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Diversity and distribution of algae in cave ecosystems of Meghalaya: An overview

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Abstract
Meghalaya has a good number of caves and each cave has unique features and habitat, can hold a diverse group of living organisms. The diversity, distribution and chlorophyll a content of algae from seven caves namely Arwah cave, Mawsmai cave, Mawjymbuin cave, Krem Dam cave, Krem Puri cave, Krem Traw cave and Siej Tyra cave of Meghalaya was carried out. In order to study the distributional pattern of algae, study sites of the caves were divided into three zones; entrance area, artificially lighted area and complete dark area. A total of 95 species have been recorded from seven caves spreading over 10 classes. Maximum number of species was recorded from Bacillariophyceae member (39 species) followed by Cyanobacteria (31 species), Chlorophyceae (13 species) and Zygnematophyceae (6 species), whereas only a single species each of Euglenophyceae, Trebouxiophyceae, Chrysophyceae, Klebsormidiophyceae, Xanthophyceae and Eustigmatophyceae was recorded. Entrance area has maximum algal species followed by artificially lighted area and complete dark area. The species diversity and species richness was high in Krem Dam cave. The chlorophyll a content was also high in Krem Dam cave as compared to other selected caves. The present investigation revealed that entrance area and artificially lighted zones harbor a good number of algal species as compared to dark area. It also revealed that Bacillariophyceae (diatom) and Cyanobacteria can survived in extreme environmental conditions.

Keywords: caves, algae, diversity, species richness and chlorophyll a

Introduction
Caves are a complex world, with four major habitat zones based on light penetration and intensity, i.e. entrance, transition, dim light zone and complete dark zone. Caves have a characteristic of having negligibleto no natural light, uniform temperature, high humidity and also in terms of bio-diversity caves have a relatively low species richness, biomass and density. They are also characterized by the formation of stalactite and stalagmite.
Algae are phototropic microorganism which grows well in sufficient photon radiation. They are abundantly distributed in freshwater, sea and terrestrial environment but from cave the growth of algae is restricted. Caves are environment where light is limited or completely dark. It is a place where nutrient concentration in the soil and water are very low which inhibited the growth of many microorganisms like algae, fungi, vertebrate to invertebrate organism. Particularly talking about algae, light and nutrient are limiting factors for colonization and luxuriant growth (Kuehn *et al* 1992; Sanchez *et al* 2002) [1,2]. The installation of artificial illumination inside the cave allowed the colonization of phototrophic alga. Algae found on the entrance of the cave are completely independent of artificial illumination since they have received regular sunlight unlike inside the cave (indoor). Cave is characterize as one of the environment where input of nutrient is very low that is change with the introduction of artificial light energy which lead to a drastic changes affecting the cave flora and fauna (Simon *et al* 2007) [3]. When input of nutrient is increased in the cave the newcomers become more competitive than the originally present organism. As a result the primitive cave dwellers organism become threatened and has a possibility to get extinct (Pipan 2005) [4]. Caves are the centre of biodiversity especially for Cyanobacteria which can grow even in the extreme environment (Joanna and Andrzej 2018) [5].

Study Sites
Meghalaya is located in North Eastern part of India, the state is hilly and blessed with quite a good number of caves, which attract lots of tourist. The state has got chilly winter and dry to wet summer. To document the diversity and distribution of algae in the caves of Meghalaya, seven caves were selected; six caves from East Khasi Hills district (Arwah cave, Mawsmai cave, Mawjymbuin cave, Krem Dam cave, Krem Puri cave, Krem Traw cave) and one cave from West Khasi Hills district (Siej Tyra cave) Fig.1.

1. Arwah cave: This cave is located in khliehshnong area of Cherrapunji at an altitude of 649.22 m asl, with the geographical coordination of latitude of 25º28'884"N and longitude of 91º 72' 636" E. This cave is popular among the tourist and known for its fossils and limestone formation. Stalagmites and stalactites were seen inside the cave and apart from that a stream was also flowing inside the cave. Artificial light were fixed at different point inside the cave.
2. Mawsmai cave: This cave is a limestone cave situated at an altitude of 647.70 m asl, with the geographical coordination of latitude of 25º 24'473" N and longitude of 91º 73'241" E. In this cave a stream was also seen flowing inside and formation of stalagmites and stalactites were observed. Artificial light were fixed at different point for illumination. This cave is also a well-known tourist spot in Meghalaya.
3. Mawjymbuin cave: This cave is located at Mawsynram village with an altitude of 1437.74 m asl, with the

geographical coordination of latitude of 25º29'489" N and longitude of 91º58'490" E. This cave is famous for naturally formed Shivalinga, which is made of stalagmite. The cave is visited by both the tourist and pilgrims. No artificial light were fixed as the cave is quite exposed to natural sunlight.

4. Krem Dam cave: This cave is located at Mawsynram village at an altitude of 1432.25 m asl, with the geographical coordination of latitude of 25º 30'706" N and longitude of 91º 59'257" E. The entrance area is wide and most of the area is filled with water. A stream is also seen entering the cave. No artificial lights were fixed inside the cave.
5. Krem Puri cave: This cave is located near Laitsohum village in Mawsynram, an altitude of 1402.08 m asl, with the geographical coordination of latitude of 25º 27' 890" N and longitude of 91º 55'963" E. It is the world longest sandstone cave which was recently discovered in 2016. The entrance of the cave is narrow and complete dark. There are number of streams inside the cave, some of them had water current with high velocity. Stalagmites and stalactites were also formed in some part of the cave. No artificial light were fixed.
6. Krem Traw cave: This cave is located at Lawbah village in East Khasi Hills, at an altitude of 1447.45 m asl, with the geographical coordination of latitude of 25º 25' 857" N and longitude of 91º 58' 857" E. The cave is dark with narrow cemented entrance. A waterfall was also seen inside the cave. No artificial lights were fixed.
7. Siej Tyra cave: This cave is also known as U Tirot Sing Cave located in West Khasi Hills, Meghalaya at an altitude of 1428.90 m asl, with the geographical coordination of latitude of 25º 70'062" N and longitude of 91º 63' 576" E. No stream was seen flowing inside the cave and no artificial lights were fixed.

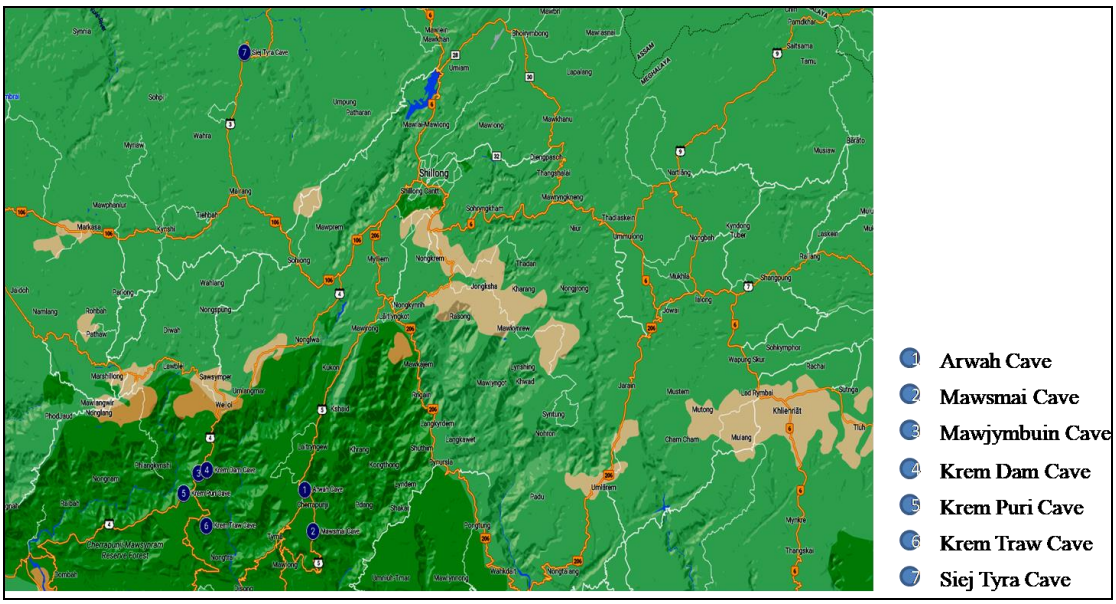

Fig 1: Map of the study sites

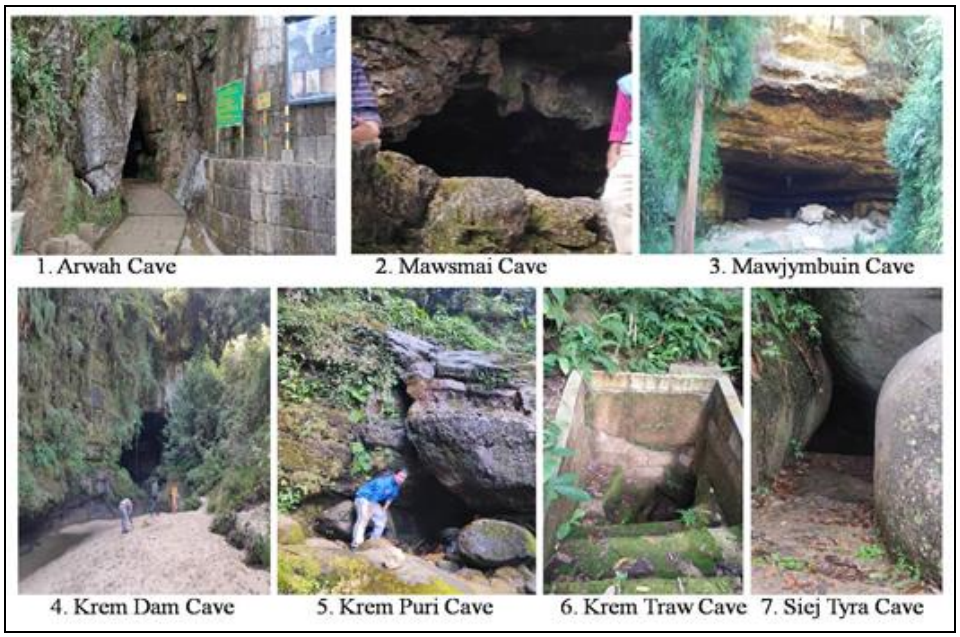

Fig 2: Photographs of the caves selected for study in East Khasi Hills District and West Khasi Hills District of Meghalaya.

Material and Methods

Sample collection and analysis

Collections of the samples from seven caves were done from October 2019 to March 2020 following a standard method (APHA, 2012) [6]. Algal samples were collected from soil, moist area, water, rock wall, stalagmites and stalactites and divided into three zones i.e. entrance area, light zone and dark zone. Samples were collected with the help of scalpel and tooth brush. Taxonomic identification was mainly carried out with the help of Floras and Monographs like Tiffani and Britton, (1952) [7]; Prescott, (1982) [8]; Desikachary, (1985) [9]; Gandhi, (1998) [10]; John *et al*., (2002) [11].

Taxonomy was also updated using the online database Algae Base (GuiryandGuiry, 2019) [12]. Algal crust samples were collected from caves wall, rocks and stones (1cm^2) and transferred to centrifuge tube and kept in ice box under complete darkness and transported to laboratory for Chlorophyll a estimation by using the spectrophotometer method (Strickland and Parson, 1972)[13].

Data analysis

Species diversity Index was calculated by using Shannon-Wiener diversity index following the formula:

$$H' = \sum_{i=1}^{s} Pi \ln Pi$$

Where; s = total number of species.
Pi is ni/N, ln Pi is normal log of Pi
Ni = Number of individuals belonging to the ith species.
N = total number of individual of all the species.
Species richness was calculated as the total number of species present in a given sample.

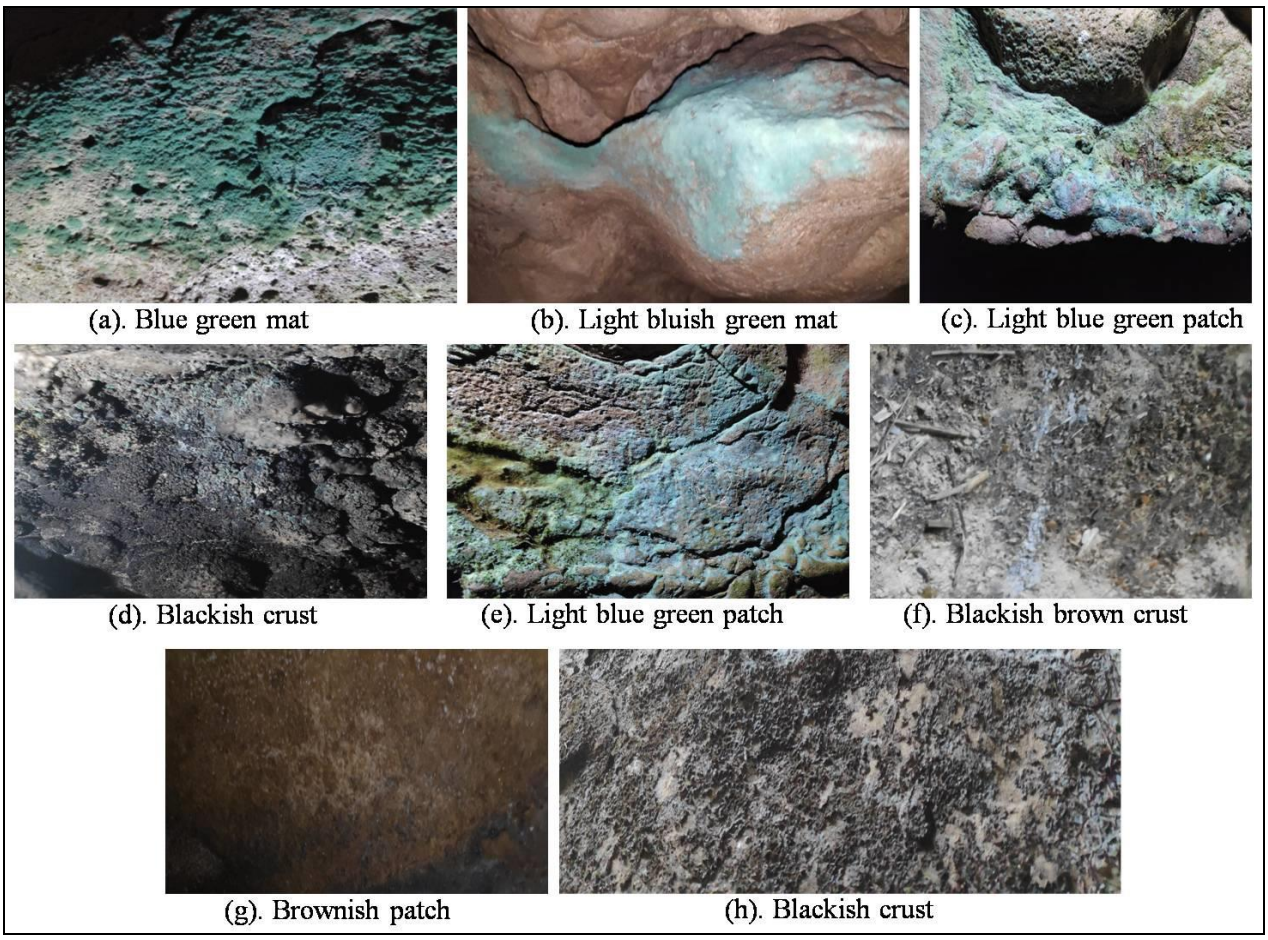

Fig 3: Photographs of biological crust (a) Blue green mate, (b) Light bluish green mat, (c) Light bluish green patch (a to c from Arwah cave), (d) Blackish crust, (e) Light blue green patch (d and e from Mawsmai cave), (f) Blackish brown crust (from Mawjymbuin cave), (g) Brownish patch (from Krem Puri cave) and (h) Blackish crust (from Krem Dam cave).

Results

A total of 95 species have recorded from seven caves spreading over 10 classes (Table 1). Maximum number of species was recorded from Bacillariophyceae (39 species) followed by Cyanobacteria (31 species), Chlorophyceae (13 species) and Zygnematophyceae (6 species), whereas only a single species each of Euglenophyceae, Trebouxiophyceae, Chrysophyceae, Klebsormidiophyceae, Xanthophyceae and Eustigmatophyceae was recorded. A total of 87 species has been recorded from entrance area, 21 species from artificially lighted area and 23 species from complete dark area. Species found only in dark area are *Amphora coffeaeformis*, *Pinnularia mesolepta*, *Pinnularia bicep*s and *Eustigmatos* sp.

Table 1: Distribution of algal flora in seven caves (Arwah cave = C1; Mawsmai cave = C2; Mawjymbuin cave = C3; Krem Dam cave = C4; Krem Puri cave= C5; Krem Traw cave = C6 and Siej Tyra cave = C7) of Meghalaya at three difference zones (Entrance area, Artificially lighted area and Complete dark area; + indicate present; - indicate absent).

Algal taxa	**Entrance area (Natural light)**	**Artificially lighted area**	**Complete dark area**
Bacillariophyceae			
Achnanthes minutissimum Kutzing	+C4	-	-
Achnanthes sp	+ C7	-	-
Amphora coffeaeformis (C.Agardh) Kutzing	-	-	+ C5
Cocconeis placentula Ehrenberg	+C7	-	-
Cyclotella meneghiniana Kutzing	+C7	-	-
Cymbella affinis Kutzing	+C4, C5	-	+ C5
Cymbella cistula (Ehrenberg) Kirchner	+ C4	-	+ C4
Cymbella turgid W.Gregory	-	-	+ C5
Cymbella turgidula Grunow	+C4	-	-
Eunotialunaris (Ehrenberg) Grunow	+C4	-	-
Eunotia minor (Kutzing) Grunow	+C1	-	-
Fragilaria capucina Desmazieres	+C4	-	-
Gomphonema gracile Ehrenberg	-	+C1	+C1
Gomphonema lanceolatum Kutzing	+C2, C6	-	+C2
Gomphonema parvulum (Kutzing) Kutzing	+C1	-	-
Gomphonema sphaerophorum Ehrenberg	+C1	-	-
Gyrosigma scalproides (Rabenhorst) Cleve	+C4	-	-
Hantzschia amphioxys (Ehrenberg) Grunow	+C5	-	+C5
Melosira varians C.Agardh	-	+C2	+C2
Navicula capitatoradiata H.Germain ex Gasse	+C4	-	+ C4
Navicula cryptocephala Kuetzing	-	-	+C2
Navicula gracilis Ehrenberg	+ C2, C3, C6	+C2	+C2
Navicula lanceolata Ehrenberg	+ C1, C4, C5	-	-
Navicula salinarum Grunow	+C6	-	+C6
Navicula sp	+ C2, C7	-	-
Neidium hitchcockii (Ehrenberg) Cleve	+C1	-	-
Nitzschia acicularis (Kuetzing) W.Smith	+C1	+C1	+C1
Nitzschia amphibian Grunow	+ C1	-	+C1
Nitzschia linearis W. Smith	+C6	-	-
Nitzschia sp	+ C2	-	-
Pinnularia biceps W.Gregory	-	-	+C4
Pinnularia braunii Cleve, nom.illeg	+ C3	-	-
Pinnularia brebissonii (Kutzing) Rabenhorst	+ C4	-	-
Pinnularia interrupta W.Smith	+ C3	-	-
Pinnularia mesolepta (Ehrenberg) W.Smith	-	-	+C5
Pinnularia sp	+ C5	-	-
Surirella elegans Ehrenberg	+C4	-	-
Surirella robusta Ehrenberg	+C4	-	-
Synedra sp	+C3	-	-
Zygnematophyceae			
Closterium dianae Ehrenberg ex Ralfs	+C4	-	-
Closterium leibleinii Kutzing ex Ralfs	+C4	-	-
Cosmarium connatum Brebisson ex Ralfs	+C4	-	-
Hyalotheca sp	+C5	-	-
Pleurotaenium ehrenbergii (Ralfs) De Bary	+C4	-	-
Staurastrum connatum (P.Lundell) J.Roy and Bisset	+C4	-	-
Chlorophyceae			
Ankistrodesmus falcatus (Corda) Ralfs	+C4	-	-
Ankistrodesmus spiralis (W.B.Turner)	+ C5	-	-
Chlamydomonas reinhardtii P.A. Dangeard	+ C4	-	-
Chlorococcum sp	+C2	-	-
Chlorococcum humicola (Nageli) Rabenhorst	+C3	-	+ C3
Coelastrum astroideum De Notaris	+ C4	-	-
Gloeocystis sp	+C3	-	-
Golenkinia radiate Chodat	+ C1	-	-
Pediastrum duplex Meyen	+C1, C4	+C1	-
Scenedesmus abundans (Kirchner) Chodat	+C2	-	-
Scenedesmus acuminatus (Lagerheim) Chodat	+ C4	-	-
Scenedesmus acutus Meyen	+C2	-	-
Scenedesmus obliquus (Turpin) Kutzing	+C1, C4	-	-
Cyanobacteria			
Anabaena constricta (Szafer) Geitler	+C1	+C1	-

Anabaena spiroides Klebahn	+ C1,C4	+ C1	+C4
Anabaena subcylindrica Borge	-	+C2	-
Aphanocapsa annulata G.B.McGregor	+C3	-	-
Aphanothece sp	+ C3	-	-
Calothrix sp	+ C5	+C2	-
Chroococcus sp	+ C4	-	-
Gloeocapsa punctata Nageli	+ C4	-	-
Hapalosiphon sp	+ C3	+C1	-
Leptolyngbya sp 1	+C1, C3	+ C1	-
Leptolyngbya sp 2	+ C1, C2	+ C1	+C1
Lyngbya limnetica	+ C3	+C2	
Lyngbya sp 1	+ C7	-	-
Lyngbya sp 2	+C2	+C2	-
Microcoleus sp	+C3	-	-
*Nostoc commune*Vaucher ex Bornet and Flahault	+C5	-	-
Nostoc linckiaf. muscorum (C.Agardh ex Bornet and Flahaut) Elenkin	+ C1	+C1	-
Nostoc sp 1	+ C6	-	-
Nostoc sp 2	+C4	+	
Oscillatoria limosa C.Agardh ex Gomont	+C3, C4	-	+ C4
Oscillatoria curviceps C.Agardh exGomont	+C1, C6	+C1	-
Oscillatoria prince Vaucher ex Gomont	+ C1	-	-
Oscillatoria sp 1	+C6	-	-
Oscillatoria sp 2	+C6, C7	-	-
Phormidium retzii Kutzing ex Gomont	+C2, C5	+ C2	-
Phormidium sp 1	+C3	-	+ C3
Phormidium sp 2	+C4	+	-
Phormidium tenue Gomont	+C3, C6	-	+ C3
Sytonema sp 1	+C1	+C1	-
Sytonema sp 2	+C2	+ C2	-
Tolypothrix sp	+ C1	+ C1	-
Trebouxiophyceae			
Chlorella vulgaris Beyerinck (Beijerinck)	+C1, C5	+C1	-
Euglenophyceae			
Lepocinclis playfairiana (Deflandre) Deflandre	+C4	-	-
Chrysophyceae			
Mallomonas sp	+ C3	-	-
Eustigmatophyceae			
Eustigmatos sp	-	-	+ C5
Klebsormidiophyceae			
Klebsormidium sp	+C1	-	-
Xanthophyceae			
Tribonema minus (Wille) Hazen	+C4	-	-

The algal community structure varied among the caves (Table 2). Algal community structure of Krem Dam cave was contributed by Bacillariophyceae (13 species), followed by Chlorophyceae and Cyanobacteria (6 species each), Zygnematophyceae (5 species), Euglenophyceae and Xanthophyceae (1 species each). In Arwah cave, the algal Community structure was contributed by Cyanobacteria (9 species) followed by Bacillariophyceae (8 species), Chlorophyceae (3 species), Trebouxiophyceae and Klebsormidiophyceae (1 species each).

The algal community structure in Mawsmai cave was contributed by Cyanobacteria (6 species) followed by Bacillariophyceae (5 species each) and Chlorophyceae (3 species). In Mawjymbuin cave the algal community structure was contributed by Cyanobacteria (9 species), Bacillariophyceae (4 species), Chlorophyceae (2 species) and Chrysophyceae (1 species). In Krem Puri cave the algal community structure was mostly contributed by Bacillariophyceae (7 species), Cyanobacteria (3 species), Chlorophyceae, Zygnematophyceae, Eustigmatophyceae and Trebouxiophyceae (1 species each). In Krem Traw cave the algal community structure was contributed by Bacillariophyceae and Cyanobacteria (4 species each). In Siej Tyra cave the algal community structure was contributed by Bacillariophyceae (4 species) and Cyanobacteria (2 species).

Among the seven caves, members of Bacillariophyceae, Chlorophyceae and Zygnematophyceae were maximum in Krem Dam cave.

Cyanobacteria was maximum in Arwah cave and Mawjymbuin cave. Euglenophyceae and Xanthophyceae were recorded only from Krem Dam cave. A member of Eustigmatiophyceae was recorded only from Krem Puri cave. Members belonging to Chrysophyceae and Klebsormidiophyceae were recorded only from Mawjymbuin cave and Arwah cave respectively.

Table 2: Composition of algal species belonging to different algal classes collected from the seven caves.

Study sites	Arwah cave	Mawsmai cave	Mawjymbuin cave	Krem Dam cave	Krem Puri cave	Krem Traw cave	Siej Tyra cave
Chlorophyceae	3	3	2	6	1		
Bacillariophyceae	8	5	4	13	7	4	4
Zygnematophyceae				5	1		
Cyanobacteria	9	6	9	6	3	4	2
Euglenophyceae				1			
Eustigmatiophyceae					1		
Trebouxiophyceae	1				1		
Xanthophyceae				1			
Chrysophyceae			1				
Klebsormidiophyceae	1						

Among the seven cave, maximum species diversity and species richness was recorded from Krem Dam cave with 2.8 and 32 respectively and minimum species diversity and species richness was recorded from Siej Tyra cave with 0.36 and 6 respectively (Fig.1 and Fig. 2.).

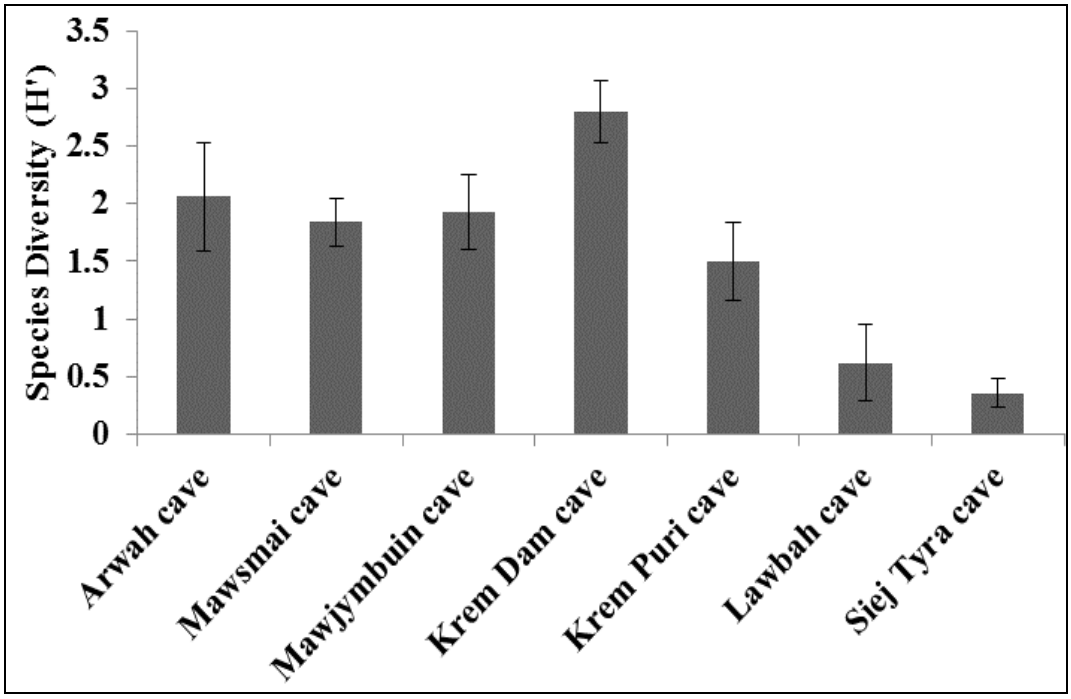

Fig 4: Species diversity of algae from seven caves of Meghalaya

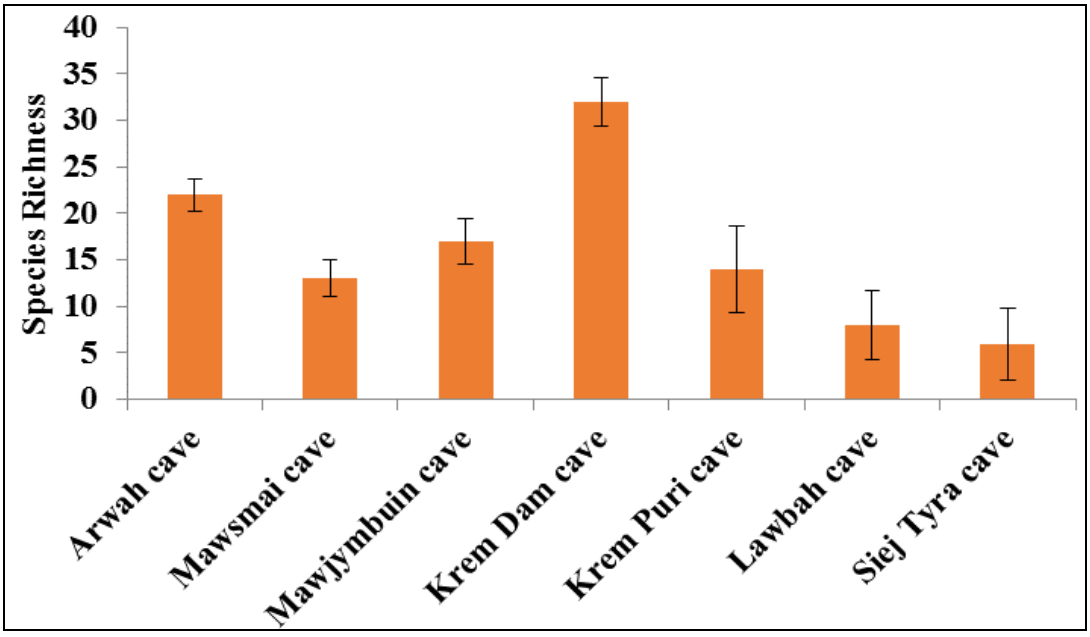

Fig 2: Species Richness of algae from seven caves of Meghalaya

The Chlorophyll a content was varied among the caves (Fig.3) and found higher in Krem Dam cave (0.0087 mg/cm^2) followed by Mawjymbuin Cave (0.0058 mg/cm^2), Mawsmai cave (0.0041 mg/cm^2), Arwah Cave (0.0032 mg/cm^2), Krem Puri Cave (0.0025 mg/cm^2), Krem Traw Cave (0.0016 mg/cm^2) and Siej Tyra Cave (0.0012 mg/cm^2).

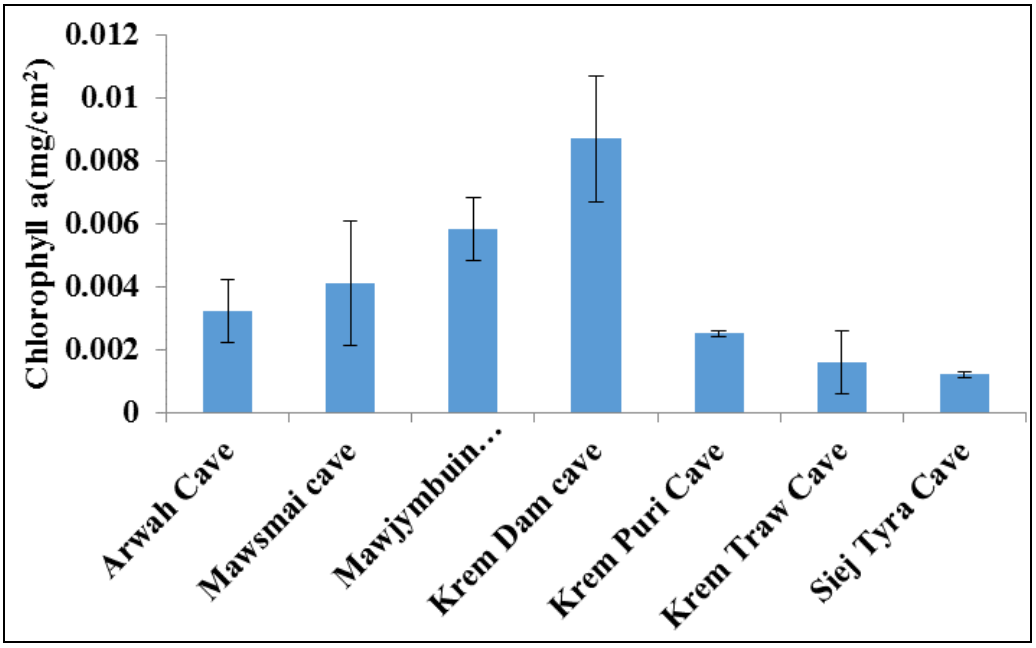

Fig 3: Chlorophyll a content from the seven caves of Meghalaya

Discussion

In our present investigation, algae grow abundantly in the illuminated areas like entrance of the cave and areas where artificial lights were fixed, however algal growth was very scanty in the dark areas. This could be contributed to the fact that the distribution of algae in the cave primarily depend on the availability of light (Mulec *et al* 2008) [14] and optimum temperature. Bacillariophyceae (diatom) and Cyanobacteria was dominant algal flora in the cave as compared to the other classes of algae, which could be due to their ability to grow and colonized in the harsh environment with minimum light availability. According to Vinogradova *et al* (1998) [15] Cyanobacteria could grow well in the cave entrance area specially the deepest part of the entrance where other algal community were unable to colonized. Diversity of algae was also rich in entrance area than that in the dark area. The algal composition change with shift in the photon radiation level when approaching in the deepest interior part of the cave (Mulec and Kosi 2008) [16], we have observed the similar pattern in the present investigation. The patch of cyanobacteria was found in the artificially lighted area. Diatom and very few cyanobacteria were found in dark area of the caves. This could be due to the adaptability of these algal communities (Bacillariophyceae and Cyanobacteria) in stress condition (No light).

Among the seven caves species diversity and species richness was higher in Krem Dam cave which could be because of wider entrance area. The stream entering into the cave received maximum sunlight which makes a suitable environment for the algae to grow. The large entrance area in Krem dam cave and Mawjymbuin cave possibly able to Received a good amount of organic materials from the surrounding which act as a food source and therefore entrance area can facilitated the colonization of algae. Van Vuuren *et al* (2019) [17], reported maximum species richness in lighted and twilighted zone and low species richness in dark zone. According to Round (1981)[18], the distribution of algae can be differentiated depending on the access to either natural or artificial light. The microclimate in cave is affected by air circulation, hydrological conditions and cave isolation from the outside thermal influences (Martinez and Asencio 2010) [19]. In our present investigation we notice that those algae (*Amphora coffeaeformis, Cymbella affinis, Cymbella cistula, Cymbella turgid, Gomphonema gracile, Gomphonema lanceolatum, Hantzschia amphioxys, Melosira varians, Navicula capitatoradiata, Navicula cryptocephala, Navicula gracilis, Navicula salinarum, Nitzschia acicularis, Nitzschia amphibian, Pinnularia biceps, Pinnularia mesolepta, Chlorococcum humicola, Anabaena spiroides, Leptolyngbya* sp 2, *Oscillatoria limosa, Phormidium* sp 1, *Phormidium tenue* and *Eustigmatos* sp) recorded in the dark area may have potential to deal with extreme environmental conditions (no sunlight and constant temperature) and evolve to survive and grow with very less energy.

Chlorophyll a content was high in Krem Dam cave possible reason could be the cave is not disturbed and present of diverse algal taxa particularly in the dark and brown patches on cave wall of entrance area as well as availability of light and microclimatic condition of the cave influence the higher Chlorophyll a content in the Krem Dam cave. Low Chlorophyll a content was recorded in other caves. The algal structural and functional attribute of the cave system is greatly affected by the human activities like construction of pathways and illumination of light for the comfort of the tourist which led to the degradation of its natural habitat. Similar result were also reported by Lamprinou *et al* (2014) [20]; Saiz-jimenez *et al* (2012) [21] and Elliot (2006) [22] where disturbance by human activities resulted in change of the oligotrophic state of the cave to mesotrophic state altering both the food web, the abundance and its (algal) distribution in the cave.

Conclusion

The diversity and species richness was high in Krem Dam cave as compared to the other caves. The distribution of algae was high in entrance area and followed by artificially lighted area and dark area. The natural light received by the entrance area plays a significant role in supporting diverse group of algae. The cave ecosystem is greatly affected by the human activities like construction of pathways and illumination of light which led to the degradation of its natural habitat and hence change the algal composition of the caves.

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