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SHORT COMMUNICATION

Differential Aerosolization of Algal and Cyanobacterial Particles in the Atmosphere

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Abstract Aeroalgal sampling at short height (2.5 m) over natural aquatic and terrestrial algal sources revealed that despite of being similar in size (<1 mm), algal groups vary in their atmospheric abundance. Cyanobacteria were the most abundant, while chlorophytes and bacillariophytes though present, but rare. Statistical analysis (Akaike Information Criterion) showed that climatic factors (temperature, relative humidity, rainfall, wind velocity and sunshine hours) acted in concert, and mainly affected the release and subsequent vertical movement (aerosolization) of algae from natural sources. Variation in aerosolization may affect the atmospheric abundance of algae. These findings have important implication as dispersal limitation may influence the biogeography and biodiversity of microbial algae.

Keywords Abundance · Aerosolization · AIC · Biogeography · Climatic factors · Dispersal limitation · Microalgae

Algae are an ancient group of oxygen producing photosynthetic organisms with vast morphological diversity. They constitute the basic component of food chain in the world's ecosystems contributing about 40% of global photosynthesis, and 50% of oxygen; we inhale [1]. Use of algae as a food supplement has a long history. In recent times, exploitation of microalgae as a source of wide range of structurally novel and biologically active compounds has attracted considerable interest [2].

Algae have been reported from almost all biotopes; and are mainly dispersed passively via water, air and organisms. Pedrós-Alió [3] related dispersal distance with population abundance and dispersivity. Species with abundant population may reach far extent compared to their rare counterparts. Despite of its immense ecological significance aerial dispersal of algae is least studied [4–6]. Also, measurement of aerial dispersal of microbes is a tough task especially, when no standard guideline is available. A successful aerial dispersal depends on the distance traveled and tolerance of algae to the stresses such as desiccation, UV radiation and atmospheric pollutants. Aerial dispersal is a complex phenomenon subject to various physical and biological factors and, it is almost impossible to assign a maximal distance in case of aerial dispersal [5]. Even, the most remote areas such as Kerguelen and other Antarctic islands have flora similar to that of the mainland and Arctic islands [5].

According to the 'ubiquitous dispersal' theory of microbes because of small body size all microbes are essentially cosmopolitan in their distribution [7]. However, recent use of molecular techniques for biodiversity analysis have revealed that microbes vary in their spatial distribution and some, if not all show endemic distribution [8, 9]. The only question is that of taxonomic unit at which endemism occurs [10]. Biodiversity of the community can be measured both at morpho- and geno-typic level; and generally, genotypic diversity exceeds to that of morphospecies diversity [8]. Role of allopatry (i.e., geographical barriers preventing gene flow) in microbial speciation is a highly debated issue [11]. Finlay [7] and many believes that because of their size microbes are dispersed globally

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and, not subjected to allopatric speciation. However, in case of microalgae, Telford et al. [12] reported that dispersal limitation does occur and decides spatial distribution of microalgae at morphospecies level.

Compared to moulds and bacteria, algae are less frequent in air [13], and different algal groups vary in their atmospheric abundance. Cyanophytes dominate in tropics whereas chlorophytes in temperate atmosphere [5]. Earlier, Sharma et al. [14, 15] reported a strong correlation between aeroalgal diversity and climatic factors. Here, we analyze the effect of climatic factors on abundance of algal particles in the atmosphere. We hypothesize that among different stages of aeroalgal pathway (Fig. 1), the process of aerosolization (release from the source and vertical up-lift) is crucial in deciding the atmospheric abundance of algae. And, different set of factors controls the aerosolization of different algal groups. Since, direct estimation of algal abundance over two surfaces (i.e., soil–air and water–air) has technological limitations. Therefore, statistical tools were used to assess the impact of different climatic factors on aerosolization process.

Aerobiological sampling was performed weekly for a period of 24 months (March 2003 to February 2005) at three sites in Varanasi city (24°43′ to 25°35′ N latitude and 82°11′ to 83° E longitudes): Gymkhana ground, Institute of Technology, Banaras Hindu University, Lanka and Durgakund. Sites differ in the extent of exposure, vegetation cover, algal source, distance from the source, and degree of pollution [15]. Aeroalgal samples were taken at short height (2.5 m) using Tilak Rotorod samplers and scattered plate technique. For detail about sampling procedure see Sharma et al. [14, 15]. After sampling, samplers and exposed petri plates were brought to the laboratory. Impacted tapes stucked over the samplers' arms were removed, cut into small pieces and placed face down on new petri plates containing 20 ml solid BBM. Both

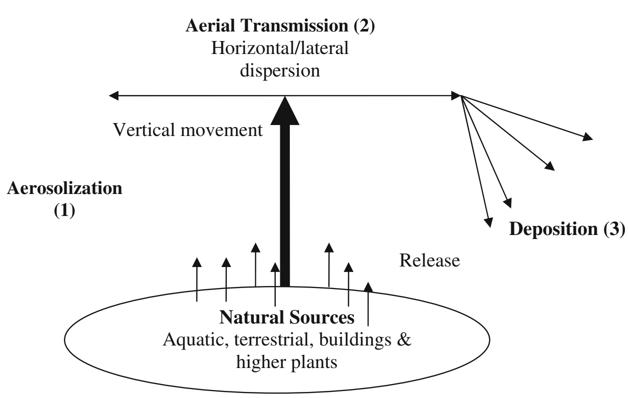

Fig. 1 Schematic presentation of the steps involved in aerial movement of algae (aeroalgal pathway)

exposed and tape-containing petri plates were incubated under standard growth conditions (temperature 28 ± 1°C, average light intensity of 70 μE $m^{-2}s^{-1}$, 14:10 L:D).

After 3–4 weeks of incubation, algal colonies developed onto the culture plates were counted using light and compound binocular microscope with magnification of 600×. Identification of algal taxa was made according to the monographs mentioned in Sharma et al. [14]. A total of 72 plates per month were counted; numbers of colonies were summed up to get total number of colony for the particular

Table 1 Thallus structures and size of algae recovered from the atmosphere of Varanasi City, India

Organisms	Thallus organization	Body/cell size; in μ
Blue–green algae		
Chroococcus sp.	Unicellular/colonial	4–11 broad
Aphanocapsa spp.	Unicellular/colonial	4–6 diameter
Gloeocapsa spp.	Unicellular/colonial	5–8 diameter
Gloeothece sp.	Colonial	25–41 diameter
Synechocystis spp.	Unicellular	5–6 diameter
Synechococcus spp.	Unicellular	>5 broad
Microcystis sp.	Colonial	3–7 diameter
Merismopedia sp.	Tubular colony	3.5–10 broad
Spirulina sp.	Trichomes spirally coiled	2–22 broad
Oscillatoria sp.	Trichome	2–29 broad
Phormidium spp.	Trichome	1–12 broad
Lyngbya spp.	Filamentous	1–24 broad
Microcoleus sp.	Trichomes in bundle	2–10 broad
Cylindrospermum spp.	Trichome	4–5 broad
Nostoc spp.	Trichome	2.2–5 broad
Anabaena spp.	Trichome	2.5–12 broad
Plectonema spp.	Filamentous	2–10 broad
Scytonema spp.	Filamentous	3–36 broad
Tolypothrix spp.	Filamentous	4.2–35.7 broad
Microchaete spp.	Filamentous	4.5–20 broad
Calothrix spp.	Filamentous	4–12.5 broad
Haplosiphon spp.	Filamentous	3–24 broad
Green algae		
Chlorococcum sp.	Unicellular	2–20 diameter
Chlorella sp.	Unicellular	5–10 diameter
Closterium sp.	Unicellular	6–32 broad
Selenastrum sp.	Unicells/colonial	2–15 broad
Stichococcus spp.	Colonial; cells in chain	3–4 diameter
Scenedesmus sp.	Colonial	3.5–7 broad
Hormidium sp.	Filamentous	5–15 broad
Oedogonium sp.	Filamentous	8–45 broad
Diatoms		
Navicula sp.	Unicellular	25–40 broad
Nitzschia sp.	Unicellular	20–30 broad
Pinnularia sp.	Unicellular	15–30 broad

month. To measure aerosolization efficiency, monthly average colony number per plate was calculated by dividing the total number of colonies for specific month with the total number of plates. Multiple stepwise regression analysis between predictor variables (climatic factors) and response variables (monthly colony counts for total algae, blue-green, green algae and diatoms) did not provide any conclusion. Therefore, Akaike information criterion (AIC) was used to get the most parsimonious model [16]. A set of eight ($R = 8$) linear regression models were developed using temperature (T), humidity (Rh), rainfall (Rf), wind velocity (Wv) and sunshine (Ss) as variables.

A total of 33 algal genera consisting mainly of cyanophytes have been reported. Other forms include chlorophytes and diatoms (Table 1). Aeroalgal abundance showed seasonal variation characterized by fluctuation in two major algal groups i.e., cyanophytes (also known blue-green algae) and chlorophytes. June was the most favorable month for the total algal counts (980 and 1004) as well as blue–green colonies (882 and 910). Contribution of green algae increased during the cold months of the year (Nov–Jan) with the highest number recorded during December 2003 (285) and January 2005 (316), respectively. Diatoms, with maximum colony count in the month of October 2003; remained rare with more or less uniform presence in the atmosphere (Table 2).

A positive correlation was found between total algal colonies and total number of genera (generic diversity) ($r^2 = 0.64$). Except during February and July (2004), where total number of genera increased despite a decrease in the total number of colonies. While during October (2003 and 2004), increase in total colony number was not proportionate with that of the increase in total number of genera (Fig. 2a). In case of cyanobacteria, correlation was positive ($r^2 = 0.78$) except for October 2003 and 2004 (Fig. 2b). In general, r^2 value (0.70) was positive for green algae (exp. June, August, November 2003; June, July, September 2004 and January 2005). In November (2004), increase in the number of green algal colonies was much sharp compared to that of total number of genera (Fig. 2c). For diatoms ($r^2 = +0.57$), a negative correlation was observed in June (2003), July (2004) and February (2005) (Fig. 2d).

Seasonal variation in the atmospheric abundance of algal particles could be attributed to the fluctuations in the meteorological factors of the area. However, no definite

Table 2 Sum total and the monthly average number of colonies plate^{-1} for total and individual algal groups

Month and year	Total algae		Cyanophyta		Chlorophyta		Bacillariophyta	
	C_T	C_A	C_T	C_A	C_T	C_A	C_T	C_A
March 2003	360	5.0	320	4.4	32	–	8	–
April 2003	384	5.3	362	5.0	14	–	8	–
May 2003	432	6.0	388	5.4	30	–	14	–
June 2003	980	13.6	882	12..3	88	1.2	10	–
July 2003	768	10.7	584	8.1	169	2.3	15	–
August 2003	672	9.3	538	7.4	121	1.6	13	–
September 2003	600	8.3	432	6.0	156	2.1	12	–
October 2003	614	8.5	313	4.3	276	3.8	25	–
November 2003	528	7.3	254	3.5	270	3.8	4	–
December 2003	528	7.3	238	3.3	285	3.8	5	–
January 2004	456	6.3	190	2.6	260	3.6	6	–
February 2004	408	5.6	221	3.1	178	2.5	9	–
March 2004	370	5.1	250	3.5	112	1.5	8	–
April 2004	410	5.6	324	4.5	78	1.0	8	–
May 2004	456	6.3	382	5.3	60	–	14	–
June 2004	1004	13..9	910	12.6	81	1.1	13	–
July 2004	780	10.8	716	10.0	52	–	12	–
August 2004	682	9.4	620	8.6	53	–	9	–
September 2004	607	8.4	556	7.7	42	–	9	–
October 2004	602	8.4	503	7.0	93	1.3	6	–
November 2004	540	7.5	245	3.4	290	4.0	5	–
December 2004	466	6.4	158	2.1	302	4.2	6	–
January 2005	410	5.6	86	1.2	316	4.3	8	–
February 2005	355	4.9	170	2.4	178	2.4	7	–

C_T total number of colony, C_A monthly average number of colonies plate^{-1}

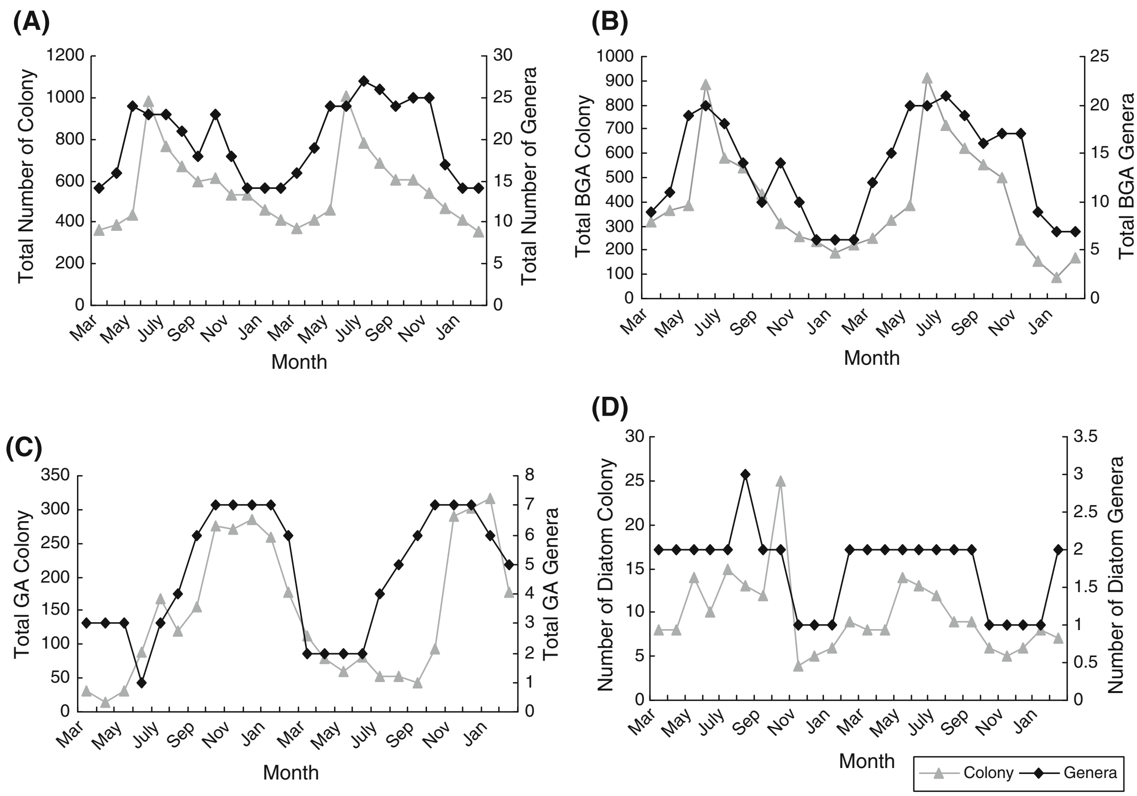

Fig. 2 Relationship between number of genera and abundance for total (**a**), blue–green (**b**), green algae (**c**) and diatoms (**d**) during different months of the sampling period (March 2003–February 2005)

conclusion emerged from multiple stepwise regression analysis between individual climatic factor and abundance of total and individual algal group. Factor statistically significant one year was not in other year (exp., humidity for blue–green algae and wind velocity for diatoms). Consequently, Akike information criterion (AIC) was used to select the most parsimonious model. Results indicate that among whole set of candidates ($R = 8$), model two ($ID = 2$) is 69 and 72% more likely to be the best model for total and blue–green algae. Likewise, it was model six ($ID = 6$) for green algae (35%) and five ($ID = 5$) for diatoms (28%). Values of AIC and its associated measures are given in Table 3. Aerosolization ability of different algal group varied. For entire sampling period the average algal colony count per plate was 7.6 (range 4.9–13.9). For cyanophytes, the value was 5.5 colonies plate^{-1} (range 1.2–12.6) and ranged 0–4.3 for green algae. The value was negligible for diatoms (Table 2).

Results indicate that atmospheric abundance of cyanophytes depends upon concerted effort of temperature, wind velocity, sunshine and relative humidity. Since, cyanophytes contributed in bulk to the total atmospheric algal load, the same factors appeared to be important for the overall abundance of algae in the atmosphere. Incase of other algal forms, besides temperature and wind velocity, relative humidity (for green algae) and sunshine (for diatoms) were found to be important. Though belonging to same size range i.e., <1 mm (Table 1) different algal groups (BGA, green–algae and diatoms) varied in their atmospheric abundance, acted upon by different climatic factors. In general, high temperature, extended sunshine and high wind velocity favors the suspension of algal particles in the atmosphere. A possible reason for the dominance of terrestrial algae in the atmosphere could be their prior acclimatization to dryness. As soil algae can withstand the atmospheric hazards better than their fragile aquatic counterparts [17]. Moreover, aquatic algae are hard to get entrained in the atmosphere compared to that of terrestrial algae [15].

The precise reason(s) for the dominance of cyanobacteria in the air is not well known. Generally, cyanobacteria have broad ecological amplitude and survive well in fluctuating environmental conditions like that of tropical regions. While, chlorophytes are abundant in temperate atmosphere [6]. Rarity of diatoms is surprising since, they possess hard silica covering conferring an additional advantage to tolerate the vagaries of atmosphere. Atmospheric presence of algae depends on various interacting and interdependent factors such as community dynamics and physiological state of the source vegetation i.e., algae

Table 3 AIC and its associated measures for total and individual algal groups

Model ID	Model	Residual sum of square	AIC	Δ AIC	Akaike weight (w_i)	Evidence ratio
	Total airborne algae					
1	T, Rh, Rf, Wv, Ss	188012.0	225.19	1.75	0.29	w_1/w_2 0.29/0.69 = 0.42
2	T, Wv, Ss, Rh	189982.6	223. 44	0	0. 69	
3	T, Wv, Rf, Ss	480393.9	245.70	22.26	1.02	
4	T, Wv, Rf, Rh	290935.5	233.67	10.23	0.00	
5	T, Wv, Ss	545449.8	246.75	23.31	6.06	
6	T, Wv, Rh	316198.9	233.67	10.23	0.00	
7	T, Wv, Rf	494611.3	244.40	20.96	1.96	
8	T, Wv	549256.1	244.92	21.48	1.52	
Blue–green algae						w_1/w_2 0.23/0.72 = 0.32
1	T, Rh, Rf, Wv, Ss	203635.5	227.10	1.97	0.23	
2	T, Wv, Ss, Rh	203847.8	225. 13	0	0.72	
3	T, Wv, Rf, Ss	408588.1	241.82	16.67	0.00	
4	T, Wv, Rf, Rh	326829.9	236.46	11.33	0.00	
5	T, Wv, Ss	465954.2	242.97	17.84	9.67	
6	T, Wv, Rh	345843.1	235.82	10.69	0.00	
7	T, Wv, Rf	444428.6	241.84	16.71	0.00	
8	T, Wv	467887.7	241.07	15.94	0.00	
Rest i.e., green algae + diatoms						w_3/w_6 0.05/0.35 = 0.14
1	T, Rh, Rf, Wv, Ss	75926.13	203.43	3.58	0.06	
2	T, Wv, Ss, Rh	76815.40	201.71	1.86	0.14	
3	T, Wv, Rf, Ss	83671.28	203.76	3.91	0.05	
4	T, Wv, Rf, Rh	76836.58	201.71	1.86	0.14	
5	T, Wv, Ss	83913.03	201.83	1.98	0.13	
6	T, Wv, Rh	77279.95	199. 85	0	0.35	
7	T, Wv, Rf	88582.36	203.13	3.28	0.07	
8	T, Wv	95078.50	202.83	2.97	0.08	
Green algae						w_3/w_6 0.05/0.35 = 0.14
1	T, Rh, Rf, Wv, Ss	72320.57	202.26	3.66	0.06	
2	T, Wv, Ss, Rh	73036.00	200.45	1.90	0.13	
3	T, Wv, Rf, Ss	79668.37	202.59	3.99	0.05	
4	T, Wv, Rf, Rh	72955.85	200.47	1.87	0.14	
5	T, Wv, Ss	79984.68	200.68	2.08	0.12	
6	T, Wv, Rh	73337.48	198. 60	0	0.35	
7	T, Wv, Rf	83727.68	201.78	3.18	0.07	
8	T, Wv	89824.84	201.47	2.87	0.08	
Diatoms						w_{1-4}/w_5 0.06/0.28 = 0.21
1	T, Rh, Rf, Wv, Ss	295.227	70.23	3.18	0.06	
2	T, Wv, Ss, Rh	304.671	68.99	1.94	0.10	
3	T, Wv, Rf, Ss	300.458	68.65	1.60	0.12	
4	T, Wv, Rf, Rh	319.916	70.16	3.11	0.06	
5	T, Wv, Ss	305.461	67. 05	0	0. 28	
6	T, Wv, Rh	322.230	68.33	1.28	0.15	
7	T, Wv, Rf	340.989	69.69	2.64	0.07	
8	T, Wv	347.311	68.13	1.08	0.16	

from old and dry substrates can easily be entrained into the atmosphere than that from young and wet substrates (Fig. 1).

Release of algae from substrate into the air is a passive process [18], and algae show an initial inactivation during the process of aerosolization. For long-term survival in the air inactivation should be minimum [17], which in turn is related to the physiological state of the cell or organism and relative humidity of the environment. Cyanophytes have broad humidity range compared to that of eukaryotic microbial algae [17]. However, why certain algae have broad humidity tolerance is not clear. In addition, due to hygroscopic mucilaginous covering aerosolized cyanophytes particles might have absorbed atmospheric moisture, became heavy and dropped down onto the exposed culture plates with increased settling velocity. Resulting in high cyanophytes counts. Jenkins et al. [19] opined that for long distance dispersal size is important for active, but not for passive dispersers.

Though preliminary in nature results clearly indicate that size is not necessarily linked to the ubiquitous dispersal of microalgae. As, despite of being similar in size range algal groups differ in their atmospheric abundance. Modeling the aeroalgal pathway especially in the vicinity of the source may redress two important questions: how for will they travel and what concentration. Knowledge of dispersal and biogeography (i.e., geographical distribution) is key to understand the diversity and evolution of organisms.

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