MATERIALS AND METHODS

Study area

Assam is located in the north eastern part of India surrounded by state of Arunachal Pradesh in the north, Nagaland in the east, Mizoram and Tripura in the south and West Bengal in the west. The countries viz. China and Bangladesh form international borders with Assam. Assam is surrounded by a ring of blue hills. It is an amalgamation of plains and river valleys. Its principal geographical regions are: Brahmaputra valley in the north; the Barak plain in the south; and the
Mikir and Cachar Hills divide the two regions. These two valleys in Assam are the main lands where a large mass of human population live. Climate of Assam is tropical monsoon type and is a temperate region which is uncomfortably humid especially during rainy season. Winter season starts from the end of October and lasts till late February. The minimum temperature during this period varies between 6 to 8°C. Nights and early mornings are foggy, and rain is scanty. Summer starts in mid May, accompanied by high humidity and rainfall. The maximum temperature reaches 35-38°C. The monsoon season starts from mid-June and continues till August. Thunderstorms known as Bordoicila are frequent during the afternoons. Spring and autumn seasons are characterized by moderate temperatures. The state has six agro climatic zones which are divided based on soil, rainfall and crop characteristics. These climatic zones are Upper Brahmaputra Valley, Central Brahmaputra Valley, Lower Brahmaputra Valley, Hill Zones, Barak Valley and North Bank Zones. Barak valley in Assam consisting of three districts, Cachar, Karimganj and Hailakandi, a geographical area of 6922 sq. km., (according to 2001 census). The valley has an undulating topography characterized by hills, hillocks, wide plains, and low-lying water bodies, locally known as beels, some of which, however, dry up in the winter, termed as haors. Most of the hills have a north south spread interspersed by the strips of plains. The land is alluvial and naturally fertile. The principal river, Barak origins from Angami Naga hills in Manipur and travels in curved route cutting through the heart of Cachar district. Jiri, Chiri, Madhura, Jatinga, Dhalesweri, Ghagra, Katakhal, Longai, Shingla, Sonai are the major rivers in
Barak Valley. Barail, Bhuban, Panchgram, Chatakerra, Mohonpur, Saraspur are the major hills with numerous hillocks in their vicinities. This plain track of Barak valley is a geographical extension of Gangetic Bengal. The valley is predominantly inhabited by the Indo-Aryan population, and the demography is formed in early times by integrating the Indo-Mongoloid, Austric and other non-Aryan ethnic groups in a long historical process. With the formation of two more districts truncating Cachar, the area in the south of Assam is collectively termed as Barak valley.

The study area comprises of Cachar district (Fig 1) of Assam located in the southern North eastern region of India, lies between 24° 49' N and 92° 48' E longitude on the left bank of the river Barak. The geographical area of Cachar district is 377610 ha. and is bounded on the north by Barail hills, Manipur on the east, Hailakandi and Karimganj district on the west and Mizo hills on the south. The district covers an area of 3786 sq.km. at an altitude of 36.5 msl.

**Geology and rocks**

Cachar district of Assam is a huge storehouse of limestone. Limestone, which is basically calcium carbonate, is primarily a sedimentary rock which is used in a plethora of purposes namely construction, interior decoration etc. Karbi Anglong District and North Cachar hills have substantial reserves of coal. Of the four types of coal namely Peat, Lignite, Bituminous and Anthracite, the third kind is readily available out here. The most important characteristic of this area in relation to Brahmaputra Valley is its geological newness and water stagnation.
Fig. 1 Map showing the locations of collection site in different zones of Cachar district.
Land use and forestry

The topography of the district varies from small hillocks to plain areas and low lying areas locally known as haors, beels, etc. about 33% of total geographical area was used for cultivation of various agricultural purposes. More than 20% of the geographical areas of the district can not be grown crop during April to September due to water stagnation. On the other hand lack of rain from November to April, most of the cultivable land remain fallow during the period. There are 2037 ha cultivable wasteland in the district. Hence, tanks/ponds check dams and storage reservoirs can be thought of for the purpose of water management and water conservation. Although Govt. attaches considerable importance to improving the quality and productivity of our land and soil resources by reclamation of degraded and fallow lands as well as problem soils under its National Watershed Development Project for Rain fed Areas, the progress/benefits derived from the project is yet to be noticeable in the district.

Farm mechanization is one of the tools to accelerate the growth of agriculture productivity. Farm mechanization not only help in increasing the productivity of agriculture sector by best use of the lands but also saves valuable time of labour force for other productive purposes. The National Policy on Agriculture seeks to actualize the vast untapped growth potential for agriculture, strengthen rural infrastructure to support faster agricultural development, promote value addition, accelerate the growth of agro business, create employment in rural areas, secure a fair standard of living for the farmers and agricultural workers and their families, discourage migration to urban areas and face the challenges arising out the
economic liberalization and globalization and it aims to attain a growth rate in excess of 4% per annum in the agriculture sector over the next two decades. Hence, mechanization of farms viz. uses of tractors, power tillers, etc. is necessary to increase production and productivity of agriculture. There are large utilized tilah lands in the district where bamboo, jatropha, etc. can be grown profitably. Further existence of Cachar paper mill in the adjacent district of Hailakandi is added advantage for bamboo growing. The mill requires 5.00 lakh MTG of bamboo for its full capacity utilization. To produce 5.00 lakh MTG bamboos it is estimated that there is a requirement of 10000 ha of land. At present, the mill is fed by bamboo grown mostly in the forest of Barak Valley, Mizoram, and N.C Hills. The mill has taken initiatives for large scale bamboo plantation in the valley. Accordingly, NABARD, in consultation with mill and bankers, prepared a banking plan for cultivation of bamboo in Barak Valley. Credit requirement for the year 2007-08 towards this sector has been estimated at Rs. 50.90 lakh. The landscape of Cachar tea areas is composed of small hillocks (called tilahs), plains (flat & plateau), and low land (the bheels). The most important characteristic of this area in relation to Brahmaputra Valley is its geological newness and water stagnation. The stagnation of water in low lying areas led to the formation of 'Bheel' i.e., bog or true peat. The tilah (small hillocks) of Cachar shows considerable variation in nature of the underlying rocks. The approximate percentages of tea under different topographies are: tilahs 35%, flat & plateau 60% and bheels 5%.

Soil
The soils in most of the tilahs are coarse, sandy loam in texture and most plateau and flat areas are silty clay loam. The 'bheel' soil (peat soil) classified as mineral soil is with high proportion of organic matter. The mineral fraction mainly constitutes fine particles of silt and clay. The soils are acidic (about 33% with pH < 4.5 and 65% between pH 4.5 and 5.5), with a large percentage showing low organic matter and potash. Wide variability in yield is observed while comparing hot (south facing) and cold (north facing) slopes on teelas. Yield of cold slopes is generally higher; however under good stand of shade with proper drainage crop in the hot slope can also be improved.

**Topography and agro-climatic characteristics**

Cachar district comes under Barak valley zone. This zone comprising 8.9% of the state area and 11.7% of the state population. Apart from the tea gardens located at the hill slopes, the entire zone is growing rice as major crop occupying about 93% of the net crop area. The climate is warm and humid during the summer and the lowest temperature is recorded during December and January. The humidity ranges from 65-70% during winter and 85-90% in the rainy season.

**Irrigation and ground water**

The district experience sufficient rainfall as a result of which most of the crops are grown in rainfed condition where irrigation is done manually with low lift pump. As a result assured irrigation was available to 2% of cultivable land in the district. There is plenty of scope to boast up the production and crop area if
irrigation facilities are provided effectively. Assured irrigation can obviously raise the regular Ahu cultivation under controlled flood situation.

**Inter-district growth differentials**

The district is divided into five agro ecological situation (AES) which are namely

- AES-1 Alluvial flood free
- AES-2 Alluvial flood prone
- AES-3 Piedmont and plantation crop growing
- AES-4 Beels and Haors
- AES-5 Hills and forests.

Silchar is the district headquarters of Cachar district in the State of Assam in India. It is the economic gateway to the state of Mizoram and Manipur. It is situated 420 kms south east to Guwahati. The town of Silchar has tremendous commercial importance and is the second largest in the state of Assam. It consequently, witnesses the settlement of a sizeable population of trades from distant parts of India. Being a very peaceful place in the otherwise disturbed north east earned it the bon mot of “Island of Peace” from former Indian Prime Minister Indira Gandhi. Silchar is situated on bank of the river Barak popularly known as Barak valley. Rice is the staple cereal. Over the past few years, the city
is constantly witnessing a huge influx of people from nearby smaller places due to city's increasing future prospects and other developments in the field of education, medical facilities and the more recently booming real estate market and other commercial business, making the city quite an overcrowded one. It has now the second highest population in the state, although the difference with the highest populated city, Guwahati the capital of Assam, is quite huge. Silchar is located on the southern part of Assam situated on the Barak river near the Bangladesh border, it is trade and processing centre for tea, rice and other agricultural products. There is limited industry, principally papermaking and tea-box manufacturing. It has an elevation of 22meters. At Silchar, the wind generally blows from the northeast in the morning and from the southeast in the afternoon. Summer is hot humid and interspersed with rain and thunderstorms. Winter generally towards the end of November and lasts till February. Towards the start of mid-April the rain clouds start covering the skyline. Silchar is inundated frequently due to excessive rainfall and flooding by the river Barak. In the last three decades, Silchar and the Barak valley have been ravaged by four major floods- in 1986, followed by the one in 1991, 2004 and more recently in 2010.

**Survey and collection**

The research work initiated with the segregation of lichen taxa collected from the Cachar district. To perform an intensive and extensive collection of the specimens, the study area has been divided into five zones Central, East, West, North and South zone. Each division was further divided into 1×1 sq. km. In each
Materials and Methods

zone, twenty sites of 1 x 1 sq. km should be selected to perform the exploration of lichen (Table 2). Collection of lichen specimens and recording the data from all the selected sites of the district were frequently visited in the first two years. A stratified random sampling method was adopted for ecological studies. A total of 500 quadrates were laid down, five quadrate of (5 x 5 sq. m) each in 20 sites of five different zones of the study area. The lichens were collected from the bark of the *Areca catechu* as a host tree. To record the ecological details of the different species growing on the trunk of the betel nut tree, sampling were performed at three different heights- base height (0-65cm), chest height (65-110cm) and head height (110-165cm). The lichen specimens were collected from he betel nut tree with the help of hammer, chisel and knife. Along with the collection of lichen specimens, the details of locality, altitudes and other ecological notes were also recorded (Table 3). The loosely attached lichens were removed from the substrate with the help of sharp knife, the due care was taken that the fixing organs (rhizines, holdfast) remain attached to the thallus as much as possible. The closely adnate forms were collected along with the substratum. The collections were made during the day time and were placed in separate polythene bags, on reaching home or laboratory the specimens were sorted out, packed in newspaper and left to dry. As before well dried the samples were separated from the bark with the help of blade or knife and glued it to the hard sheet, otherwise the samples were broken up in to smaller pieces. In the laboratory, the dried specimens brought from the field were placed in lichen herbarium packets, with the details of locality, date of collection, field number, collector name and other ecological
notes. The labeled and dried specimens were lodged in Assam University, Silchar (AUE) and Lichen Herbarium of National Botanical Research Institute, (LWG) Lucknow.

**Table 1** Month wise temperature data during sampling period.

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Table 2 Collection sites within different zones of the Cachar district.

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<th>South Zones</th>
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Fig 3 Altitude variation of the study area

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Identification


The external morphology has invariably been studied under dissecting binocular microscope. The anatomy of the thallus and apothecia were studied under compound microscope. The external morphology was examined generally in dry condition but dark brown to bluish specimens of Leptogium were studied in wet condition. Anatomical details of the thallus and fruiting bodies were studied in free hand sections with water as mounting medium under compound microscope. The anatomical structures were studied after cutting the sections of dry material by microtome and with the help of safety blade. The thin dry sections of the thallus and ascocarp were immersed in 90% ethyl alcohol to drive off the intercellular or inter-hyphal air bubbles and the sections were mounted in water or in cotton blue in lactophenol. The colour of medulla, epithecium, hypothecium and ascus were recorded. The shape and size of the asci, ascospores and conidia were measured in the sections mounted in water. The measurement of the thallus, medulla, epithecium, hymenium were generally taken in the sections mounted in cotton blue. The thallus size was measured in centimeter, lobe size and ascocarps in millimeter and thallus, medulla, epithecium, hymenium thickness asci and ascospores in millimicron. Chemistry of the specimens includes colour tests and thin layer chromatography.
Colour tests

Colour tests has been performed by chemical reagents by applying it on thallus and medulla resulting the changes in colour. A positive change is denoted by a positive symbol (+), followed by the colour produced and no change in colour is denoted by a negative symbol (-) symbol. The chemical reagents used are as follows.

a) K test: 10-25% aqueous solution of potassium hydroxide, applied to cortex, medulla and part of apothecium.

b) C test: A freshly prepared aqueous solution of calcium hypochlorite or bleaching powder or modern commercial bleaching fluid containing active chlorine by dissolving calcium hypochloride in distilled water in 2% ratio.

c) KC test: At a particular spot of thallus, K applied first and immediately followed by C.

d) PD test: Solution of paraphenylenediamine is prepared in ethanol or alcohol in a small quantity for the use of a day. It is unstable and can not be used for the next day. A more stable solution called Steiner’s PD is prepared by dissolving 1.0 gm paraphenylenediamine and 10 gm of sodium sulphite in 100 ml of distilled water with 1.0 ml of a liquid detergent. This reagent keeps well for about a month.

e) I test: 2-5 gm of iodine is dissolved in water with 0.5 gm of potassium iodide. The reagent keeps well for several days and is to be renewed when colour fades.
Other colour tests

A dilute solution of nitric acid and an aqueous solution of ferric chloride are sometime used for identification of Melanelia and Buellia species. The spot test can be done on any part of the thallus but younger parts give better results. Colour test is done to a small fragment of the desired lichen thallus part or thallus or ascocarp. A definite colour comes showing the presence of any lichenic acid.

Micro crystallography

Micro-crystallography was introduced by Asahina (1936). A small fragment of lichen to be investigated was placed on the middle part of a microscopic glass slide and one-two drops of acetone or any other organic solvent were dripped on to the fragment by means of dropper or pipette. Lichen substances if present gets dissolved in the solvent and extracted on the slide as residue in a ring form around the fragment as soon as the solvent evaporates. The thallus fragment was blown off. A micro-cover glass was placed over the residue and a drop of one of the crystallizing fluids (detailed below) was placed at the edge of the cover glass. The fluid gradually seeps in. The slide is then heated gently over a spirit lamp. The residue dissolves in the fluid and lichen substances gradually crystallize into their characteristic shapes on cooling. These crystals are under low power of microscope and identified by comparison with the photographs or line diagram published by Asahina (1950, 1952), Hale (1967)). Identification of depsides, depsidones and dibenzo-furans is usually usually confirmed by this method. The crystallizing fluids used were:
a) G.E. Glycerol: acetic acid, 1:3


Chromatography

Earlier in chromatography, paper was used for spotting the lichen substances. Use of paper has been substituted by thin layer chromatographic plates. Glass sheets were either used in the laboratory by coating with silica gel or precoated aluminium plates are purchased from the market for this purpose. Chromatographic plates are prepared in the laboratory by taking ordinary smooth glass plates of 20x20 cm size. They are thoroughly cleaned by keeping them in glass tank, dried in a low temperature in (ca. 30-35°C) oven. About 9-10 ml of silica gel paste is spread over glass plates into a uniform thin layer. After the silica gel paste has set the plates are dried at 35°C and can be left at that temperature till they are needed for chromatographic purposes. Generally Parmelinella wallichiana is used as a control for atranorin and salazinic acid (Rf class7and 2 respectively) and Usnea baileyi or Pyxine philippina for norstictic acid (Rf class 4) have been used when silica gel plate has been fully spotted with the desired number of extracts. It is placed in a jar, internally lined by filter paper and containing a specific solvent, level of which is about 1.0 cm below the spotting places of the lichen extracts. The solvent gradually rises up in the silica gel coating and is allowed to rise up to 14 cm mark. The plate is taken out dried
in air and observed under ultra violet lamp; any fluorescence observed is mark or noted. For spotting the different fatty acids, distilled water is sprayed on the plates and spots are marked with pencil. A 10% aqueous solution of sulphuric acid is finally sprayed over the coated surface of the plate which is then placed in oven at a temperature of $110^\circ\text{C}$ for about 5-15 minutes or until the different coloured spots at different levels become clear. The plate is then taken out, allowed to cool. The colour of the spots their position for each extract are noted, and again observed under ultra violet light and finally Rf value are calculated. Identification of lichen substance is made on the basis of the position and colour of the spots by comparison with the charts published by Culberson (1972), Walker and James (1980), White and James (1985).

The Rf value is calculated by using the formula:

$$Rf \text{ value} = \frac{\text{Distance travelled by lichen substance (indicated by spot)}}{\text{Distance travelled by solvent (solvent form)}}$$

The following three solvent systems usually used for the chromatography are:

Solvent A or BDA- Benzene: 1,4 dioxane: acetic acid: 90ml: 25ml: 4ml.


Solvent C or TA- Toluene: acetic acid: 85ml: 15ml.

The most common solvent system used for chromatography is:

T.O.A – Toluene 180 ml, dioxane 60 ml: 8-acetic acid.
Fluorescence analysis

The presence of certain lichen substances was also indicated when the lichen thallus containing them was exposed and observed under 254nm or 366nm UV light.

Ecological methods

The relative importance of organisms in a community is not determined by its taxonomic position but rather by the number, size and other relationships. The degree of importance of a species is usually expressed by an index of dominance. Community analysis may be carried out in any given location on the basis of distinct zones or gradients that may exits in that area. Generally, the steeper the environmental gradient, the more distinct are the communities since sharp boundaries are formed by the abrupt changes in the physical nature of the environment.

The ratio between the numbers of individuals in a community is termed as species diversity. This is related to the stability of the environment and it varies with different communities. Species diversity is of great importance in assessing the extent of damage done to natural systems by human interference.

Species diversity may be taken to denote the number of species in a given area or as the number of species among the total number of individuals of all species present. This relationship may be expressed as diversity index. The number of species in a community is important ecologically since the species diversity seems to increase as the community becomes more stable. Several disturbances
cause a marked decline in the diversity. A great diversity also indicates the availability of a large number of niches.

Study of species

With the aid of sampling techniques like transect, quadrat, bisect, etc. the organization and structure of communities can be studied and expressed and quantitatively, both in absolute terms of species and with respect to all other plants species of the area. The important parameters for describing community structure in precise quantitative terms are as follows.

Frequency (%)

This term refers to the degree of dispersion of individual species in an area and is usually expressed in terms of percentage occurrence. It can be defined as the chance or probability of an individual of a given species to be present in a randomly placed quadrate. This can be studied by sampling the study area at several places at random, or in a desired pattern to cover the site adequately, and recording the names of species that occur in each sampling.

The formula to analyze frequency is:

\[
\text{Frequency (\%)} = \frac{\text{Number of quadrates in which a species occurs}}{\text{Total number of quadrates sampled}} \times 100
\]

A species most abundantly spread all over the area will have chance of occurring in all the samplings and therefore, its frequency will be hundred percent. A poorly spread even with large number of species in one corner will have a chance
of occurrence in only few quadrates and its frequency value will be low. Thus, a higher frequency value shows greater uniformity of its spread or dispersion.

Frequency of a species may also be studied in terms of its dispersion relative to that of all the rest of the species. Relative frequency is determined by the use of following formula using the data obtained by the quadrate method:

\[
\text{Relative frequency (R.F.)} = \frac{\text{Frequency of the individual species}}{\text{Total frequency of all the species}} \times 100
\]

The relative values are more useful than absolute ones in computing the ecological importance of individual species in community of plants.

**Density**

Density is also an expression of the numerical strength of a species where the total number of individuals of each species is divided

\[
\text{Density} = \frac{\text{Total number of individuals in all the quadrates}}{\text{Total number of quadrates studied}}
\]

Relative density is the study of numerical strength of a species in relation to total number of individuals of all species and can be calculated as:

\[
\text{Relative Density} = \frac{\text{Number of species in an area}}{\text{Total number of Individuals}} \times 100
\]
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Abundance

Abundance is an appreciation of the relative number of individuals of each species entering into constitution of the plant population of the territory under study, but thus obtained in quantitative terms gives little idea of the distribution of the species. It is calculated as:

\[
\text{Abundance} = \frac{\text{Total number of the individuals of the species}}{\text{Total number of quadrate used in sampling}}
\]

Important Value Index (IVI)

As it is not possible to measure the basal area of every lichen specimen encountered in the quadrate technically therefore, the IVI (Important Value Index) cannot be given but the sum of relative frequency and relative density and Relative abundance is provided.

Alpha diversity

Species richness measures have great intuitive appeal and avoid many of the pitfalls which can be encountered when models and indices are employed. Species richness measures provide an instantly comprehensible expression of diversity. Species richness, as a measure of diversity, has been used successfully in many studies (Abbott, 1974; Connor and Simberloff, 1978; Harris, 1984).

A number of simple indices have been derived using some combination of S (the number of species recorded) and N (the total number of individuals summed
overall species). These include A) Margalef’s diversity index (Clifford and Styephenson, 1975).

\[ D_{mg} = R1 = (S-1)/\ln N \]

B) Menhinick’s index (Whittaker, 1977)

\[ D_{Mn} = R2 = S/\sqrt{N} \]

Simpson Index (D)

Simpson (1949) gave the probability of any two individuals drawn at random from an infinitely large community belonging to different species as:

\[ D = \sum p_i^2 \]

Where \( p_i \) is the proportion of individuals in the ith species. In order to calculate the index, the form appropriate to a finite community is used

\[ D = \sum \left( \frac{n_i(n_i-1)}{N(N-1)} \right) \]

Where \( n_i \) is the number of individuals in the ith species and \( N \) is the total number of individuals

Shannon-Weiner Index

Shannon and Wiener independently derived the function which has become known as Shannon index of Diversity. It is sometimes incorrectly referred to as Shannon Weaver index (Krebs, 1985). The Shannon index assumes
that individuals are randomly sampled from an infinitely large population (Pielou, 1975). The index also assumes that all species are represented in the sample. It is calculated from the equation

\[ H' = - \sum p_i \ln p_i \]

The quantity \( p_i \) is the proportion of individual found in the \( i \)th species

**Evenness indices**

When all the species in a sample are equally abundant, it seems intuitive that an evenness index should be maximum and decreased toward Zero as the relative abundance to the species divert away from evenness.

Evenness index (E1): Hurlbert (1971) proposed the most common evenness index

\[ E_1 = H'/\ln (S) \]

Evenness index (E2): Sheldon (1969) proposed an exponential form as

\[ E_2 = e^{H'/S} \]

Evenness index (E3): Heip (1974) proposed the following formula

\[ E_3 = (e^{H'} - 1)/S-1 \]

**Beta diversity**

The term \( \beta \) diversity was coined by Whittaker (1960, 1977). \( \beta \) diversity is essentially a measure of how different (or similar) a range of habitats or samples are in terms of the variety of species found in them. One purpose of \( \beta \) diversity
measures is to ascertain the degree of turnover in species composition along a
gradient or transect. A further method of estimating $\beta$ diversity employs
similarity measures. This technique looks at the similarity of pairs of sites, either
in terms of species presences and absences

$$\beta \text{ diversity} = \frac{\text{No. of species in site A only} + \text{No. of species in site B only}}{S}$$

Jaccard measure $\beta J = \frac{S}{(A+B-S)}$

Where $S =$ No. of species found in both sites.

Sorenson measure: This measure is similar to the Jaccard index and uses identical
variables

$$\beta S = 2S (A+B)$$

Substrate Analysis

Water holding capacity (WHC)

To measure the Water holding capacity, bark samples of similar sizes (triplicate)
for each tree were oven dried at $105^\circ C$ for 24 hrs., weighed. Samples were again
weighed after removal of excessive water with paper towel.

Water Holding Capacity was expressed as,

$$\text{WHC} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$
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pH:

The outermost layer of bark pieces were removed from the surface of the bark (0.5 g) on tree using a knife or a chisel. Amount of sample placed in 10 ml. beaker with 5 ml. of distilled water, and sealed to prevent Carbondi-oxide contamination. The samples were left for four hours with occasional shaking. The mixture was then filtered and the pH measured with Digital pH meter.

Transplant:

During survey and collection of lichens in the district, most of the localities in the area are devoid of trees and lichens. In such areas where lichens exhibit their absence, the transplant technique was employed for determining the levels of metal accumulation of the area. Five monitoring sites (Premtala, Sadarghat, Sonai road, Hospital road and Shillongpatty) were selected to perform the lichen transplant experiment. These sites are situated in the heart of the district, major business centre, densely populated and having heavy traffic load. The foliose lichen *Dirinaria aegialita* was used for the transplant study. It is one of the most suitable species for transplant as it is widely distributed and most common lichen available in the surrounding areas of the Cachar district, found growing luxuriantly on *Areca catechu*, *Mangifera indica*, *Artocarpus heterophyllous* and other trees growing on the district. Thalli of similar sizes of *Dirinaria aegialita* along with their substratum were collected from an area with low pollution level. The samples were collected from Chengkuri Bagan, 20 km away from the monitoring sites. The untreated samples of *Dirinaria aegialita* were stored in the
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laboratory at room temperature. Five patches of samples were glued along with substrate on cardboard of 20x20cm size and were fixed on exposed pole of two different heights (upper and lower) at all monitoring sites. The exposure duration was for four weeks and first day of the week, a patch of lichen was collected from each monitoring sites to estimate their physiological responses. After an exposure of four weeks the transplant samples for metal estimation was collected. The collected lichen thalli (approx. 4 gm) were carefully removed from the bark with a snapper blade or a knife. The samples were oven dried for 12 hrs to a constant weight at 90°C. The dried lichen samples (3-replicates) were grinded to powdered (1.0 g each) and digested in a mixture of concentrated HNO₃ and HCLO₄ (v/v 9:1) for 1 hr. Residues were filtered through Whatman Filter Paper No. 42 and diluted to 15 ml. with double distilled water. Analysis was done with Flame Atomic Absorption Spectrophotometer (Perkin Elmer, model analyst 300). Stock standards were from Merck India and traceable to NIST (National Institute of Standard Technology). Working standards were prepared from the stock using demonized water for dilution.