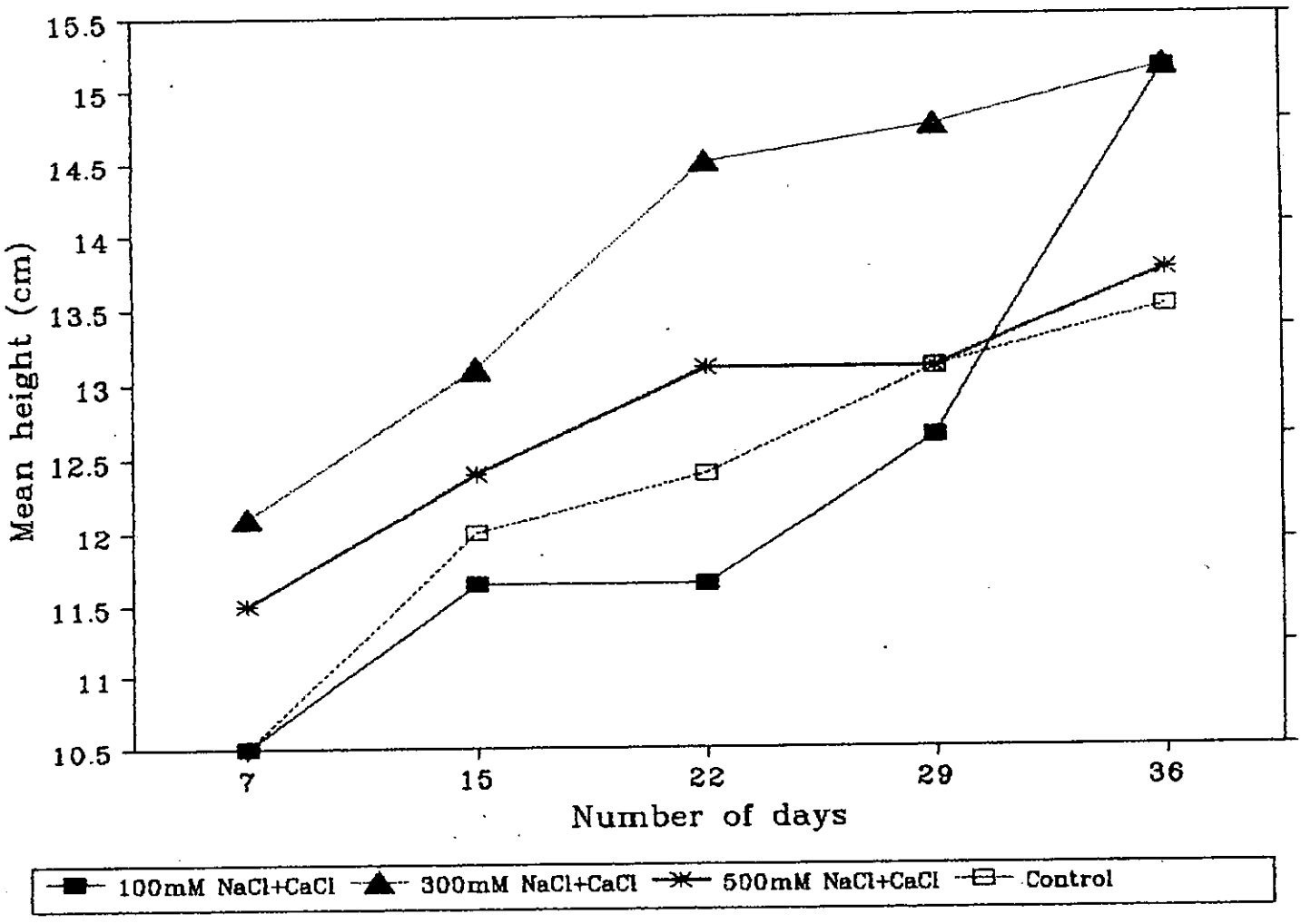


Fig.8 Variation in mean height of Avicennia with NaCl+CaCl treatment



maximum in 500 mM treatment on an average.

In the NaCl + $CaCl_2$ treatment, plants responded very well with the 100 mM treatment both in the rate of growth and in mean height attained (Fig) followed by an almost equal response from the plants treated with 300 mM and 500 mM concentrations. Like in NaCl treatment, the number of leaves formed were only 3 sets and that too the growth of internodal length was rather slow. The response to the treatment in the experiment was almost similar to that of NaCl alone.

Treatment with a mixture of salts

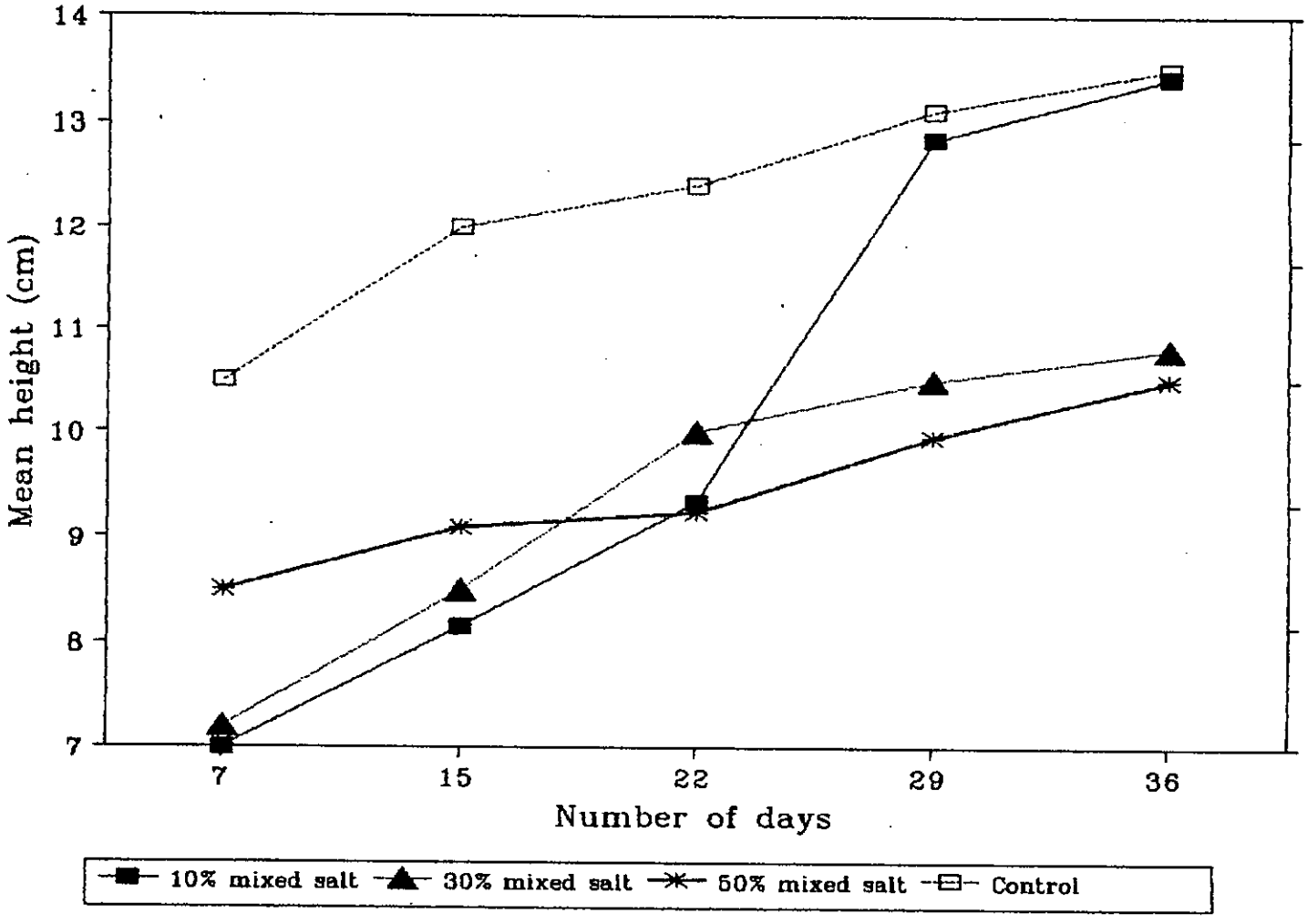
The effect of the combination was studied on the growth of Avicennia plants which gave an interesting pattern. Maximum rate of growth was observed in the 10% treatment followed by 50% then by 30% treatments (Fig 9). In the plants treated with 10% mixed salts the growth went up to 18 cms while in the 30% treatment it stopped at around 9 cms. The 30% and 50% treatments also showed to considerable difference with that of the control in terms of the rate of growth.

Rate of growth of internodes in various treatments

ASW treatment

Interestingly the total number of leaves formed were 4 sets in the plants treated with 10% ASW. Also the rate of growth in terms of internodal length was maximum with this treatment. While the plants treated with 10% ASW showed up 3 sets of leaves

Fig.9 Variation in mean height of Avicennia with mixed salt treament



while the plants grown under other treatment conditions had only 2 sets and in control the total length never exceeded 5 cms on an average (Fig 10).

NaCl treatment

Contrary to ASW treatment, the response of plants to NaCl treatment was much uniform. Plants treated with 100 mM NaCl treatment showed up only 2 sets of leaves while those treated with 500 mM showed 3 sets and the rate of growth is also faster. The plants grow with fresh water (control) also estimated a similarity with 100mM treated plants (Fig. 11)

NaCl + $CaCl_2$ treatment

The combination of both salts (NaCl and CaCl) had shown a slower growth response. But in this experiment maximum amount of internodal length was observed in 300 mM treatment while the growth in control and 100 mM treatment were more or less similar until 29 days from the beginning of experiment. Maximum internodal elongation was observed in 100mM treatment (Fig 12).

Mixed Salt

Mixed salt treatment had a detrimental effect on the growth internodes especially in 10% combination. But maximum internodal length was found in 30% treatment. The time taken for the second internode to appear is maximum in this treatment when compared to all other treatments (approximately 5 days). Only in the 50%

Fig. 10. Growth of internodes in plants treated with ASW

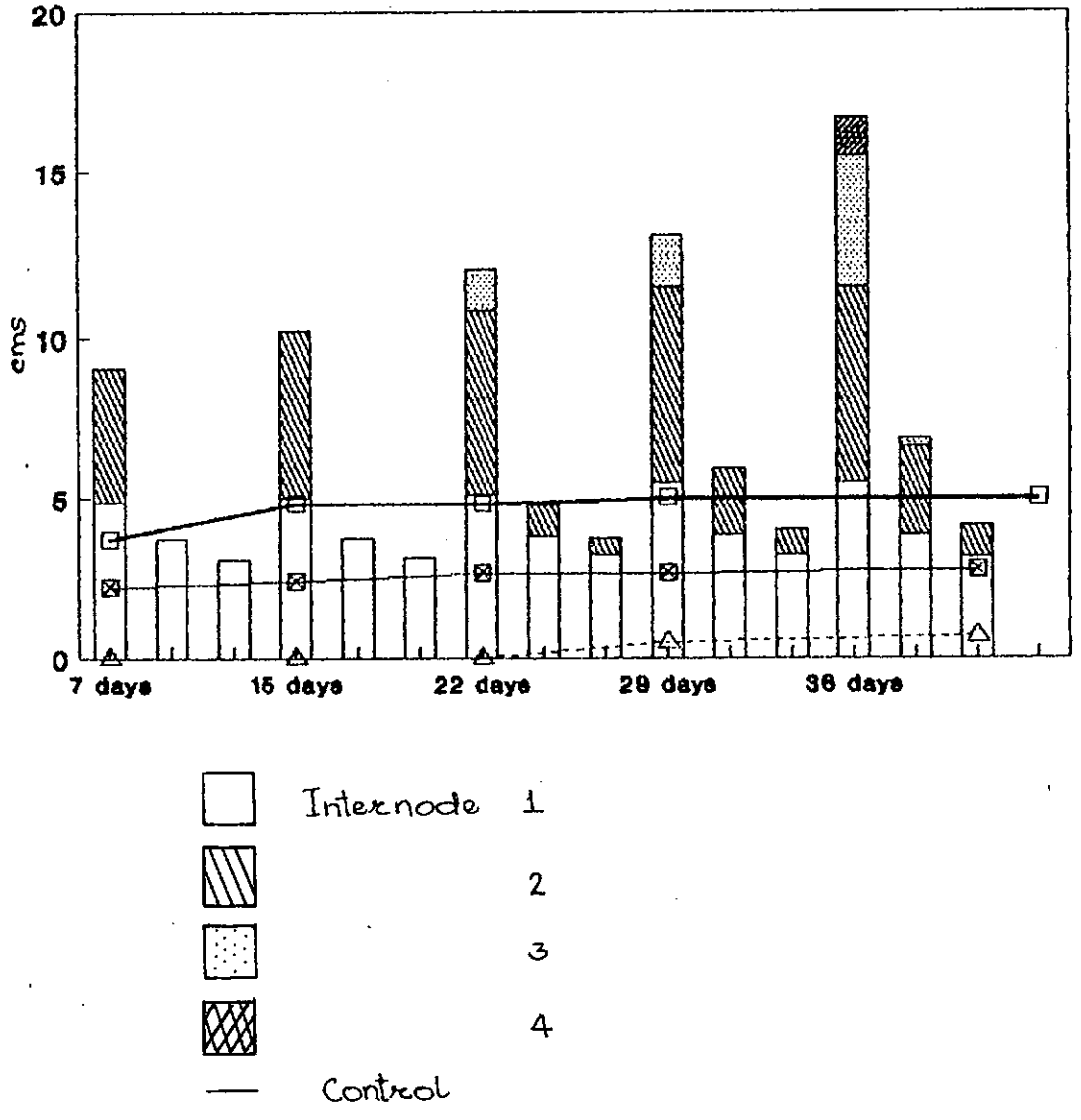


Fig. 11. Growth of internodes in plants treated with NaCl

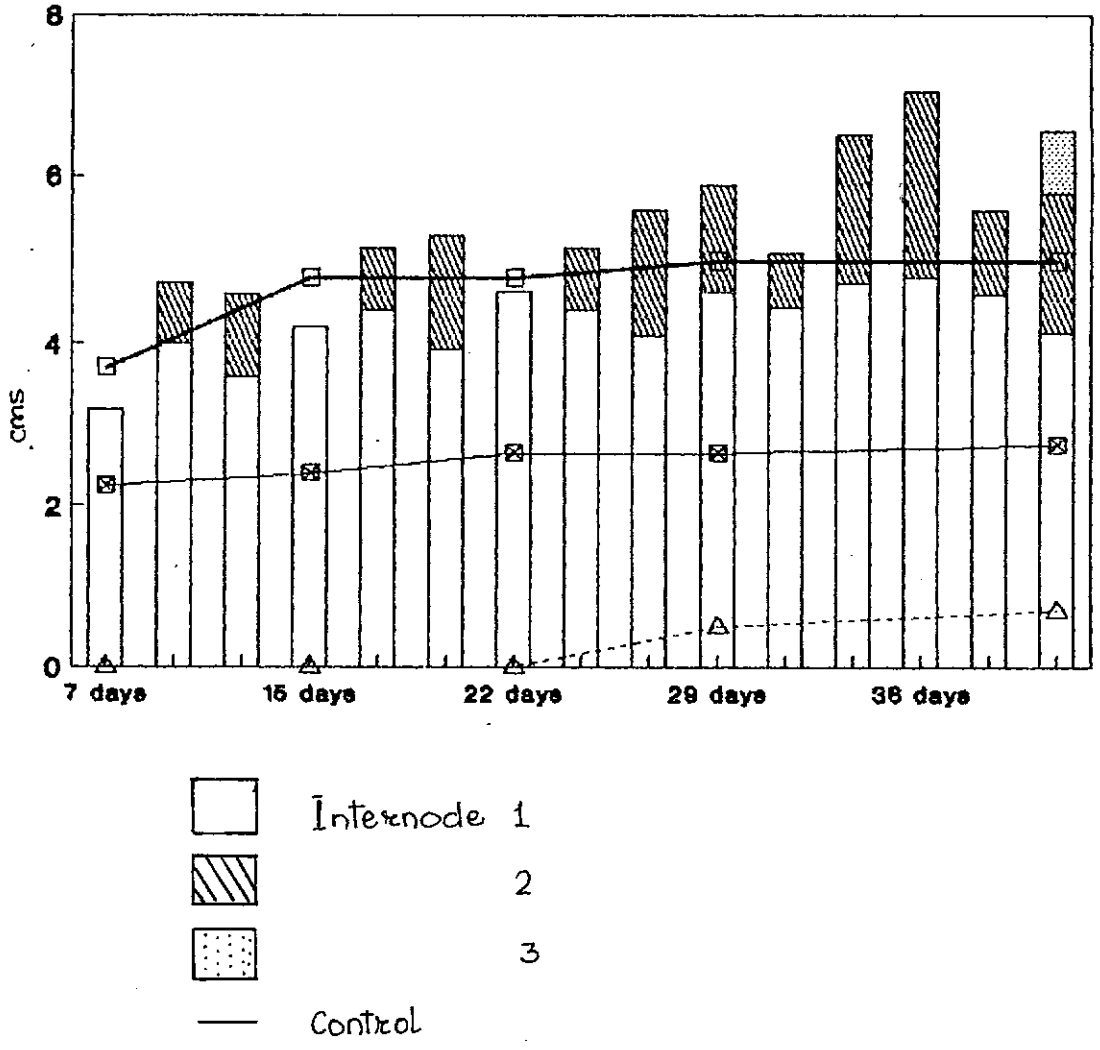
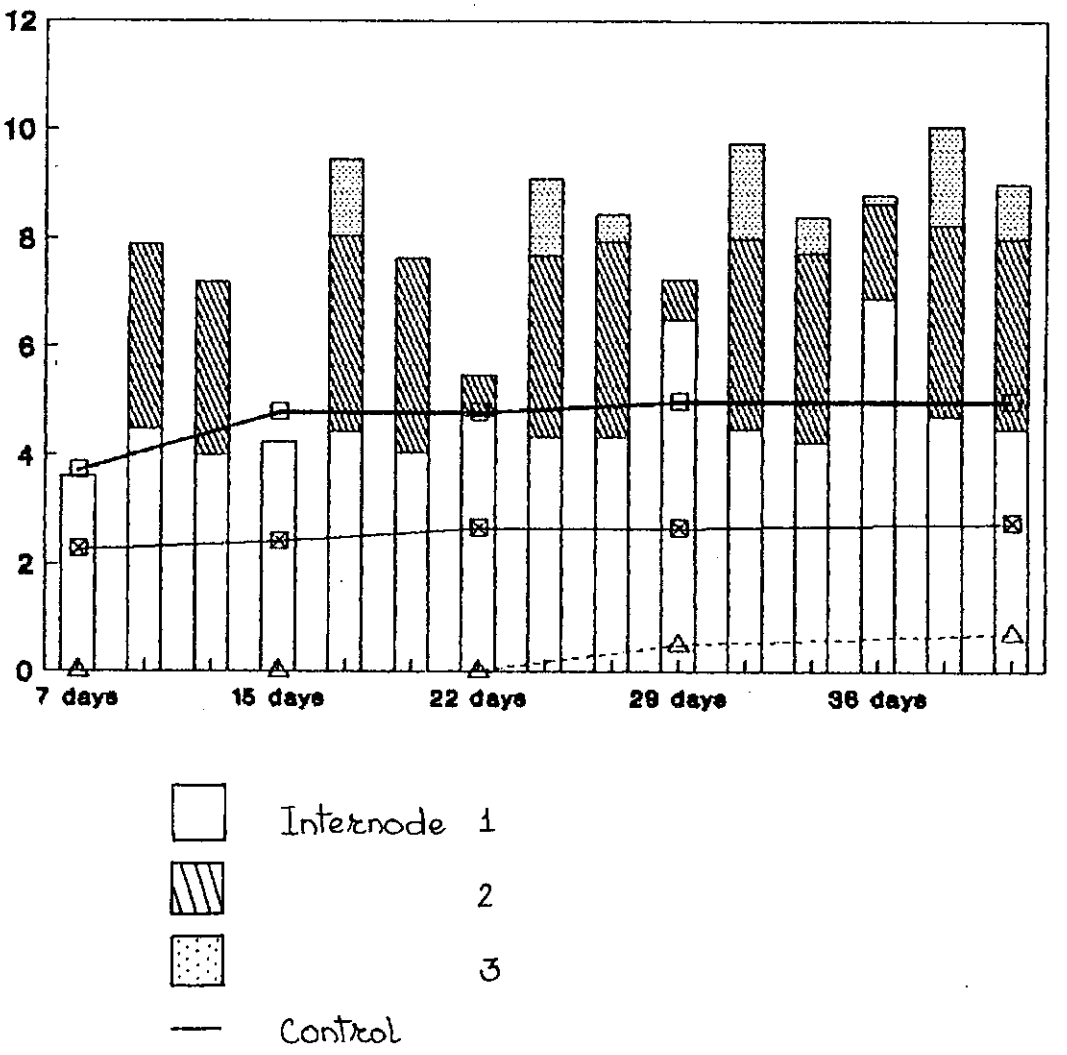


Fig. 12. Growth of internodes in plants treated with NaCl + $CaCl_2$



treatment a third internode appeared but the growth of the same is very minimal (Fig13).

Electron microscopy

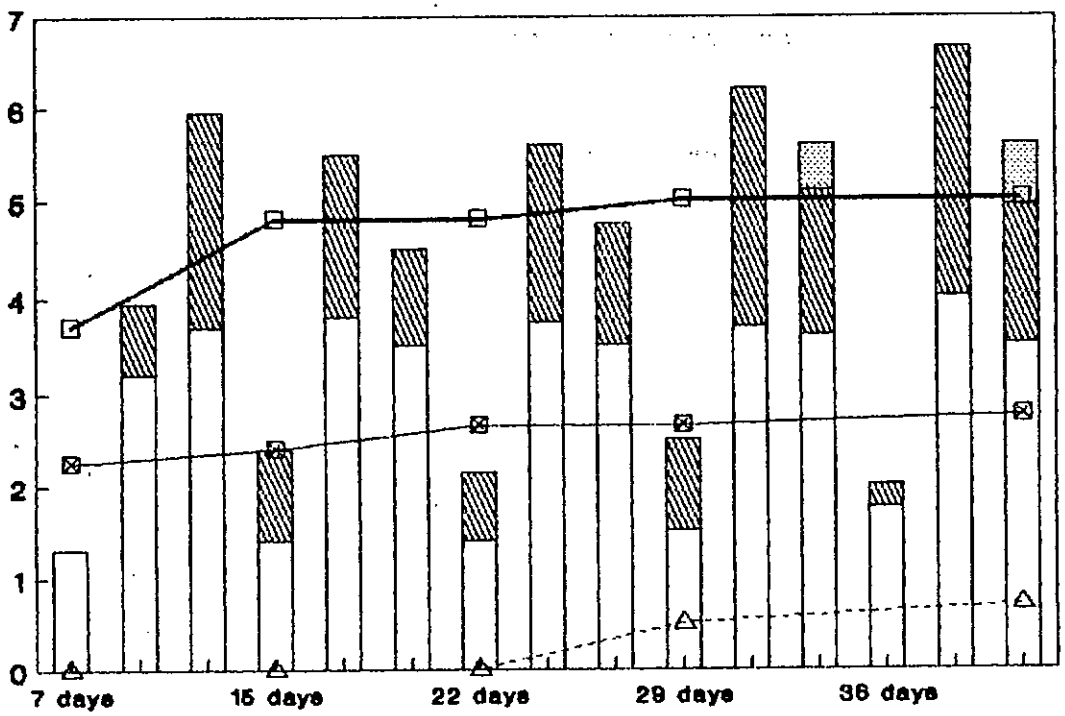
Scanning Election Microscopic studies of the surface of Avicennia marina leaves of different treatments were carried out. Both adaxial and abaxial surfaces of the leaves were scanned for salt glands.

Structure of salt glands

It was reported that in most of the mangroves including Avicennia, the salt glands are present both on the upper and lower surfaces of the leaves and more conspicuous presence is on the upper side. Stomata are distributed on the lower surface covered by many trichomes that protect and prevent the stomata from losing any water by the way of transpiration. A thorough study was undertaken by analyisng the electron micrographs of the salt glands and the trichomes besides assigning the reasons for their structural differences which is a result of the varying treatments of salt solutions and fresh water to the plants. Figures represent the details which are discussed in the later part.

Many halophytic plants have epidermal glands on their leaves and stems which secrete salt and plays an important role in salt tolerance (Metcalfe and Chalk , 1950). These glands have been

Fig. 13. Growth of internodes in plants treated with mixed salt



considered efficient devices for the secretion of excess salt which accumulates in the tissue (Scheolander et al., 1965, 1966). One of characteristic features of the salt glands is that they are almost entirely enclosed by a cuticular layer. This feature was demonstrated in the electron microscope examination of Avicennia. To substantiate the role of salt glands and their secretion in Avicennia, the plants were treated with different salts and their combination in varying concentration. The suggestion of Haberlandt (1914) that the salt glands function in regulating the salt content by secreting excess salt from the tissue is also documented in the study.

Many traits contribute to the resistance of salinity in mangroves. One of these is the structure and distribution of salt glands on the leaf surface. It is very essential to study the nature, distribution of the glands if any experiment relating to aspects of regeneration is to be undertaken. It is but inevitable to make sure also that the plants grown in nursery are grown quickly but is also important to ascertain how they can fit back to a different but original habitat, once they are transplanted. This experimentation gave a few findings of the above and also recommends the best possible nutrient treatment to maintain a successful nursery of mangrove trees.

Concentration of salts in the plant can be altered by lowering the sodium transport to the shoot. But this character is largely controlled by plant vigor. Vigor and water use efficiency are associated with "plus" plants while the

productivity of such plants increases largely (Richards 1983). The mechanisms increasing salt tolerance are distinguished as tolerance and avoidance. Interestingly Avicennia exhibit both the mechanism. Breckle (1990) have studied the effect of different concentrations of salts on leaf growth in Limonium sinatum and reported similar results as presented above. Tomlinson (1986) described the salt glands of Avicennia and reported that the salt glands are abundant on the leaves but are not equally distributed which is also recorded in the study.

Fahn and Shimony (1977) and Shimony et al., (1973) reported that the hairs are abundant on abaxial side of the leaves where they are not sunken. Several trichomes in the present study are observed on the lower surface of the leaves which cover the stomata and prevent transpiration. The investigation also revealed that the stress of salinity, the influence of varying salts in different concentration also have an influence on the shape and number of these trichomes besides influencing the shape and abundance of salt glands (Fig. 14).

The variation in the study can be attributed to the effective intake and outflow of salts. In fresh water treated samples, the glands are not efficiently used by the plants and hence are few in number per unit area while the plants treated with ASW have more number of salt glands (Fig.15). The salts glands of Avicennia plants collected from Pichavaram and Muthupet also differed which is mainly due to the fact that the Muthupet

Figure 14 a - Trichomes of Avicennia in ASW

b - Trichomes of Avicennia treated with Fresh Water

c - Trichomes of Avicennia collected from Muthupet

d - Trichomes of Avicennia collected from Pichavaram

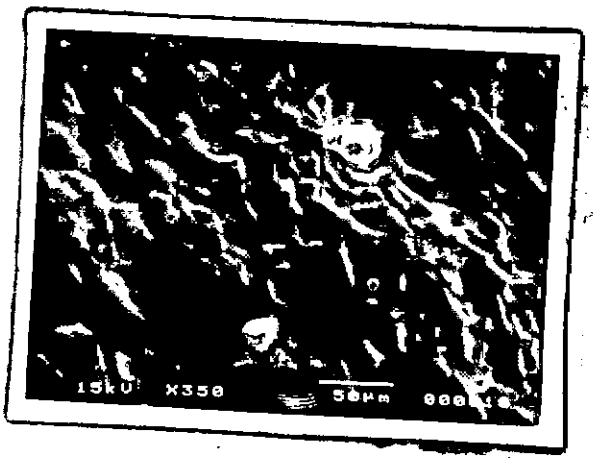
a

b

c

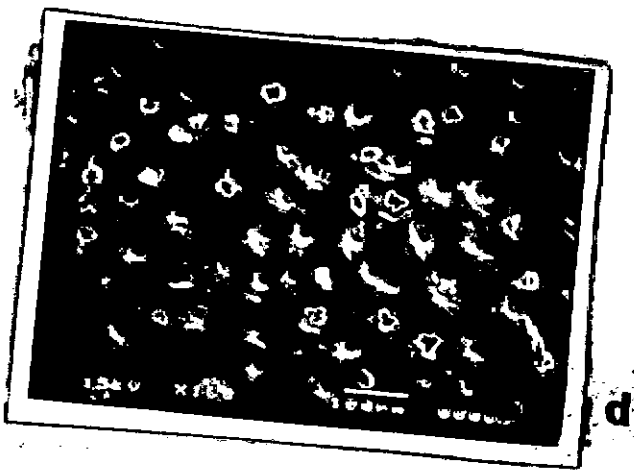
d

a



b

c



d

have higher salinity compared to Pichavaram, since Pichavaram receives more of fresh water inflow (Selvam 1992). Higher salinity obviously leads to selective pressure, negative, which can invariably lessen the growth of plants in the area. Even in the different salt concentrations used in the study, maximum growth was observed only in 10% ASW, 100mM NaCl treated plants. These results are being consolidated to be published shortly.

Work on RFLPs and Generic DNA library would be initiated as soon as their facilities become available.

2. Probes labled and tested for polymorphism
3. Non-radioactive detection methods including chemiflourescence
4. PCR, RAPD - in genetic diversity measurement.
5. Preparation of training manual.

Work on the above four objectives would be taken up during the subsequent phases of the project.

6. Bioindicator studies - ex situ or lab conditions

9. Bioindicator in relation to forest disturbance, water and air pollution and land quality

Lichen flora as useful early warning systems (Bioindicators) of atmospheric pollution

This work has been carried out as a part of objective 9 of this project. The scope of the bioindictor study under this objective includes many ecosystems of South India, most of which requiring immediate attention, since they face threats from varied anthropogenic pressures.

Biological early warning systems, are simple tools, which could forecast a potential pollutant threat, so that necessary precautions may be taken in protecting that ecosystem. Indigenous plant or animal species which show positive sensitivity to a pollutant, but remain an integral part of that ecosystem, exhibiting observable variation in their morphology, growth or population density are generally employed for bioindication purpose. This method of monitoring ecosystem health are superior to physical and chemical analytical methods since they are energy efficient and less costly, since no sophisticated instruments are needed. Moreover rarely a pollutant effect goes unnoticed since these organisms are the targets of these perturbations ultimately. To begin with this study it was proposed to develop some bioindicators useful for Pichavaram mangroves, since mangroves are the most threatened

ecosystem besides ever green forests of Western ghats.

Mangrove ecosystems are unique in that they are deltaic formations occurring in the sheltered coastal regions, rich in biological diversity and productivity, harbouring varied fauna and flora, most of which ecologically and economically important. These ecosystems, once abhorred for they are thought to harbor harmful insects and mosquitoes, besides being hot and humid, are doomed as waste lands and hence been cleared for varied purposes. But these ecosystems have been getting much attention of the scientific community, off late, since their potential values have been realized.

The remaining patches of these ecosystem that have not faced the brunt of the anthropogenic pressures must be guarded from any threat, considering their gene pool value and biological productivity. Erosion of these species due to indiscriminate felling for timber and fire wood and grazing by animals could be curbed by imposing stringent laws and constant guarding by forest personnel. But threats from fast industrialization along the coastal region could pose a major threat for these ecosystems. Pichavaram mangroves have 24 plant species out of which about 12 species are true mangroves. This ecosystems have been particularly guarding this coastal belt from soil erosion which every year faces cyclonic threats from the activity of South west monsoon.

Although these ecosystems presently face no immediate

threats from pollutants from air or water, one has to be concerned about the recent developments in the Bay of Bengal and surrounding of Pichavaram. Offshore oil rigs near Cuddalore (about 35 km North of Pichavaram) SIPCOT industrial complex in Cuddalore and recent oil findings in Narimanam (70 km South of Pichavaram) have lead to fast industrialization in this coastal strip. A potential threat from air and water from these industrial activities cannot be overruled, if not now, in future.

With this idea, it was proposed to identify or develop some biological early warning system for these ecosystems. These ecosystems since they face threats both from water and atmosphere identifying appropriate indicators for each case may be necessary. Exploring the possibility of employing some organisms useful under marine polluted conditions is underway.

For monitoring atmospheric pollution lichen species have been chosen to begin with, because of their frequent and persistent occurrence on the mangrove species. Moreover their value as potential indicators of atmospheric pollution has been proved beyond doubt (Hawksworth and Rose, 1976).

Lichens are unique organisms. which are born out of a symbiotic association between free living alga(e) and a fungal species. The phycobionts (algal partner) performs photosynthesis and the mycobiont is involved in reproduction and dissemination of these species. They are ubiquitous in their distribution and inhabit extremes of climatic conditions from hot deserts to

arctic zone and from tall mountains to tidal zones of the oceans and can colonize on any substratum provided there is enough light and moisture. Lichens are mainly grouped based on their morphology. Crustose lichens are those having simple morphology and foliose lichen having leaf like margins and fruticose lichens have a pendant thallus.

In spite of their widest distribution they are very sensitive to atmospheric pollutants and hence generally disappear with the increase in atmospheric impurity. Because of this high sensitivity they are proven indicators of clean atmosphere. Lichens generally show mixed responses to the nature of a particular pollutant and hence sôme species may exhibit high or moderate or low sensitivity to a particular pollutant, depending upon the lichen-pollutant interaction, as the case may be.

In addition, many lichen species are known to accumulate heavy metals, radio active elements and particulate matter in their thalli and hence could enable assessment of the toxic chemicals in the atmosphere especially in the case of radio active fallouts for monitoring atmosphere for human health.

Hence a study of the lichen flora of Pichavaram mangroves, will lead to useful biomonitoring for atmospheric pollution. Moreover the lichen flora of the mangrove ecosystems have not been studied so far and no information is available on the number of lichen species occurring on the mangrove trees.

Methodology

Frequent surveys were made to the Pichavaram mangroves, to study, pattern of general distribution of lichen species on various islands like Bungalow thittu, Nadu Odai, Bungalow thittu saragam, Entrance of Peria guda area, Periaguda, Channels adjacent to periaguda area, Kannagi Nagar, etc.

Lichen species from these areas were collected from the trees Crustose and foliose species were scrapped off with a sharp knife along with the bark and foliose species wee scrapped with their holdfast intact, without injuring the specimens. Herbaria were prepared before sending them to the experts for identification (Prof. D.D. Awasthi, Retd. Prof., NBRI, Lucknow, Dr. G.N. Hariharan, Senior Research Fellow, Bharathidasan University, Trichy). Numerical data regarding colony size, frequency of occurrence on a host, distribution among the mangrove species will be collected soon which will form a sound basis for biological monitoring. The over all distribution pattern based on empirical observations together with identification of lichen species has been done so far.

Observations

General Pattern of Distribution on Mangrove Species

Lichen species are generally distributed over 4 mangrove species. They are Rhizophora apiculata R. mucronata R. lamarckii and Exoecaria agallocha. One non-lichenised fungal species is

occurring on Avicennia officinalis. All these mangrove species are big trees and another important tree species A. marina is barren when lichens are concerned. Infrequently some crustose species may occur on Brugiera cylindrica. Hence only these tree species were mainly taken up for the survey.

There are as many as 7 individual crustose lichen species are known to occur on the mangrove species. They are listed as follows.

1. Pyrenula species
2. Pyrenula alboostiolata
3. Buellia montana
4. Lecanophora allophana
5. Buellia isidiophora
6. Graphis scripta
7. Graphis dumastii

In addition there are two foliose lichens species.

1. Dirinaria confluens
2. Dirinaria consimilis

and one fruticose species Roccella montagnei are known to occur on the above stated mangrove species.

All these 10 lichen species differ in their pattern of distribution in the over all mangrove area also. There appears to be a gradation in the pattern of distribution of the three different lichen groups, based on the wind pattern and availability of sun light.

On mangrove areas which directly face sea breeze, the fruticose species (R. montagnei) dominates and entire surface of the stilt roots are covered by these species (See plate 16). They are followed by the foliose species Dirinaria consimilis (Plate)

are followed by the foliose species Dirinaria consimilis (Plate) and less often by Dirinaria confluens.

In the midway to interior region, the sea breeze becomes slightly less and hence mostly foliose species and some crustose species are seen. Here the fruticose species are limited to the top branches of the trees if at all they are present. Still interior regions of the mangroves, the foliose species become limited in number and crustose lichens dominate. Even among the crustose lichens group. Pyrenula species are the most competent and entirely cover the surfaces in some instances. The foloise group here again limited to the top of the trees. Fruticose species are totally absent in these areas.

In the dense formations of mangroves with a thick canopy and greatly abutting stilt root systems, even the crustose lichen group become rare. (Plate 17).

This dominance of fruticose species (Roccella montagnei) followed by foloise species (Dirinaria consimilis) on the mangrove trees facing direct sea breeze may be with the fact that the sea breeze brings in copious amount of moisture. This moisture content is vital for the lichens to become metabolically active. In the absence of enough moisture. They become dry and suspend their matabolic activities. Hence when sufficient mositure is avaialable, they absorb this water perform, metabolic functions essential for their growth and dissemination (Richardson 1992). Under these conditions, the fruticose species simply outnumber other lichen groups in their competition. This

Figure 16 a - Crustose and fruticose lichen species on Rhizophora apiculata stilt roots

b - Fruticose and foliose lichen species on Rhizophora apiculata stilt roots, facing sea breeze

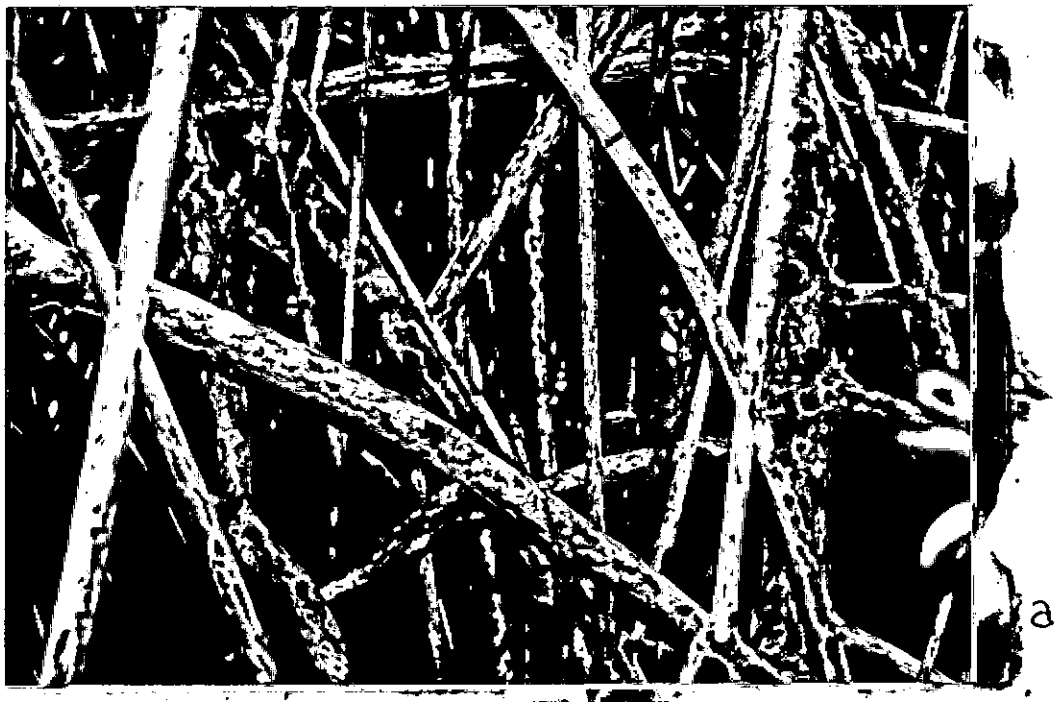
a

b

Figure 17 a - Crustose species diversity on *R. mucronata* and *R. apiculata* in the interior areas

b - Crustose, foliose & fruticose lichen groups on *E. agallocha*

c - Dense mangrove formations (Keerikadu) showing little lichen growth



a

b

c

may not be essntially the same in the interior regions since free wind flow is restricted by the dense formations and moreover there is a decrease in the availability of sunlight.

This view is fruther supported by the following observations :

1. Fruticose and foliose species are mainly limited to the top branches, in the inward regions of mangroves since the breeze often blows above the tree.

2. In dense areas wherever the inner regions are pruned or clearly felled on that side of the trees, fruticose and foliose species are seen occurring frequently.

These emperical observations clearly reveal the role of sea breeze and sunlight in influencing the distribution pattern of these lichen groups. This study with numerical data using transects for frequency of occurence on individual trees should form a baseline data for biomonitoring atmosphere using lichens and this work is being carried out presently.

7. Green house and laboratory evaluation of speceis for the sensitivity to oraganochlorines, heavy metals, trace elements, SO_2, flourides.

8. Titer levels of pollutants in eliciting a response.

Work on the above two objectives would also be taken up in the subsequent phases.

FUTURE PLAN OF WORK FOR 1993 - 1994

Work has been initiated already on the collection of other rare and endangered plants. The priority areas will be to identify and characterize the candidate rare plants and evaluate them. Probe preparation of RFLP work would also be taken up simultenaously, firstly the available probes would be used and then work would be initiated to construct a genomic DNA library. RAPD based identification of variations would be taken up soon and data collected.

Work on identifying and developing bioindicators for other ecosystems of South India with apropriate orgnisms will be carried out in the course of time. Work is in progress already in studying the occurrence of maximum number of endangered species in a particular ecosystem so that suitable bioindicators can be developed.

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AN I

APPLICATION OF BIOTECHNOLOGY IN THE CONSERVATION OF ENDANGERED PLANT SPECIES FOR GENETIC ENHANCEMENT

Sponsored by
DEPARTMENT OF BIOTECHNOLOGY, GOVERNMENT OF INDIA

REPORT

Submitted to
THE REVIEW COMMITTEE

18 DECEMBER 1992

PERIOD OF WORK - MARCH 1992 to NOVEMBER 1992

M.S. SWAMINATHAN RESEARCH FOUNDATION
MADRAS

TECHNICAL REPORT

WORK INITIATED

OBJECTIVES 1-4

Collection, propagation, field evaluation, RFLP studies of Mangroves, Genetic library construction.

The collection, propagation and field evaluation of plant species forms a prelude for the project whose baseline envisages a strong hold of the collection.

The objectives of the project include the saving of plant species in Peninsular India which are to be done in a phased manner, through micropropagation and other appropriate methods also. The 'Hotspot' locations for Mangroves are being identified now. Besides, the endangered plant species, mangroves are also being identified and collected. The list is appended as Table 1.

Collections are being done on a regular basis at the following sites (Table 2).

1. Pichavaram mangrove forest area
2. Ennore estuary area

The collections are aimed at finding,

a. The presence of a particular species, if consistent or otherwise.
b. The relative species richness

c. The value and relationship between different edaphic factors at the site.

The collection trips were also done at the following sites (Table 3).

1. Western coast
2. Eastern coast
3. Andamans (prior to the initiation of the project)

The material collected was brought both as plants and as propagules, in sufficient numbers so that a nursery can be raised in the foundation and the site. They were planted in mixed compost and were maintained with fresh water. We are also using ASW (artificial sea water) as a substitute to fresh water and to simulate the conditions of natural growth.

The field evaluation studies are aimed at seeing, if fresh water could successfully be used in the nursery establishment.

If this is possible, the plants would be tested for their morphological, physiological and biochemical adaptations, if any. If the result of raising using fresh water is not very useful then the components of the plant which comes in the way would be tested for.

If the experimentation with ASW is successful, we are aiming to see the difference in the plants in relation to structure, function and biochemical changes. These experiments would also let us know the optimal concentrations of different salts required in collection and separately and we would also be able to evaluate the necessity of sea water in the successful establishment of the mangrove species, if introduced into a different place. Studies are in progress to measure the genetic diversity in Rhizophora species using techniques of isozymes and RAPD based markers. The work was done using facilities at SPIC Science Foundation.

Data has been collected from 3 species, Rhizophora apiculata, R. mucronata and R. lamarckii with the aim to study the intraspecific variability.

Isozyme studies

Out of seven enzymes, only three (Esterases, Peroxidases and Polyphenol oxidases) enzymes resolutions could be seen within populations. However, variablity between Pichavaram and Andaman populations is refleted in the banding pattern. Each species shows distinct banding pattern except between R. mucronata and R. stylosa.

RFLP and PCR based marker, RAPD systems are to be used in the project for the evaluation of the germplasm. The

detailed methodology for the above analyses are being worked out. The work will be taken up as soon as all the requisite laboratory facilities become available in the new laboratory building.

Molecular Variability

Studies on molecular variability were carried out using RAPD markers. The essential step in this experimentation is to extract the DNA in a pure form. Methods are standardized for the same using CTAB extraction buffer, after treatment of the tissue with chemicals like Triton X 100.

Studies on the molecular variations were done using 55 different primers. The following results were obtained by this.

(i) All the 4 species studied showed distinct banding patterns,

(ii) Variability within and in between populations (collected from Pichavaram and Andamans) are evident at the DNA level. But no isozyme variability could be found within the populations,

(iii) Hybrid nature of R. lamarckii was shown by RAPD analysis,

(iv) Species relationship could be clear with the statistical analysis of the data and the construction of a dendrogram.

OBJECTIVE 5

A training programme on the "Application of Bioindicators to the Conservation of Biodiversity" was organised jointly by M.S. Swaminatha Research Foundation on behalf of the Department of Biotechnology.Twenty two candidates,including Scientists, Research Scholars, Lecturers and Pollution Control Board Personnel were selected from all over India. Faculty included, 4 specialists from abraod, apart from experts from India and many local lecturers. The course was conducted for a duration of 3 weeks from May 4th to 22nd 1992, with the aim to popularize Bioindication Technology in the Conservation of Biodiversity. The course content comprised of introduction, selection and sampling criteria for bioindicators, examples of different types of pollution and use of appropriate bioindicators in relevant cases. Some practical classes and demonstrations and field trips were also conducted. (See Brochure for details).

The highlight of the training was the demonstration of sampling and use of Insect species and Lichen populations (Table 5) as litmus test for forest disturbance and quality of atmosphere and also field trip to the mangroves of

havaram in which candidates were trained in the use of sects and quadrats in sampling and measuring biological rsity.

The candidates were distributed background materials he beginning of the course, containing the lecture notes relevant published papers. Compilation of a manual from e materials is underway for the use of condidates in re courses.

CTIVE 9

Study of the lichen population has been taken up tially with the mangrove forests of Pichavaram, with the to identify a few species useful as bioindicators in this system. The use of lichen population as bioindicators of quality has been well established.

Lichens are unique organisims which comprise free ls of a phycobiont (algal partner) embedded in the mat of mycobiont (fungal partner). The mode of survival is by ng photosynthetic products of the algal partner and pagation is achieved through the production of fungal res. Lichens are worldwide in distribution and are unique their tolerance to extremes of climatic conditions. spite of these facts they are very sensitive to atmospheric

Rhizophora species have been taken up for biomonitoring the atmospheric quality.

Almost similar lichen flora has been observed on all the three species of Rhizophora. There are about 5 different crustose lichens species, one foliose lichen Pyxine cocces and one fruticose lichen Roccella montagnei.

All the lichen species occur on the relatively young barks and not on the dried old barks. Foliose and crustose species are distributed all along the length of the tree. Fruticose species are generally observed above 10 feet from the surface up to maximum height of the trees (30 feet).

The fruticose species are interestingly found to occur frequently on the trees facing the sea front. The role of wind direction and velocity whether have any role in their establishment has to be studied.

The general occurrence of fruticose and foliose lichen species freely above the surface indicates the purity of the atmosphere. Hawksworth (1989) has made a similar study in London and demonstrated the quality of air based on the location of lichen population on the barks. Pichavaram Mangroves, though face a major threat from biotic stresses like grazing by cattle and clear felling, do not at present face any threat from the air pollutants. Even in the event of such a pollutant threat, monitoring these lichen species

could serve as early warning systems so that necessary precaution can be taken to protect this precious ecosystem.

Further study includes the survey of other islands of Pichavaram, identification of already observed lichen groups and comparison of these lichen flora with the flora of other mangrove ecosystems.

With reference to Biomonitoring of marine pollution, so far, these ecosystems have not been facing any threat from any marine pollutants. Hence a comparative study of the degraded mangrove of Ennore and Adyar, (former polluted by industrial chemical effluents and the latter by organic pollution) with the mangroves of Pitchavaram was taken up. In Ennore the A. marina grows to a height of 3 feet only but at Adyar and Pitchavaram they grow upto 30 feet. The cause for stunting has been ascribed to high salinity in Ennore. It was thought more than salinity, influence of some pollutant might play a role in stunting of A. marina but studies reveal, such stunting occurs even in Pichavaram where other mangroves are absent especially Rhizophora. This aspect is being investigated further. The other herbaceous species Suaeda monoica exhibits pigment variation from the normal plants of the same species. The whole plant shows dark pink to pinkish green colouration. This aspect is also being investigated further.

Monitoring the meiofauna and macrofauna have been initiated, making a comparative study of "hot spot" location with relatively clean spots along the coasts of Tamil Nadu. (Appendix 1).

Under macrofauna Perna viridis has been identified as indicator species in coastal ecosystem,and can be used as an early warning system for marine pollution. Perna viridis a bivalve filter feeder, is well known for its bioaccumulation and biomagnification. Hence these species are also being monitored for marine pollution.

FUTURE WORK PLAN

OBJECTIVE 1

The survey and collection and the evaluation of the species would be done in a phased manner. The collection and survey would be done in conjunction with studies on different biotic and abiotic factors influencing the growth of the species. The work on mangrove germplasm collection would be done also in

a) Bhitar Kanika

b) Chorao islands

c) Andaman islands

besides Pichavaram (Table 4). The morphological variations that are occurring are not well documented (Table 3). It is

intended to evolve a suitable key for field identification based on as amny morphological characters as possible. This key would substantiate ways of finding out '+' (plus) trees more reliably.

Marker based identification of germplasm is a relatively easier and better system. The markers that could be used include those of isozymes besides RFLPs and RAPDs.

ISOZYME STUDIES

We have embarked on a programme for studying the isozyme patterns of mangroves and their associates. Work is progressing on studying the patterns of peroxidases, amylases, MDH, isomerases and other enzymes. The protein patterns among and in between a species collected from different sites are also being evaluated. The main aim is to find out the intraspecific variations present in the sample species.

RFLPs

The major handicap with the experiments concerned with the isolation of total genomic DNA is the presence of phenolics and tannins within the leaf tissues of mangroves. Methodologies are being standardized with respect to the isolation of DNA in Rhizophora species. The DNA isolation techniques for different species of mangroves will be done as soon as the first complement of equipment arrive.

Besides DNA isolation, work in the next phase would include the initiation of the work for the creation of a cDNA library.

OBJECTIVE 2

Probe preparation is an innate component of the project proposed. But it is possible for the experiments to be done only after the cDNA or genomic DNA is constructed, first. But the probes available from non-mangrove species would be used for the RFLP analysis to begin with. In this regard, a simple procedure for probe preparation is being attempted. If this work is successful, it will be possible for the probe preparation to be done within a period of one year.

OBJECTIVE 3

Non-radioactive labelling work could be done only after the probes for the RFLP analysis is ready.

OBJECTIVE 4

Compared to the RFLP exeprimentation, PCR based marker system RAPD is more easier, cheaper and effective. Keeping this in mind we are attempting to use this marker for an effective and efficient analysis. Already some work on the use of RAPD markers on Rhizophora have started. Different

species collected from different sites would be subjected to the RAPD analysis using the four sets of primers.

Tissue culture forms an intricate part of the work that is being attempted. It would not only help in the multiplication of the plants in numbers but also would enable a continuous supply of the plant material. The conditions of growth in in situ conditions would be evaluated. The endangered plant species identified would be effectively propagated in aseptic conditions besides studying the physiological processes that occur during the culture procedures. A detailed report is being submitted to the Department of Biotechnology, Government of India which includes details of methodlogies, results and a thorough discussion.

OBJECTIVE 5

Preparation of a training manual useful for candidates attending Bioindicators training programme and others pursuing research in the use of Bioindicators is underway and is being compiled from the background materials (lecture notes and relevant literatures) supplied to the condidates. The manual is being released in parts and this manual will essentially comprise aspects of biological diversity and the necessity to conserve them, use of Bioindicators in Conservation, introduction, selection and

sampling methodology and a few examples of pollution and use of bioindicators under such circumstances.

OBJECTIVE 6

It is proposed to survey and select a few species, amenable to laboratory studies (ex situ conditions) from the forests of Western ghats. In additon to that collection of base line data on their natural variation, temporally and spatially upon which any drastic change detected could indicate the imminent danger from any stress.

OBJECTIVE 7

Under simulated conditions of pollution using special growth chambers, these selected species can be studied for their sensitivity to the various pollutants.

OBJECTIVE 8

The sensitivity studies could be evaluated by studying different concentrations, (titres) of various pollutants.This could enable in locating a maxium sensitive species (Rapid sensitivity) to lowest dose possible so that necessary precaution may be taken to save the ecosystem from further damage by using these species as early warning systems.

OBJECTIVE 9

The work has been initiated using lichen population as early warining system of mangrove forests. Work on marine pollution, land quality will have to be started once the laboratory has received entire and full complement of the equipments.

Appendix 1

Identification of Pollution Indicators From the Intertidal fauna.

Introduction: It is possible to detect even traces of pollutants in the environment by chemical analysis of air, water and soil samples. Biomonitoring is important since the effect of the pollutants on the living communities has to be understood. In the present context,however,the aim is to find intertidal faunal species or groups of species which can aid in the easy detection of pollution so that further monitoring, both chemical and biological can be carried out and the necessary control measures can be undertaken.

Bioindicators have been grouped under 5 types

1) Sentinels.
2) Detectors.
3) Exploiters.
4) Accumulators.
5) Bio-Assay Organisms.

In the present study the main interest is to identify groups of species of the type detectors and exploiters, by whose relative abundance the pollution status can be understood. This has been termed Community Bioindication.

The first step towards the identification of indicator species or groups of species from the intertidal fauna, is to find out the species composition and relative abundances from different sites. There are several methods which uses relative abundance, total number of species, number of individuals per species and biomass data to indicate the pollution status of an area. Diversity indices (Comparison between polluted sites and unpolluted sites), Frequency Distributions (fits to log-normal curve) and the Aundance Biomass Comparison method are examples for such methods for detection of pollution.

The present sampling is aimed at finding out the species composition and abundances in three different estuarine habitats and to test the ABC method to see whether it can be used in the detection of organic pollution in tropical estuarine habitats.

The Abundance Biomass Comparison (ABC) Method

By plotting the percentage dominance (cumulative scale) on the Y-axis against the species abundance rank (Logrithimc scale) on the X-axis the K-dominance curve of a faunal

assemblage can be plotted. In the ABC method the combined K-dominance curves for biomass and abundance is plotted on the same graph. If the biomass curve is above the abundance curve the biomass is less than the numerical diversity and then an unpolluted site is indicated. If the biomass curve and the abundance curve are close together and intercept at one or more places a moderately polluted site is indicated. When the abundance curve is above the biomass curve the numerical diversity is less than the biomass, and this indicates a grossly polluted area. (See figure paper 7).

The Theory Behind The ABC Method. Warwick (1986) has used Huston's Theory (Gray, 1974) of the relationship between the frequency of disturbance and diversity to explain the ABC method. This method was originally proposed for subtidal macrobenthic assemblages. Later it was seen to be applicable to intertidal areas also despite the higher physical disturbance seen in the intertidal area.

In unpolluted benthic area a few K-selected (conservative, longlived species) become the biomass dominants through competitive displacement. These biomass dominants are few in number. Present in the habitat along with the K-selected species are numerous r-selected species (short lived, oppurtunistic species). The number of individuals are more or less evenly distributed among these. So in unpolluted

areas the numerical diversity is more while the biomass is dominated by a few species.

In a moderately polluted area the K-selected species are greatly affected. So the numerical dominants become the biomass dominants also.

In a grossly polluted area most of the r-selected species are also affected by the pollutants. A few very small species thrive in such situations. Even this case there will be some of the larger r-selected species contributing to the biomass. In such situations the biomass is more than the numbers diversity.

Advantages of Using This Method: By interpreting the ABC curve from just one representative sample the pollution status of a site can be estimated. It need not be compared to controls along spatial and temporal gradients to be interpreted.

Disadvantages:

1) Great care has to be taken to ensure adequate and representative sampling. If the few K-selected species that are present in the habitat is missed out in the sampling a wrong pollution status will be indicated.
2) This method can only detect organic pollution.

3) After finding out occurrence of disturbance it can be said to be due to pollution only after verifying that it is not due to biological or physical disturbances. This has to be carefully checked out whenever a moderately polluted state is indicated.
4) This method is relatively new and has not been adequately tested.

Aim:

1) To use ABC method for three sites - unpolluted, organically polluted and industrially polluted.
2) To find out the species composition and relative abundance of the fauna in these areas and to use this to calculate the diversity indices and frequency distributions.
3) To select a few species to observe in the laboratory their reaction to the different doses of pollutants to which they are likely to be exposed in their habitats.
4) Collection of soil samples for meiofaunal studies - sorting and identification techniques.

Area: Three sites have been chosen- (1) Adyar estuary (organically polluted), (2) Ennore estuary (industrially polluted) and (3) Pichavaram (relatively unpolluted). Three field sites will be selected in each of these three area - one at the mouth of the estuary, another at the outlet of

pollutants and another near the mangrove vegetation. These field sites will be further stratified into high tide, mid tide and low tide areas where ever tidal action is evident.

Methods and Equipment: Each sample will consists of according to the site and the tidal zone as described by Holme and McIntyre so that the sampling is adequate. Separate samples will be taken using a corer (2 cm diameter and depth of 10 cm) for qualitative studies of the meiofauna. Each site will be sampled once in three months. Every time a site is sampled the following environmental parameters will also be measured :- 1) Grain size, 2) Sorting co-efficient 3) Salinity, (4) pH, (5) Redox potential, (6) Oxygen content, (7) BOD. Information about nature and amount of pollutants will be collected from Pollution Control Boards. The samples will be sieved at the site itself since a lot of water is necessary. The Fauna will be preserved and sorted in the lab. The macrofauna will be sorted according to morphological characteristics counted, weighed and preserved for identification. The meiofaunal samples will be used to try and sort out species in to groups based on morphological characteristics.

The data obtained will be as follows for macrofauna (1) Number of species, (2) Number of individuals per species, (3) Total number of individuals, (4) Biomass of each species,

(5) Total biomass. Using this data, diversity indices, relative abundance, frequency distribution can be calculated in addition to the ABC curves. It may also be possible to find individual species or a small group of species which may be conspicuous is unpolluted area and little or absent in pollued areas or Vice versa, as found in freshwater systems.

TABLE 2

SITE EVALUATION

CRITERION	CHARAO	PICHAVARAM	BHITAR KANIKA
Genetic Aspects	5	5	1
Ecological Aspects	4	5	2
Neighbouring flora/ Fauna	4	5	4
Land Size	3	4	2

Scale : I - Most desirable, 9 - Least desirable

TABLE 3

MORPHOLOGICAL VARIATION (HEIGHT) AMONG MANGROVE SPECIES AND ASSOCIATES

SPECIES	CHARAO	PICHAVARAM	BHITAR KANIKA
Avecinnia officinalis	H	H	M
A. marina var. marina	M	H	-
Brugiera cylindrica	L	L	L
Ceriops decandra	-	L	-
Excoecaria agallocha	M	M	M
Lumnitzera racemosa	-	L	L

L - 0 - 5m

M - 5 - 10m

H - - 10m

TABLE 4

SITE SELECTION FOR COLLECTIONS

S. NO	NO. OF SITES	COLLECTION SPOT
1	5	Pichavaram
2	5	Bhitar Kanika
3	5	Charao

TABLE 5

POLLUTION MAPPING OF MADRAS CITY BASED ON THE OBSERVED TRENDS IN THE POPULATION OF LICHEN SPECIES

Lichen group	Number of species observed			
	Ennore	Madras University campus	IIT campus	Theosophical Society
Fruticose (shrubby)	0	0	0	1
Foliose (leafy)	0	0	1	1
Crustose	0	0	7	10
Leprose (powdery)	0	0	1	1
Total	0	0	9	13

SENIOR LEVEL TRAINING PROGRAMME

ON

THE APPLICATION OF BIOINDICATORS IN THE CONSERVATION OF BIODIVERSITY

MAY 4 – 22, 1992

MADRAS, INDIA

M.S. SWAMINATHAN RESEARCH FOUNDATION

CENTRE FOR RESEARCH ON SUSTAINABLE AGRICULTURAL AND RURAL DEVELOPMENT

Madras, India

DEPARTMENT OF BIOTECHNOLOGY

Government of India

New Delhi

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Mr. P. Balakrishna	
Miss. Sheela	

OBJECTIVES

" The Earth mourns and withers.,
The Earth lies polluted
under its inhabitants;
for they have transgressed the laws,
violated the statutes,
broken the everlasting covenant"

(Isaiah 24: 4-5)

Biological diversity is the very foundation of biological productivity. Present day estimates of the number of species on earth, ranging from 5 to 10 million and shooting upto 80 million, are indicative of both our rich genetic heritage and of the extent of our ignorance of species identification and distribution. Several scientists have hazarded a guess that perhaps one quarter of the world's total biodiversity is at serious risk of extinction over the next 20 to 30 years due to human inflicted damage to the habitats of flora and fauna. It is in this context that steps for inventoring and monitoring biodiversity assume urgency.

Bioindicators are organisms expressing particular symptoms or responses indicative of changes in some environmental influence, usually in a qualitative manner. If *in situ* conservation programmes are to be effective and placed on a secure scientific base, some objective method of measuring that effectiveness is a prerequisite. It is impractical to monitor on a regular basis the populations of all but the most conspicuous macrorganisms. Hence, it is necessary to identify particular organisms, or groups of organisms, that can serve as bioindicators of the health of the communities that we are striving to conserve.

The bioindication technology involves the identification of organisms belonging to any systematic group possessing the following characteristics.

- Show a prompt and accurate response to particular discrete causes of pollution or other forms of environmental perturbation.
- Be readily identifiable to the level necessary for bioindication in the field.

- Reflect some aspect of ecosystem function.
- Be amenable to the application of portable and standardised sampling methods.

Bioindication methodology is low in cost but high in accuracy. It can be operated by School children, after proper training. It can become a trigger for community involvement in monitoring environmental health. Yet its application has been very minimal in our country. The present senior level training course was therefore initiated for the purpose of developing a cadre of specialists well versed in the science and art of identifying and using bioindicators as instruments of early warning of ecosystem health. The course was particularly designed to promote the use of this technology in studying threats to habitats rich in biological diversity.

STRUCTURE OF THE COURSE The course consisted of (a) Lectures and Practicals (b) Demonstrations and (c) Field visits

The details are given below

Date	*Topic*	*Faculty*
04.05.1992	Conservation of Biodiversity	Prof. M.S. Swaminathan
	Carrying Capacity of the Environment	Prof. C.A. Sastry
05.05.1992	Practical Examples of Biomonitoring	Dr. Staffan Holmgren
	Floristic diversity of Penisular India	Dr. A. Raman
	Application of Bioindicators in Conservation of Biodiversity	Prof. V.M. Meher–Homji
	Bioremediation of Industrial Wastes	Prof. C.A. Sastry
06.05.1992	Bioclimatology	Prof. V.M. Meher–Homji
	Bioindicators of Pollution	Prof. C.A. Sastry
	Microbial diversity : opportunities and pitfalls	Dr. Carl Heden
	Marine Plankton diversity (practicals)	Dr. L. Kannan
07.05.1992	Vegetation maps of India	Prof. V.M. Meher–Homji
	Biodiversity of Marine Algae	Prof. V. Krishnamoorthy
	Plant indicators of water resources and Minerals	Dr. E.A.V. Prasad
08.05.1992	Water quality management in aquaculture	Dr. S. Chandra Prakash
	Biodiversity and its conservation	Mr. John Joseph
	Biodiversity of Marine plankton	Dr. L. Kannan
	Metabolic fingerprinting technique	Prof. Rolland Mollby
	Chemical ecology of protozoa, protozoan indicators.	Dr. R. Gopichandran
09.05.1992	Air pollution and its effects on ecosystems.	Prof. G. Oblisami
	Microbiology of the atmosphere	Dr. B.P.R. Vittal
	Air sampling devices (Practical)	Dr. B.P.R. Vittal
	Bioindicators – an introduction	Prof. S.B. Chaphekar

Date	*Topic*	*Faculty*
11.05.1992	Air pollution and plant indicators	Prof. S.B. Chaphekar
	Industrial waste water recycling	Prof. G. Oblisami
	Epiphytes as indicators of pollution	Prof. D.D. Awasthi
	Criteria for selection of bioindicators	Prof. S.B. Chaphekar
12.05.1992	Indicators of Aquatic ecosystems	Prof. S.B. Chaphekar
	Pollution occuring due to agricultural practices	Prof. S. Suryanarayanan
	Pollution due to mining	Prof. G. Oblisami
	Air pollution and plants (practicals)	Prof. S.B. Chaphekar
13.05.1992	Lichens as indicators of pollution	Prof. D.L. Hawksworth
	Environmental Biotechnology	Prof. P. Khanna
	Demonstration at IIT Campus	Prof. P.M. Hammond &
	Biodiversity of insects and lichens	Prof. D.L. Hawksworth
14.05.1992	Field trip around Madras city covering polluted and unpolluted areas	
15.05.1992	Invertebrates (Insects) as bioindicators of wetlands and lakes	Prof. P.M. Hammond
	Indicators of Land degradation	Prof. S.B. Chaphekar
	Practicals – Analysis lichen samples collected during field trip	Prof. D.D. Awasthi Prof. D.L. Hawksworth
	Practicals – Extraction from the insect traps	Prof. P.M. Hammond
16.05.1992	Potential of Bioindicators in monitoring ecosystem health	Prof. D.L. Hawksworth
	Bioindicators in Forest and Agroecosystems	Prof. P.M. Hammond
	Field trip to Pichavaram mangroves 17th	

Date	*Topic*	*Faculty*
18.05.1992	Biomonitoring of aquatic ecosystems	Prof. K.S. Bilgrami
	Practicals on sampling and estimation of Phytoplankton	Dr. L. Kannan
	Sampling and analysis of insect fauna obtained from the traps at IIT Campus (Practicals)	Prof. P.M. Hammond
19.05.1992	Algae as indicators of water quality	Prof. K.S. Bilgrami
	Practicals bacterial sampling from Cooum river and their estimation	Dr. S.S. Sahay
	Xenobiotic compound indicators	Prof. C.B.S.R. Sharma
	Bacteriological indicators of drinking water quality	Prof. R. Pitchai
20.05.1992	Ecology and diversity of Mangroves	Prof. A.N. Rao
	Bacteria as indicators of organic pollution and faecal load	Prof. K.S. Bilgrami
	Estimation of total bacterial density and total Coliforms (Practicals)	Dr. S.S. Sahay
21.05.1992	Indicators of heavy metal accumulation in water bodies	Prof. K.S. Bilgrami
	Pollution affecting fish resources	Dr. V.K. Pillai
	Practicals – Test for E. Coli and faecal coliform estimation	Dr. S.S. Sahay

A highlight of this programme is a study of the impact of pollution on lichens in different parts of the Madras city, starting from Ennore and extending upto Vandalur

A visit was also organised to the Pichavaram Mangrove Forests near Chidambaram, in order to stimulate interest in the application of bioindicators in measuring threats to Mangrove ecosystems.

PARTICIPANTS

Dr. Abha Sinha,
Officer on Special Duty
EPCO
Paryavaran Parisar
E-5 Area Colony
Bhopal - 456 016

Mrs. D.Anbu
Environmental Scientist
Tamil Nadu Pollution Control Board (AEL)
25, Radhakrishnan Road
Mylapore,
Madras - 600 004

Mr. G.K. Ahuja
Junior Scientific Assisstant
Central Pollution Control Board
Parivish Bhavan,
East Arjun Nagar, Shahdra,
Delhi - 110 032

Mr. S. Balaji
Deputy Conservator of Forests
Social Forestry, Periyar Building
Nandanam,
Madras - 600 035

Dr. S. Chandra Prakash
Technical Officer
Central Institute of Fisheries Education
Versous, Bombay - 400 061

Dr. Gunwant Joshi
Scientist , Central Laboratory,
E-5, Paryavaran Parisar,
Area Colony,
Bhopal - 456 016

Dr. K.O. Joseph
Scientist
Central Institute of Brackishwater
Aquaculture (ICAR)
12, Leithe Castle Street
Santhome,
Madras - 600 028

Dr. L.Kannan
CAS in Marine Biology
Annamalai University
Parangipettai - 608 502

Dr.D.G. Kshirsagar
Scientist 'C'
Environmental Biotechnology Division
National Environmental Engineering
Research Institute
Nehru Marg,
Nagpur - 440 020

Dr. V. Kunjukrishna Pillai
Senior Scientist
Central Marine Fisheries Research Institute
P.B.No. 2704, Dr. Salim Ali Road,
Cochin - 682 031

Dr. M. Mukundan
Senior Scientist
Central Institute of Fisheries Technology
Matsya puri (p.o.)
Cochin - 682 029

Dr. (Mrs) Neelam Verma
Lecturer
Department of Biotechnology
Punjabi University, Patiala

Mr. C.N. Paupiah
Marine / Environmental Department
University of Mauritius
Reduit,
Mauritius

Dr. P. Perumal
Research Associate
CAS in Marine Biology
Annamalai University
Parangipettai - 608 502

Dr. Prabodh Ghosh
Senior Research Fellow (CSIR)
Environmental Toxicology Laboratory
Department of Zoology
Visva-Bharathi University
Santhiniketan - 731 235

Dr.S.Ramakrishna Rao
Scientist ' SE '
Eastern Regional Environment and Forests
Government of India
194, Kharavela Nagar
Bhubaneswar - 751 001

Dr.C.R. Ramesh
Scientist S-3
Head, Plant Pathology and Entomology
Central Agricultural Research Institute
P.B.No. 181,
Port Blair - 744 101

Dr. P.K. Singhal
Reader
Department of Biological Science
Rani Durgavati University
Pachpedi,
Jabalpur - 482 001

Mr.A.Sivakumar
Research scholar
Centre for Bioscience and Biotechnology
Indian Institute of Technology
Madras - 600 036

Dr. (Mrs) V. Sobha
Senior Lecturer
Department of Aquatic Biology and Fisheries
University of Kerala
Trivandrum - 382 043

Dr. (Mrs) Sudha Nair
Research Associate
Microbiology Dept.
Central Leather Research Institute
Adyar,
Madras - 600 020

Mr.K.B.Vaghela
Junior Scientific Assistant
Gujarat Pollution Control Board
Sector - 10 -A
Gandhi Nagar - 382 043

FACULTY

INDIA

Prof D.D.Awasthi
38, Khurshed Bagh
Lucknow - 226 004

Prof.K.S.Bilgrami
Prof and Head
University Dept. of Botany
Bhagalpur University
Bhagalpur - 812 007

Dr.R. Gopichandran
Entomology Research Institute
Loyola College
Madras - 600 034

Mr.John Joseph
M.S.Swaminathan Research Foundation
14, II nd Main Road
Kottur Gardens,
Kotturpuram
Madras - 600 085

Prof. S.B. Chaphekar
Prof and Head
Dept. of Botany
University of Pune
Maharashtra - 411 007

Prof. P.Khanna
Director
National Environmental Engineering
Research Institute
Nehru Marg,
Nagpur - 440 020

Dr. Maria Saleth
Scientist
M.S.Swaminathan Research Foundation
Madras - 600 085

Prof.V.M.Mehr Homji
French Institute of Pondicherry
P.B.No.33
No.10, St. Lui Street,
Pondicherry - 605 001

Prof.G.Oblisami
Head, Dept of Environmental Science
TNAU Campus
Coimbatore - 641 003

Prof.R.Pitchai
Director
Centre of Environmental Studies
Anna University
Madras - 600 025

Dr.E.A.V.Prasad
Dept of Geology
Sri Venkateswara University
Tirupati - 517 502

Dr. A.Raman
Entomolgy Research Institute
Loyola college
Madras - 600 034

Prof.C.A.Sastry
Chemical Engineering Department
Indian Institute of technology
Madras - 600 036

Prof.C.B.S.R.Sharma
Salim Ali school of Ecology
University Campus
R.Venkatramana Nagar
Kalapet
Pondicherry - 605 014

Dr. Staffan Holmgren
Environmental Advisor
Bay of Bengal Programme
N0.91, St. Marys Road,
Abhiramapuram
Madras - 600 018

Prof. S.Suryanarayanan
Emeritus Scientist
CAS in Botany
University of Madras
Madras - 600 025

Dr. B.P.R.Vittal
Reader,
CAS in Botany
University of Madras
Madras - 600 025

Prof.V.Krishnamoorthy
Emeritus Professor
Dept of Botany
Presidency College
Madras - 600 005

Prof M.S.Swaminathan
M.S.Swaminathan Research Foundation
14, II Main Road,
Kottur Gardens
Madras 600 085

SINGAPORE

Prof.A.N.Rao
Dept. of Botary
National University of Singapore
Singapore

SWEDEN

Prof. Carl Goral Hedan
Biofocus Foundation
Stockholm
Sweden

Prof.R.Mollby
Karonlinska Institute
Stockholm
Sweden

UNITED KINGDOM

Prof. P.M.Hammond
Entomologist
Natural History Museum
London
U.K.

Prof.D.L.Hawksworth
Director
International Mycological Institute
CAB International,
Ferry lane, Kew,
Surrey TW 9 3AF
London U.K.

FOLLOW-UP

The outputs of this Training Programme will be the following

a. Preparation of a Training Manual for use in similar courses in the future.

b. Organisation of courses on bioindicators at the state level, particularly for school and college teachers, and

c. Development of an All-India coordinated Network on Bioindication technology, with particular reference to the health of ecosystems rich in biodiversity.

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