



Trade Science Inc.

Environmental Science

An Indian Journal

Current Research Papers

ESAIJ, 5(6), 2010 [378-395]

Airborne algal bio-particulates in Jharia coalfields: Prevalence, characterization and their allergen city

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Received: 28th March, 2010 ; Accepted: 7th April, 2010

ABSTRACT

Abiotic and biotic particulates constitute the airspora of the coalfield region, which, singly and/or in association with fine coal dust particles, after coming in contact with human beings cause different kinds of allergic ailments. Among different bio-allergens found in coalfield environments, algae, with different compositions and in varying concentrations, have also their share for causing various allergic disorders in humans. Notwithstanding the prevalence of different types of algae in the atmosphere of Jharia Coalfields owing to the most favorable climatic conditions (rainfall, temperature, Relative Humidity, light) and also the occurrence of allergic diseases in the coalfields, surprisingly no work has been done on the coalmining and coal-based industries' area-specific bio-allergens, of which algae constitute an important allergenic constituent. Reported for the first time in the present paper are the results of our investigation on the prevalence, characterization, and seasonal variation of coal mining area-specific airborne algal flora present in the environments of Jharia coalfields. In all 13 coalfield-specific algae have been found and identified in the present study, which are prevalent in the atmosphere of Jharia Coalfields. They include : *Anabaena*, *Chlorella*, *Chlorococcum*, *Fragilaria*, *Gloeocapsa*, *Lyngbya*, *Navicula*, *Nostoc*, *Oedogonium*, *Oscillatoria*, *Phormidium*, *Plectonema* and *Spirogyra*. They are both unicellular and colonial, and diatoms, belonging to class *Chlorophyceae* and *Cyanophyceae* respectively. The results have evinced that the concentration of the algae varies with the variation in meteorological parameters (temperature, rainfall, relative humidity, light), and that the algal load (population) is subject to seasonal variation, being totally absent during summer (April, May), moderate during the months of December to March and maximum during rainy season (August to October). Two major factors, viz., physiological ability of an algal group to tolerate different types of abiotic stresses, and the climatic conditions prevailing in the area., seem to be responsible for regulating the structure of the aeroalgal community in the atmosphere of any specific area-coalfields in the

KEYWORDS

Airborne bio-allergens;
Algae;
Unicellular;
Diatoms;
Chlorophyceae;
Cyanophyceae allergenicity;
Allergic diseases;
Jharia coalfields.

present case, While most of these algae are inhalant types, some are both inhalants and contactants: (*Anabaena*, *Fragilaria*, *Lyngbya*, *Phormidium*) Almost all the diatoms have allergen city, with potential for causing allergic health hazards manifested by different skin, respiratory and other allergic disorders in humans living in and around coal-mining and coal-based industries' areas.. The inhalant algae are potent allergens, responsible for causing hazardous allergic ailments like hay fever, nasal blockage, bronchial asthma, allergic rhinitis, eczema, persistent dermatitis and other skin diseases. The necessity for systematic identification of the particular metabolite (s) from the suspected allergic disease-causing algae and their correlation with the disease through clinical investigations has been emphasised.

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INTRODUCTION

During coal mining operations, not only severe degradation of land takes place but also the ecology and environment of the area is badly disturbed, invariably simultaneously accompanied by release of dust and also abiotic particulate matter (SPM, RSPM, SO_x, and NO_x) and biotic particulates due to disturbance of flora and fauna thriving in the mined and nearby areas. According to an estimate^[1], the coal dust generated through all mining operations include : top soil removal (69.9kg/day), overburden removal (666.0kg/day), extraction of coal (365.9kg/day), size reduction of mined coal (6812.5kg/day), thus making the cumulative mining generated dust to be ca. 6812.6kg/per day. In addition to this, the amount of wind-generated dust is to the tune of 1568kg/day, depending upon the wind velocity, direction, and other micro-meteorological conditions. This cumulatively generated huge quantity of dust is not all dispersed into the atmosphere, rather the larger particles of the dust settle down and get deposited on the soil, while the small and micro-sized particles become suspended in the atmosphere along with other abiotic and biotic particulates. Thus, the atmospheric bio-particulates - alone, and in combination with fine coal dust particles, constitute the airspora of the coalfield areas. Some of the constituents of this airspora, after coming in contact with human beings, cause different allergic manifestations. A variety of bio-allergens are prevalent in coal-mining and coal-based industries' area environments, including pollen grains, fungi, algae, and house dust mites, which are of different compositions and varying concentrations. These coalfield-specific bio allergens more particularly algae have not yet been prop-

erly identified and studied.

Defined botanically, algae are thalloid prokaryotic (Cyanophycene and Prochlorophycene) and eukaryotic (Rhodophycene, Cryptophycene, Dinophycene, Heterokontophytes, Hepto-phycoaceae, Euglenophyceae, and Chlorophytes) photosynthetic organisms with chlorophyll-a and other photosynthetic pigments-releasing O₂. The body of these microscopic or near-microscopic, simple autotrophic algae, being of widespread occurrence, is unicellular, colonial, filamentous, siphonous, and parenchymatous, never with roots, stems or leaves. Their sex organs being unicellular or multicellular with all cells fertile (except Charales), these algae typically possess inherent potentialities for their distribution through the medium of atmosphere. While some algae are beneficial (e.g single cell green algae, which, apart from being endowed with such properties as detoxification, and stimulating friendly bacteria in the body, contains green photosynthetic pigment chlorophyll and chloroplast, is a potent source of food and its supplements), some other genera are potentially allergenic, causing allergic diseases in humans. It is now widely recognized that single-celled algae in a persistent resting stage or fragments of filamentous forms or even fragments of more colonial forms are usually transported in dry state by wind to different sites where they not only become airborne but also actively grow under congenial conditions of moisture, temperature and light. The role of algae in air pollution and effect of meteorological conditions on air borne algae was reported by Smith in 1973^[2], who also suggested that soil algae should also be considered along with airborne algae as the former is most likely to be disseminated by both wind currents and thermal uplifts. It was further reported that yet an-

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other source of viable algae could be house dust, mostly from contamination of indoor environment by soil and/or airborne algae. Some of these airborne algae are allergenic and, upon inhalation, can and often do, cause allergic disorders of different sorts. While the possible role of airborne algae as a potent cause of allergy and allergic respiratory diseases was suggested by Salisbury as early as in 1866^[3], the symptoms of hay fever was correlated with algae^[4]. In several other investigations^[5-9], the role of airborne algae in allergy has been thoroughly studied. In yet another systematic study, Holland et al.^[10], have reported the presence of more than 40 genera of algae present even in house dust samples in viable conditions. These researchers have ascertained the distinct possibility that some of the chemical constituents of some genera of algae are indeed responsible for causing allergenic reactions and, in turn, different allergic diseases. Through their investigations on the origin of airborne micro-algae, many researchers have identified different possible sources including dust, possible adherence to moss spores, raindrop splash on soil, airborne foam, and water through bursting of bubbles or subaerial surfaces^[11-12]. In yet another investigation, Bernstein and Safferman^[12] found that even the indoor aquaria could be possible pathways for dispersion of many algae, which finding was subsequently confirmed by Schlichting^[13], who also proposed a conceptual model for the study of airborne algae as related to micrometeorological conditions and air chemistry. Through a classical and interesting study by Overeem^[14a,b] in as early as 1936-1937, which involved sampling of airborne algae by using aircraft over Netherlands at different heights (400, 500, 1000, 2000 meters) during the months of July and August for which an aeroscope and Beltger bubbler were employed, she clearly showed the abundant prevalence of several algae. Her work is still considered a valuable contribution in Phycology, as it has provided an insight into the invasion, by algae both in quality and quantity, of even the troposphere. Though sporadic, yet a good deal of work on different types of algal spora, including diatom algae, prevalent in the atmosphere has been done during the past 20-25 years by many investigators^[15-21]. In a recent study, Gantar and Svircev^[22] have investigated and discussed the importance, usefulness, and possible toxicity (aller-

gen city) of some micro-algae and cyanobacteria. In yet other recent investigations, Sharma et al.^[23a,b,24] have not only studied the diversity and seasonal variation of viable aero-algal particles in the atmosphere of a subtropical city in India but have also compared the allergenicity of airborne cyan bacteria *Phoridium fragile* and the alga *Nostoc muscorium*. Of late, the traditional morphological-based methods of taxonomy of algae in general are being considered in a state of flux in that they sometimes lead to misidentification of different algae, as later was revealed when modern molecular techniques were employed^[25,26]. In a latest investigation on the taxonomy and bioactivity of an Egyptian *Chlorococcum* isolate, El Semary et al.^[27] have applied a polyphasic approach, which essentially involved the combination of both morphological and molecular methods coupled with phylogenetic analysis using small subunit rDNA as a marker, so as to get a clear-cut diagnostic phenotypic characters and to accurately place the isolate in its correct taxonomic position. These investigators have highlighted the importance of combining traditional classification methods with modern taxonomic techniques such as phylogenetics and chemotaxonomy and its significance in exploring the bioactive metabolites and their antimicrobial activity of microorganisms for futuristic biotechnological applications.

A careful scan of published up-to-date literature demystifies that despite having considerable ecological and economic significance, and sometimes also their possible adverse impact on human health manifested by allergic skin and respiratory diseases, airborne algae study has, somehow, not received much attention of the aerobiology and phycology researchers as it deserves and still remains rather scantily studied. Although during the past 2-3 decades sporadic investigations in different parts of the world have come into light. Notwithstanding this, little or practically no work has been done on the occurrence and/or prevalence of algal bio-particulates in the coalmining and coal based industries' areas. Since airborne algae of different types, compositions and varying concentrations, are, *inter alia*, also likely to be and indeed they are, the persistent constituent of the atmosphere of tropical/sub-tropical countries-India in particular and especially in the environments of its coal-mining and coal-based industries' ar-

As a sequel to our previous communication on the prevalence of allergic diseases caused by coalfield-specific house dust mite bio-allergens in Jharia Coalfields^[31], the results of investigations on the prevalence, characterization and possible allergenicity of airborne algae in the environments of coal-mining and coal-based industries' areas of Jharia coalfield in Dhanbad district (Jharkhand, India) are reported, for the first time, in the present paper.

EXPERIMENTAL

The experimental procedure for studying different aspects of airborne algae entailed essentially the collection of air samples at regular intervals from different pre-selected sampling sites in Jharia coalfields, examination of the algal filament samples, isolation, culturing, incubation, identification and morphological characterization of the Airborne Algae. For ascertaining the seasonal variation in the concentration of algae and preparation of a monthwise calendar for a period of two years (2006-2007 and 2007-2008). These procedures are individually described briefly below under different subsections.

Selection of sampling sites

The algae samples (algal filaments) were collected from North Tisra and Lodna collieries, CFRI colony (situated in the close proximity of Bhowrah colliery and coke plant), and Sindri (situated in the adjoining area of Fertiliser factory and ACC Cement Factory), all of which typically belong to the coal mining and coal-based industries' areas of Jharia Coalfields.

Collection of algae samples

Samples of airborne algae filaments were fortnightly collected from all the above mentioned sampling sites, for which Volumetric Burkard Air Sampler (Burkard Manufacturing Co., U.K.), shown in Fig.1, was used.

The special feature of this personal sampler, especially designed for short-term sampling directly onto glass plates, is that it can be used both in domestic or industrial environments, specially also where no power supplies are available. The sampler's sampling orifice is mounted in the vertical plane and operates at an air through put of 10 liters per minute.

Operation of burkard air sampler and analysis of samples

The glass slide (size 3" × 1"), smeared with a suitable adhesive, viz., glycerin jelly in the form of a uniform thin film, was inserted into the aperture in such a way that the adhesive slide was in upwards direction. The sampling chamber was then closed by rotating the upper ring in either direction at least one inch, after which the sampler was ready for collection of samples. After switching on the apparatus, its turbine fan starts moving and in a very short time achieves an acceleration of 10 l/min. and the exhausted air will move out through the side holes in the walls of the sampler. The sampling of the airborne particulate matter was done for 5-10 minutes depending on the air pollution load of the sampling area. At the end of the selected sampling period, the slide was removed by rotating the upper ring with a suitable spatula, after which the slide was ejected from the opposite side of the upper housing. Thus, though this procedure, the airborne bio-particulate matter of the particular area gets deposited on the glass slide. In this manner, glass slides of algal filaments from different sampling sites were collected and analysed.

Examination of the algal filament samples

After sampling and preparation of the glass slides of bio-particulates (*vide supra*), each slide was scanned under 10x optical lens and computer-controlled Trinocular Research Microscope (Nikon, Japan make), shown in figure 2, for qualitative and quantitative estimation of the algal flora. The counts were expressed as number per m³ and the observed total counts were divided by a conversion factor of 0.05.

Isolation and culturing of airborne algae

Airborne algae were isolated by the culturing method, for which two different media, viz., Bold's Basal, and Allen and Amon's media, are generally used. In the present study, the Bold's Basal Medium

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(BBM) was used, the method of preparation of which is adequately described by Tilak^[32]. This medium, for culturing the airborne algae, had the following chemical composition:

Chemical and their amount per litre

NaNO₃ (0.25g), CaCl₂.2H₂O (0.25g), MgSO₄.7H₂O (0.75g), K₂HPO₄ (0.75g), KH₂PO₄ (0.175g), NaCl (0.026g).

Micro element solution 1.0ml, and each of the following three trace element solutions

- 1 EDTA: 50g dissolved in 1 litre distilled water
- 2 KOH: 31g dissolved in 1 litre distilled water
- 3 FeSO₄.7H₂O: 4.98g dissolved in acidified water (1.0ml of H₂SO₄ dissolved in 999ml distilled water)

Chemical and their amount (dissolved in 1litre distilled water)

ZnSO₄.7H₂O (8.82g), MnCl₃.4H₂O (1.44g), MoO₃ (0.71g), CuSO₄.5H₂O (1.57g), CO(NO₃).6H₂O (0.49g).

The pH of the medium thus prepared (commonly referred to as Bold's Basal Medium or in short BBM) was adjusted to 7.3 before autoclaving. Alternatively, solid medium, when required, can also be prepared by adding 2% w/v of agar into liquid medium before autoclaving.

For the isolation of different algal forms, a loopful of algal growth (*vide infra*) from the culture flask was taken and dispersed in 1 ml distilled water in a test tube, after which this algae-containing suspension was streaked on the agar surface in petriplates. The advantage of this method is that the single cells or groups of cells are left behind as the inoculation needle moves, such that the discrete colonies are obtained on the agar surface. Then, the isolated colonies are picked up and transferred to agar slants. To avoid any external bacterial contamination, this entire procedure was conducted in an inoculation chamber pre-sterilised by ultraviolet light.

Incubation and culturing of airborne algae

Exposed petriplates were incubated in a culture room on glass plates fitted on iron racks. The whole system was well illuminated from beneath with 40 w fluorescent tube lights kept a distance of 6cm from the

glass plate. In this way, a light intensity of 1000 to 1500lux was uniformly provided at the surface of the glass plates. Constantly maintaining the temperature between 26 and 30°C, the liquid cultures were thoroughly shaken at least twice a day.

Isolation and identification of airborne algae

The exposed plates were incubated for 2 to 3 weeks, till the algal growth became clearly visible as green colonies, after which observations were made. Next, the growth of algae from the culture flasks and isolated colonies from the petriplates were carefully picked up with the help of inoculation needle.

Examination of the algal forms was done after mounting the algal material of the glass slide and examining the same under 10x optical lens of the computer-controlled Trinocular Research Microscope (Nikon, Japan make). Different algal forms were identified primarily on the basis of their morphological characters. Morphological identification was performed using light microscopy and aided by following the identification keys prescribed in detail by Prescott^[33].

Preparation of algae calendar

At all the selected sites, concentration of airborne algae was regularly recorded twice (fortnightly) every month consecutively for two years (2006-07 and 2007-08) and a month-wise calendar was prepared for each year. Monthly per cent contribution of algae in relation to meteorological parameters, viz., temperature (maximum and minimum), rainfall, and relative humidity (RF) was also calculated and recorded. In this way, each algal calendar thus prepared, showing the prevalence and seasonal variation of algae in the environments of Jharia coalfields, helped in ascertaining the seasonal variation of algae concentration and also in establishing a correlation between algae concentration and meteorological conditions like temperature, rainfall, and relative humidity.

RESULTS AND DISCUSSION

Characterisation of algal flora

The results on the identification and characterisation of airborne algae prevalent in the coal-mining and coal-based industries' area of Jharia coalfields are presented in figure 3(a), 3(b), 3(c) and 3(d). The charac-

TABLE 1 : Characteristics of the identified airborne algae in coal-mining and coal-based industries' areas of Jharia coalfields

Sl. No.	Name of algae	Color	Form of thalus	Main pigment size	Type
1	<i>Anabaena</i>	Blue green	Simple, unbranched with heterocysts	Chlorophyll-a, C-Phycocyanin, C-phycoerythrin	Inhalant cotactant
2	<i>Chlorella</i>	Green	uni-cellular, non- mobile forms	Chlorophyll a, b along with carotenes and xanthophylls	Inhalant
3	<i>Chlorococcum</i>	Green	mostly unicellular	Chlorophyll a,b along with carotenes and xanthophylls	Inhalant
4	<i>Fragilaria</i>	Blue	unicellular	Chlorophyll a,c along with carotenes and xanthophylls	Inhalant
5	<i>Gleocapsa</i>	Blue green	Multicellular, colonial form	Chlorophyll a,c, and C-phycoyanin, C-phycoerythrin	Inhalant
6	<i>Lyngbya</i>	Blue green	simple, filamentous, form without hetero- cysts	- do -	Inhalant contactant
7	<i>Navicula</i>	Brown	Unicellular	Chlorophyll a, c Carotenoids, and xanthophylls	Inhalant;
8	<i>Nostoc</i>	Blue green	simple, unbranched filament with heterocysts	Chlorophyll a, C-phycoyanin, and C-phycoerythrin	Inhalant
9	<i>Oedogonium</i>	Green	green, multi-cellular, llamentous, parietal reticulate, unbranched	Chlorophyll a,b	Inhalant
10	<i>Oscillatoria</i>	Blue green	simple, filamentous form without hetero- cysts	Chlorophyll a, c; C-phycoyanin and C-phycoerythrin	Inhalant
11	<i>Phormidium</i>	Blue green	simple, filamentous form without hetero- cysts	- do -	Contactant.
12	<i>Plectonema</i>	Blue green	hetero trichous filament with false branching	- do -	Inhalent
13	<i>Spirogyra</i>	Green	multi-cellular, filamentous, unbranched	Chlorophyll a, b	Inhalent

teristics of the identified each individual algae are included in TABLE 1. Further, from the month-wise data collected on the concentration of the identified algae, their year-wise concentrations for consecutive 2 years (viz. from April, 2006 to March, 2007 and April, 2007 to March, 2008 in each month) were regularly assessed, in order to understand the monthly prevalence of the algal flora in Jharia coalfields. The results are shown in the form of histograms in figure 4. Likewise, an attempt was also made to correlate the month-wise prevalence and % concentration of the identified algae in Jharia coalfields with those of meteorological parameters (temperature, rainfall, and relative humidity) during the above period of 2 years (2006-07 and 2007-08) and the results are included in TABLE 2 and 3 respectively. From the data on month-wise concentrations of the identified algae, an algae calendar was also prepared and the same is depicted in TABLE 4, from which the presence of algae during any particular period or month of the year can be readily made out.

From the pictures of the algae (Cf. Figure 3(a), 3(b), 3(c) and 3(d)), it is seen that in all 13 algal species, prevalent in the coal mining and coal-based industries' areas of Jharia Coalfields, have been identified and they include: *Anabaena*, *Chlorella*, *Chlorococcum*, *Frgila-*

ria, *Gleocapsa*, *Lyngbya*, *Navicula*, *Nostoc*, *Oedogonium*, *Oscillatoria*, *Phormidium*, *Plectonema* and *Spirogyra*.

Characterised by wide differences in their morphology, composition of pigments, metabolic products, and details of their life histories, algae, in general, are considered lowly evolved plants with simple thaloid plant body and are autotrophic (i.e. photosynthetic or holophytic, i.e. which feed like photoautotrophic) and O₂ evolving, chlorophyll-bearing unicellular or sometimes multi-cellular green plants. Their importance can be simply realized from the fact that out of total O₂ evolved from green plants in the biosphere, ca. 90% comes from these simple plants-algae! Characteristics of each of the 13 coalfield specific identified algae in this study are briefly described below.

Spirogyra

Derived from the Greek word *Spirogyra* (spira = coil + gyros = twisted), this alga, belonging to the family Zygnemaceae and class Chlorophyceae and being the most abundantly occurring, aquatic, bright green colored, free-floating silky masses and sobriqueted as *pond-silk*, *water-silk*, and even *water-scum*, includes about 300 species, of which ca. 94 are found in India, growing in stagnant fresh water ponds, lakes, and even

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TABLE 2 : Month- wise % contribution of algae vis-à-vis meteorological parameters in Jharia coalfields during the period April, 2006-March, 2007

2007-08	Monthly % contribution	Maximum temp. (°C)	Minimum temp. (°C)	Rainfall in mm	Average RH (%)
April	1.0	34.3	30.10	0.0	33.34
May	0.0	45.62	34.53	90.0	25.55
June	4.3	37.42	30.12	151.0	58.23
July	27.4	34.85	26.45	213.1	59.63
August	22.9	34.4	25.14	260.0	60.54
September	16.8	33.59	24.64	129.7	62.68
October	11.6	32.49	23.32	112.2	59.37
November	7.5	25.53	20.38	0.0	47.41
December	4.0	21.06	11.25	28.2	45.79
January	3.7	20.73	10.11	52.3	42.74
February	2.4	22.46	14.14	27.1	45.65
March	1.0	24.2	22.6	21.7	54.32

slow-flowing streams, though a few rare species are also known to grow on moist soil as well. The plant body of these algae is simple, long, and filamentous, the filaments being green, hair like, silky, multi-cellular, unbranched and is comprised of uniseriate row of cylindrical cells, which are usually longer than breadth and arranged one above the other. The growth of filaments takes place by division in any cell. Insofar as the cell structure of these algae is concerned, each cell of a filament is composed of a small amount of protoplast bounded by the cell wall. Characterized by the most prominently distinguishing feature of the cells of this algae by its being elongated, parietal, and ribbon-shaped, its green spiral or helical bands of chloroplasts are twisted anti clockwise direction from one end to the other. The protoplast is differentiated from plasma membrane, thin layer of cytoplasm, single nucleus, one or more chloroplasts with pyrenoids and a large central vacuole bounded by tonoplast. Another characteristic feature of this alga is that the number of chloroplast varies in different species, from one in some species to as many as fifteen in each cell in other species. Yet another distinguishing feature of this green alga is that the chloroplast can either be narrow with smooth margin or broad with seriated margin, each chloroplast possessing a large number of small and spherical pyrenoids, which are usually located equidistant from one another and are the sites of starch formation. Furthermore, each cell of this alga being uninucleate, its the nucleus can be angular, lenticular or

TABLE 3 : Month- wise % contribution of algae vis-à-vis meteorological parameters in Jharia coalfields during the period the period April, 2007-March, 2008

2008-09	Monthly % contribution	Maximum temp. °C	Minimum temp. °C	Rainfall in mm	Average RH%
April	1.2	36.4	24.86	16.5	35.25
May	0.0	45.9	25.08	38	26.63
June	6.3	36.4	24.17	195	57.67
July	24.5	35.31	20.52	232.5	55.45
August	20.3	36.01	21.76	174.5	63.46
September	18.5	35.5	20.66	38.5	65.22
October	11.2	34.51	23.18	71.5	59.14
November	5.4	25.24	18.72	20	51.74
December	5.8	21.21	12.38	0	45.67
January	5.4	21.32	10.62	0	41.88
February	2.3	21.64	12.97	0	42.67
March	1.3	22.6	17.35	22.5	54.41

flatly cylindrical in shape and remains firmly embedded in the central mass of little cytoplasm. The big central vacuole is filled with tanniferous vesicles containing tannins.

The alga *Spirogyra* reproduces by any of the three methods, viz., vegetative, asexual, and sexual methods. While the vegetative reproduction entation of filaments, the asexual method takes place through Aplanospores, which are non-motile reproductive spores produced singly in the vegetative cells, which have so far been reported in only eight species. In the sexual method of reproduction, although no sex organs are produced in *Spirogyra*, the sexual fusion occurs between the two morphologically similar gametes, called isogametes and the mode of sexual reproduction is called isogamous, with a few exception of species which show physiological anisogamy. The most characteristic reproductive feature of *Spirogyra* is that it occurs in a specific season (spring or late in the growing season) of the year, the sexual reproduction being dependent on temperature, light, humidity, increase in pH and deficiency of nitrogen in the atmosphere, the last factor being particularly important for the initiation of the sexual process.

Oscillatoria

The alga oscillatoria found in present study belongs to *O. annae* species in grows abundantly in dirty stagnant and polluted water, water channels forming blackish blue green mussels. This type of aquatic environ-

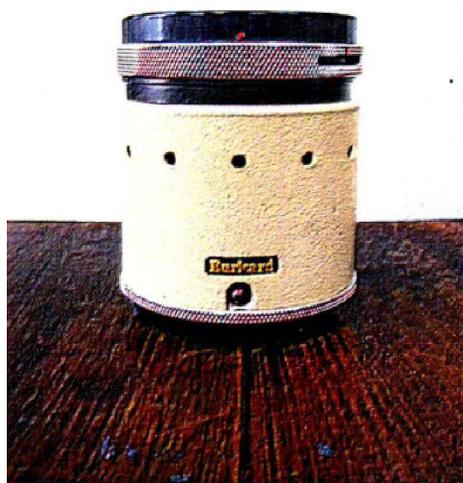


Figure 1 : Burkard volumetric air sampler

ment is typically found near the coalmining sides where water is pumped out from the mines is stored in the form of ponds. In which fine coal dust alongwith other pollutant present in mines water is also accumulated over the tank. This creates a favourable environment for this alga to grow and multiply. The plant body of this alga is filamentous, consisting of long thread like structure called trichomes the latter occurring singly or a large number of them inter woven to form flat stratum or spongy sheets. The cell trichome can be slightly cylindrical or in some cases they found to be as long as broad or longer then broad the internal structure of the cell has an outer same what rigid cell wall not surrounded by mucilaginous sheath, the cell wall enclosing the protoplasm which is differentiated into two regions; the outer coloured chromplasm and central hyaline centropiasm. The typical blue green colour is imparted to this algae due to presence of pigments and located in sac like thalakovids. viz., *C.phycocynin* and *C.phycoerythrin*.

Characteristics of trichomes of *oscillatoria* sp. In general are believed to be responsible for its spontaneous movement which may be gliding (creeping) oscillatory or bending; The oscillatory movement may be most common. Although mechanism of this movement is still mystery, yet some authors attribute the secretion of some kind of geletenous material.

Nostoc

The algae *nostoc* is colonial and filamentous uniseriate and unbranched. The filaments are usually curved, contorted and intertwined, each filament consisting of a large number of spherical cells. A large num-

TABLE 4 : Algae calendar of Jharia coalfields (shaded part of the year indicates the presence of house dust mites during the period)

Algae calendar	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
<i>Gleocapsa</i>												
<i>Lyngbya</i>												
<i>Nostoc</i>												
<i>Oscillatoria</i>												
<i>Phormidium</i>												
<i>Plectonema</i>												
<i>Chlorella</i>												
<i>Chlorococcum</i>												
<i>Anabaena</i>												
<i>Flagilaria</i>												
<i>Navicula</i>												
<i>Oedogonium</i>												
<i>Spirogyra</i>												

ber of filament being embedded within a mucilagenous envelope forming a ball shaped colony. The color of which could be either blue, green is blue green. The characteristic feature of these algae is that the size & shape of algal ball keep on chaning with age (spherical oblong, ellipsoidal are sometimes irregular.) Further more its size ranges from as small as a pin head to as large as the size of hens egg, reaching soon. Yet another characteristic feature of these algae is that its young colonies are solid but with aging and maturity they become hollow as even break open into flat or lobed expanses-paving the way for becoming air borne due to wind velocity or thermal uplift. Loosily joint with each other the vegetative cells (its structures being typically similar to cyanophycean) of the algae are typically spherical oval, cylindrical or barrel shaped.

Each cell has firm and rigid cell wall and the protoplasm is differentiated into outer colored protoplasm and central hyaline centropiasm. The outer protoplasm is colored owing to the presence of chlorophyll-a, carotene, xethophills and phycobilins, *C-phycocyanin* and *C-phycoerythrin*. While the photosynthetic pigments are located in sac like lamellae called thylakoid. The reserve food material occurs in the form of cynophycean starch. Belonging to the blue green algae family the algae *nostoc* is considered most important and responsible for fixation of atmospheric nitrogen viz conversion of atmospheric nitrogen into ammonia.

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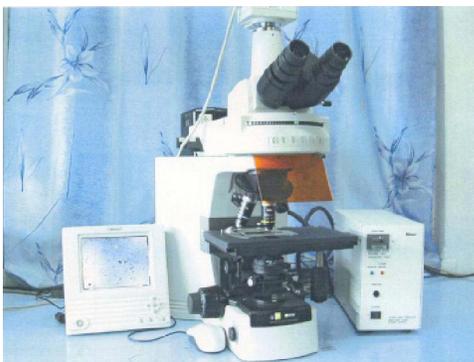


Figure 2 : A view of tri-nomial microscope (Make: Nikon, Japan) used for examination and identification of house desk mites

Navicula

The algae *Navicula* belongs to the botanical division Bacillariophyta and class bacillariophyceae the class representing the collective name of about 170 genera 5500 species. This class comprises two orders viz. Centrals (possessing frustules cells) circular pill box with radial symmetry occurring in marine water, pennales, possessing frustules like oblong boxes with isolateral symmetry occurring in fresh water. Majority of the diatom occur as phytoplanktons both in fresh in salt water, some form occurring even at the bottom of water reservoirs, some diatoms are also known to occur as terrestrial form usually on moist soil or even on moist rocks and inside the dark building / monuments, imparting it a brown color. Fossilized diatoms can be also found in large deposits and in that cases they are called diatomaceous earth are Kiesulguhr. Thus the diatoms algae are ubiquitous and cosmopolitan in distribution. The general characteristics of diatoms to which the identified *Navicula* in the present study belong, are unicellular photosynthetic organism; sometime being unicellular cells become colonial or filamentous owing to accumulation of large number of cells in a common mucilaginous envelope.

In so far as the cell structure of diatoms concerned, each cell of a diatom is called frustule which is made up of two parts i.e. the cell wall and protoplast. Further cell wall is two layered, while the inner layers is thin membranous continues and composed of pectin, the outer layer, however impregnated with silica and made up of two overlapping halves of theca. Both halves fit closely together like Petrydisc, the upper half being then called epitheca and the lower half hypotheca. The side

of two overlapping connecting bands is called girdle.

Characteristics of diatom the uninucleate protoplast i.e. internally bounded by plasma membrane, the centre being occupied by large vacuole whereas in case of pinnate diatom the central vacuole is bridged by cytoplasmic strands and the nucleus remain suspended in the centre of strand the chromophore of navicula are brown in color due to presence of large excessive amount of carotenoid in association with other pigment like chlorophyll a, chlorophyll c, carotenoids and xanthophylls (diatoxanthin, diadinoxanthine and fucoxanthin). The food reservoir diatom navicula is oil and chrysolaminarin. The most significant of feature of these algae is chlorophyll-b is totally absent.

The reproduction in diatoms take place mainly by two methods namely cell division are fission, and sexual. While the first method is the most common means of multiplication and occur at night. The sexual multiplication involve the production of autospores. Which being usually larger then the vegetative cells are derived form zygote. In this way autospores are special diploid spores produce as a result of sexual gamatic fusion of zygote and its differentiation.

Anabaena

The algae anabaena belonging to nostocaceae family with variant of 25 species occurs abundantly in India. Being aquatic it occurs in shallow, deep temporary and semi permanent water bodies either for floating or attach to submerged plant. The majority of species of anabaena are exclusively planktonic in ponds, tank, lake and rivers, imparting a blue-green coloration to water because of their blue green color some species e.g. *A gelatinicula*, grow even on moist soil and also in rice fields, thereby enriching the soil with, nitrogen fertilizer via atmospheric nitrogen fixation. Some of its species have been reported as grow even in close symbiotic association with other plant such as *Azolla*, *Cycas* coralloid roots.

The vegetative structures of anabaena is multicellular, unbranched and filamentous, the filaments, occurring either signally or in groups forming irregular thalli, the latter being blue green in color, indefinite in shape, form torn flouccose or soft gelatinous masses. The individual filaments of this diatom algae are either straight or curved with indistinct or without a muicilaginous

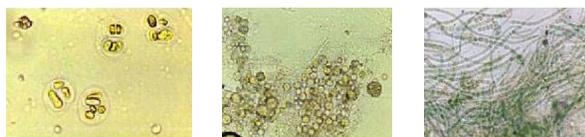


Phormidium

Lyngbya

Oscillatoria

Figure 3(a) : Airborne algal flora in Jharia coalfields, phormidium, lyngbya and oscillatoria

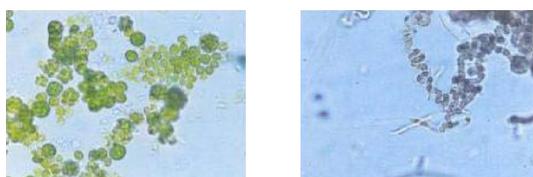


Pandorina morum

Gleocapsa

Nostoc

Figure 3(b) : Airborne algal flora in Jharia coalfields, pandorina morum, gleocapsa and nostoc



Fragilaria

Plectonema

Figure 3 : Airborne algal flora in Jharia coalfields, fraffilaria and plectonema

sheath, however in planktonic form the filaments are usually curved circinate or irregularly contorted a furthermore the gelatinous sheath if present, is diffiluent and never imparts a definite shape to the colony A typical characteristics of this algae is that its each filament is uniformly broad throughout consisting of cylindrical rectangular, spherical or short barrel shaped cells. Yet another distinguishing feature of this algae is that the heterocyst are usually intercalary are located at irregular interval thorough out the entire length of the filament. In contrast, the heterocyst is some species are both terminal and intercalary. They are either of the same size and shape as the vegetative cells or slightly broader in addition to this, the akinetes occur singly or in long chain either near or in between the heterocyst. They are cylindrical, oval or spherical in shape and are generally produced when to algae grows in nitrogen deficient medium. Anabaena reproduces by vegetative and asexual method, e.g. By herbogonia, by akinetes (or spores, by heterocyst, and by endosporces.

Chlorella

Chlorella is an algae which belongs to the class chlorophyceae, its genus being chlorella, the latter includes about 8 species out of which 4 species, are found

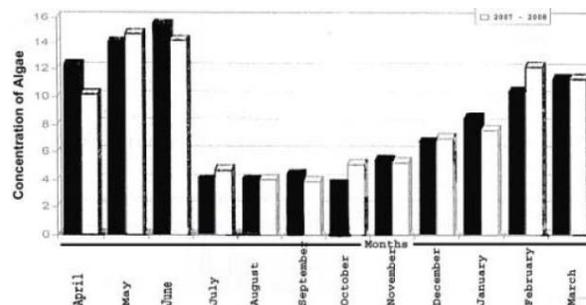


Figure 4 : Histogram showing seasonal variation of concentration of airborne algae in Jharia coalfields

in India, namely: *C-Valgaris*, *C-gonlomerata*, *C-Conduetrix* and *C-Parasitica*. Although They occur in variety of habitats, most of them occur as phytoplants in fresh water ponds, ditches and other water reservoir, afew also occurring in brackish water near the sea shore. Notwith- standing this, some species of chlorella are also terrestrial and grow on moist rocks,damp soil, wells, tree trunk etc. It has been reported that some species occurs in symbiotic association with lichens and invertebrate (*C-lichina*).

The vegetative structure of algae chlorella is a simple, small, unicellular coenocystic and non motile colonial form. It vegetative cell possess well defined cell wall single nuclear and single parietal chloroplast with single paranoid. The cells of chlorella are usually found solitary but sometimes they has been observe to farm colony of irregular out line, the vegetative cell being spherical ellipsoidal in shape, measuring about 2-10mu in diameter. Each cell is bounded by a thin cellulose cell wall. The reproduction of chlorella algae does not occur by nuclear division in reproductive cell via cytoplasmic cleavage rather it occurs by zoospores and autospores.

The importance of these algae lies in (1) its economic value in that it is an edible alga, possessing high nutritive value with contents of about 50% protein, 20% lipids, 20% carbohydrates, essential amino acids Vitamin and mineral, salts. Exploiting its high nutritive value it is finding increasingly greater use as food or food supplements for human consumption in Japan, Israel, Germany, Holland and America. (2) It is used in municipal savage disposal wherein it acts as an aerating agent through removing CO₂ and restoring O₂ by the process of photosynthesis as it helps in aerobic decomposition of sewage (3) This alga is used in physiological experiment particularly related to photosynthesis and

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respiration, wherein its properties of multiplying fast and showing all the biochemical process are akin to higher plants are exploited (4) chlorella is used in space research for providing ready source of oxygen evolved during photosynthesis (5) This alga being used in trial runs to control global warming by spraying the algae in the atmosphere from the air crafts flying it high altitudes and (6) this algae is gainfully used for the production of the antibiotic clorellin.

Notwithstanding the afore mentioned economic gains chlorella has also been reported in a few sporadic studies to be possessing some toxicity, though it still remains to be conclusively confirmed.

Fragilaria

Belonging to the order Pennales of class Bascillariophyceae, the diatom *Fragilaria* is a marine alga, golden brown in color. As stated above, the alga is a member of the araphide pinnates, which, according to Medlin and Kaczmarek^[34], are polyphyletic. It is unicellular microscopic photosynthetic organism, which may be filamentous. The cells, being regular and tapered to the poles, are known as frustules and are like oblong boxes. The frustules are comprised of two halves, the lower hypotheca and upper epitheca, both of which when fitted closely, look like a candy box or Petridish. Though it does not have a raphe, the striae produce a longitudinal gap forming a pseudoraphe. Its golden brown colour is due to the presence of xanthophyll, mainly fucoxanthin, diatoxanthin, and didinoxanthin, besides chlorophyll -a and c. It reproduces vegetatively via cell division (mitosis), asexually by autospore formation. Sexual reproduction is rare, but, if present, is amoeboid isogamy.

Oedogonium

This alga belongs to class chlorophyceae family oedogonic caeae. They are aquatic and grow in fresh water ponds, pools, shallow lakes or even slow flowing stream. They usually occur as epiphytites on submerged aquatic plants. Oedogonium is multicellular filamentous alga, the filaments of which are unbranched. Each cell of filament is long cylindrical and possesses thick and rigid three layered cell wall. The inner layer is pectic and outer most layer is made up of chitin. Each cell is uninucleate and nuclear lies in the peripheral cy-

toplasm. The chloroplast characteristically possesses pyrenoids (site of starch synthesis).

Growth of filament occurs by cell division any cell of the filament except the basal cell which is capable of division. Few intercalary cells of filament possess ring-like transverse striations, called caps. The cells, having caps, are called capces and they function as reproductive cells, doing both sexual and asexual reproduction.

Reproduction in oedogonium occurs by vegetative asexual and sexual methods (a) vegetative raped by fragmentation. (b) Asexual reproduction by Zoozpres and Akinetes formation.

Sexual, Reproduction is oogomous. The female sex, organ is oogonium and male sex organ is antheridium the sexual reproduction usually occur in the filaments growing in quite water after a certain period of vegetative growth. The external conditions, which favor sexual reproduction, are high pH (alkaline) and deficiency of nitrogen in water.

Chlorococcum

A non-motile, colonial, fresh water green alga, Chlorococcum belongs to the family *Chlorococcaceae* of order Chlorococcales and division Chlorophyta. The green color of its thallus is due to the presence of dominant pigment Chlorophyll a and b. along with traces of carotenoids. It is a subaerial alga, growing sometimes on damp soil or brick work. The most distinctive feature of this alga is the striking variation in size of vegetative cells, which are uninucleate and contain parietal chloroplast with single pyrenoid. The cells become multinucleate at the time of reproduction. Vegetative division of the cell is absent. Reproduction is both asexual and sexual. The former takes place by biflagellate zoospores, while the latter takes place by formation of biflaigillate gametes. Both zoospores and gametes are formed by cleavage of multi-nucleate protoplast into uninucleate segments and their metamorphosis.

Gloeocapsa, Lyngbya, Phormidium, Pectonema

All these are blue green algae belong to the class cynophyceae and are also commonly called cyanobacteria, the common characteristic of these algae being (1) They are cosmopolitan or ubiquitous in their distribution and occur usually in tropical part of the world and (2) Most species of this algae occur on

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moist soil and in cultivated lands, majority of this species of algae are aquatic in fresh water labial, whereas others grow in brackish (salt) waters. They have been found to occur as phytoplankton, blooms epiphytes or as benthic formation on mud white a few species is known to occur as endophyte or endozoically. While the organization of the thallus of these algae range from unicellular nonmetile forms through multicellular colonial forms to highly evolved heterotrichous form, some other intermediate forms are also known including unicellular polar thalli with a definite base and apex, simple filamentous forms without heterocysts and akinetes; simple unbranched filaments with heterocysts, unbranched heterocystus filaments with base and apex, heterotrichous filaments with false branching.

The cyanophyceac algae found in this present study can be put, as per above description of organization of thallus, in the following category: (a) Gloeocapsa-unicellular colonial form, (b) Phormidium and Lyngbya-simple filamentous form without heterocyst and akinetes, and (c) Plactonema-heterotrichous filamentous form without branching.

The common features of above mentioned algae are briefly described hereunder

- 1 The important pigments present in the cells, which are responsible for imparting different colouration to the algae, are chlorophyll-a, b Carotene, myxoxanthophyll maxoxanthin, C-phycoerythrin and C-phycoerythrin. Whilst C-phycoerythrin is blue, the C-phycoerythrin is red in color. The intensity of blue green color in the particular species depends on the predominance of any of these two pigments, e.g. of C-phycoerythrin is more in comparison to C-phycoerythrin if produces typical deep blue-green color to the algae.
- 2 Each all of the algae possess a definite cell wall generally surrounded by a mucilaginous sheath, the latter may be thin or thick hyaline are pigmented homogenous and stratified and is made up of reticulately arranged microfibrils within an amorphous matrix. The fibrils are composed of pectic and mucopolysaccharide. The cell wall is firm and rigid, and two layered characteristically followed by a plasma membrane. The inner contents of the cell are typically distinguishable into thin regions i.e. over pigmented region cell chromoplast and central hyaline centroplast. The significant cellular inclusion include viz, nuclear chloroplast, mitochondria, endoplasmic reticulum, dictyosomes etc are absent.
- 3 The significantly important feature of these algae is that the photosynthetic pigment are located in broad sheet – like lamellae, called thylakoids, which are restricted to peripheral part of cytoplasm in unicellular form (Gloeocapsa) but in filaments forms they traverse the entire cytoplasm in the form of network (as in Phormidium, Lyngbya and Plactonema) Also some lipid globules have been found to occur within the thylakoid, whereas phycobilisome particles are attached to their surfaces. It is generally believed that these lamellae provide the sites for cellular respiration too.
- 4 Most of the blue-green algae which are filamentous and heterocysts, possess the ability to fix the atmospheric nitrogen. Further more, it has been recently shown that even some non heterocystus form (M. Plactonema, Gloeocapsa found in present study are also able to fix atmospheric nitrogen either in free living condition as in symbiotic association of other plants.
- 5 Species of blue-green algae show very close family in bacteria in that (i) both are prokaryotic and both the organism devoid of true nuclear with definite nuclear envelope. The nuclear material being disposed in central region, (2) the DNA and RNA molecules with nuclear protein i.e. histone. (3) the cell wall possess characteristic mucopeptide-a.e. diaminopimelic acid and muromic acid, (4) cell organelle like chloroplast mitochondria, endoplasmic reticulum golgi bodies and membrane bound vacuoles are absent, (5) Blue green algae and photosynthetic bacteria both possess photosynthetic lamellae cell thylakoids and perform various metabolic function such as photosynthesis respiration, oxidative phosphorylation and nitrogen fixation, while fixing the atmosphere nitrogen both organism oxidized hydrogen sulphide.
- 6 Although there no meiosis and no true sexual reproduction, in blue green algae their vegetative reproduction, however, occur by fusion, fragmentation and by formation of homogonia while asexual reproduction occurs by the formation of akinetes, endospores,

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exospores, and nannocyst.

As mentioned above, during the course of the present study, 13 types of airborne algae components were trapped from the air over coalmining and coal-based industries' areas of Jharia Coalfields and, save for the months of April and May, the presence of algal forms was observed in all other months of the year, albeit in varying concentrations. The atmospheric algae found were either filamentous or colonial forms. While some are green algae, the others were diatoms (blue-green algae), with different vegetative (thallus) structure and color (*vide supra*). The observed trapped algae in the atmosphere at different sites in Jharia coalfields were in the range 75-115 colonies per m³ of air. Contrary to the findings of Hamilton^[35], who reported rare occurrence of diatoms and desmoids, in our study the prevalence of diatoms (e.g.,) are clearly seen, probably because of favorable climatic conditions (temperature, rainfall, and humidity) prevalent in the coalfields. Microscopic algae was estimated to be few to few hundred per. m³, which are prone to be occasionally deposited on the ground and grow and multiply on the soil in favorable conditions of moisture and temperature. Occurrence and distribution of two distinct types of cyanophages i.e., clear (virulent), and turbid (lysogenic) plaque-forming strains in natural habitat, and cyanophyceae, have been reported in permanent ponds, sewage and rice fields of Cuttack^[36], the Cyanophages having been reported for the first time in rice fields of India.

Correlation with environmental parameters

A careful examination of the results of TABLE 2 and 3 reveals that the concentration of the algae varied during 2 years of studies (2006-07 and 2007-08), with a variation in meteorological parameters such as room temperature, rainfall, relative humidity (RH) etc. It is observed that the occurrence of algal population was totally absent during summer (April, May). This clearly suggests that high temperature reaching up to 45°C or more during extremely hot summer experienced in the months of April and May, associated with very low relative humidity (up to 25-26%) and lack of rains, creates a highly unfavorable environment for the growth and sustenance of algae. However, the algal population was observed to be moderate during the months of December to March. Quite in contrast to this, during the rainy

season, spanning from June to October and sometime extended up to November of both the years of study. With the onset of monsoon and with the start of rains,, the growth of algal flora as observed to have started in the month of June, after which, as the rainy season progressed and more rains occurred, the population of different types of algae was observed to be gradually increasing. Maximum algal load i.e., concentration of the algae was recorded in the months of August to October, when the average temperature was 27-34°C, associated with average relative humidity in the range 50-60%. The contribution of algae during this period ranged between 90-115 colonies per m³ of air,. After this, in the month of November, a slight decline in the algal was recorded, which ranged between 65 and 80 colonies per m³ of air, when the average temperature was 25-27°C and relative humidity 50% during both the years of study. It is thus evident that a temperature range of 34-35°C with humidity around 60% is most favorable condition for the growth, multiplication, and subsequent increase in the population of algae in the coal mining and coal-based industries' areas of Jharia coal fields,. This implies that in the environments of coalfields in general, and Jharia coalfields in particular, where usually relatively high temperatures and slightly lower percentage of RH prevail during summer, are not conducive for the identified algae to thrive. Thus, in the coalfields, the frequent rains during the rainy season play an important role in maintaining proper RH (60% or more) and required moderate temperature for the algae to grow and survive. The reason for the low concentration of algae (< 30-40 colonies per m³ of air) in the month of July, when monsoon in the month of June was already in place and environmental factors were also quite favorable (average temperature 36.9°C, and RH about 58%), could possibly be that the vegetative growth of the algae species (via fragmentation or otherwise) was in the initial stages.

It is further observed from the results of TABLE 2 and 3 that, in general, there is a progressive decline in the concentration of algae during the December-March in both the years (2006-07 and 2007-08). In the month of December of both the years, for example, the algal load in the atmosphere was ca. 70-72 colonies per m³ of air, after which the decreasing trend continued till March, in which month it was at the minimum of the

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rainy season (ca. 40-45 colonies per m³ of air, which is only slightly higher than the initial load in the month of June. It is thus evident from these observations that the morphological parameters during winter, with prevailing lower temperature in the range 21-23°C and low RH (41-45%) are not very favorable for the increase concentration or load of the algae, which is why there is sudden and progressive decline in the algal population during the months of winter (December-March). It is also obvious that rains do play an important role in creating and maintaining appropriate conditions of temperature and RH during the months of July-November, for the reproduction, growth and multiplication of different types of algae in the environment atypical to coalfields. In the course of Aerophycological investigations in Aurangabad for consecutive 2 years (1985-86, 1986-87), the presence of algal forms in almost all the months was observed^[18,19], the atmospheric algae encountered being both filamentous and colonial forms. In these studies, the most common and frequently found alga was *Phormidium*, the occurrence of which was also reported in Varanasi by Singh^[37]. Sabina Anis and Javed^[18,19] further reported that the ratio between the Chlorophyceae and Cyanophyceae was about 1 : 3 and that *Phormidium*, *Nostoc*, *Plectonema*, *Chlorococcum*, and *Chlorella* are the frequently encountered algal airspore in Aurangabad. In a more recent study, Sharma and coworkers^[23a] while carrying out the sampling at a height of 2.5 m. The normal human breathing zone, characterized the airborne algal diversity in a populous subtropical urban environment, of Varanasi (U.P) and showed that both airborne and soil-born algae are the permanent constituent of Varanasi city atmosphere, and that the nature, composition, and relative ratio of constituting groups differed among sampling sites., the Cyanobacteria., because of broad ecological distribution, dominating the fluctuating climate. This finding is quite in consonance with the results of our present investigation in that here also a diversity of airborne and soilborne algal flora has been found and the soil-borne algae constitute the bulk of aeroalgal flora, plausibly due to their ability to withstand the dehydrating effect of the coalfield atmosphere. But the climatic conditions, viz., the high temperatures ranging between 35 and 46°C with RH in the range 25-34%, prevailing in the months of April and May in

the Jharia coalfields are too harsh and unfavorable for the algae to grow and sustain during summer, which is why the airborne algae are absent in the months of April and May. The wide distribution of algae in the coalfield areas could well be due to the fact that the single-celled algae in a resistant resting stage (like in soil) or fragments in filamentous forms, or fragments of more colonial forms are usually transported, in dry state, by wind to different sites, where they resume active growth under congenial climatic conditions, viz., temperature, moisture and light.

Seasonal variation of prevalence of airborne algae

From a critical appraisal of the histograms in figure 4, showing the month-wise concentration of the identified airborne algae species over 2 years, it is found that the concentration of all the alga detected is subject to seasonal variation in that they were totally absent during summer (April and May), moderate during winter (December-/March), and highest during the rainy season i.e. from July to October-November in both the years. This clearly shows that the climatic conditions have an important bearing on the growth, and sustainability and survival of the algae.. It is observed from the algae calendar shown in TABLE 4 that the Cyanophyceae members (*Chlorococcum*, *Goeocapsa*, *Anabaena*., *Oscillatoria*., *Lyngbya* *Phormidium*., *Nostoc*), are dominant forms of the aeroflora in Jharia coalfields, occurring abundantly in the atmosphere not only in the winter months (December to February) but also occurred moderately in the months of March, April and even in the month of July. Although our this finding is somewhat at variance from the results of other investigators who reported prevalence of *Cyanophyceae* species occurred during the warmer months in Aurangabad^[18,19], Pune^[38], and Varanasi^[39]. It is explicable on the basis that compared to Aurangabad, Pune and Varanasi, the winters (December-February) in Jharia coalfields are very mild, the maximum and minimum temperatures ranging between 20-22 and 10-14°C, respectively and the months of March, April, and July are quite warm, with RH in the range 47-55% and such climatic conditions are quite favourable and have seemingly well been adopted by the algae to grow and sustain.. It is further observed from the algae calendar in TABLE 4 that *Chlorophyceae* members (*Oedogo-*

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nium, *Plectonema*, *Spirogyra*, *Chlorella*, *Fragilaria* and *Navicula*) also occur abundantly in the cooler months (July-November) and moderately in the months of December to March, and also in July. Similar seasonal variation with the change in climatic parameters (temperature, relative humidity, rainfall) and the composition of the aeroalgal community in different parts of India but not in coalfields, have also been reported by other investigators^[23a,40].

From the above discussions, it is apparent that the two major factors responsible for regulating the structure of the aeroalgal community in the atmosphere of any specific area-coalfields in the present case the physiological ability of an algal group to tolerate different types of abiotic stresses, and the climatic conditions prevailing in that area.

Allergenicity of algae

Although there are reports evincing the allergenicity of some selected algae, yet clinical significance and algal metabolites are not fully understood nor are the causative effects and reasons definitively proved. But, in general, some forms of diatoms (*Cyanophyceae*) and some species of *Chlorophyceae* also are known for their allergenicity. The high concentration of protein in algae cells is well known and plays significantly important role in causing allergic reactions in sensitive humans. As such, increase in the concentration (population) of such airborne allergenic algae in the airspora is obviously prone to induce allergic disorders in sensitive individuals living in and around the coal-mining and coal-based industries' areas of the coalfields. The allergic diseases reportedly manifested by airborne algae are hay fever caused by *Chlorella*^[9,41], persistent dermatitis caused by blue-green algae *Anabaena*^[42]. Similarly, the disease 'Protothecosis', which affects lymph nodes, liver, lung, kidney, and the dermis, is reported to be caused in sheep, cattles, dogs, and beavers by the alga *Prototheca* (a *Chlorophyceae* genus)^[43]. Algae are also known to cause silicosis (caused by diatoms)^[44] and goitre^[45]. Some other kinds of toxicosis reported to be caused by algal toxins called 'casual agents' for various allergic manifestations including: dermatitis or itching, and respiratory allergy like hay fever, fungal fever, and allergic rhinitis^[9,41,42,46-53]. Different types of skin itching caused due to direct contact with various algal

material have been reported in recent years. The disease *swimmers' itch*; has been reported to be caused by a freshwater species of *Anabaena*^[42,54]. Similar types of algal allergenicity manifestations evincing allergic reactions, involving blockage of nose, itching of eyes, bronchial asthma, and urticaria, and causing of hay fever symptoms by lake swimming proved and attributed to the algae *Phormidium* and *Lyngbya*, have also been reported by other investigators^[55-58]. Various grades of algal respiratory allergies, namely, inhalant allergy in children, hay fever, Tingui or Tamandane fever, positive skin reaction, allergic rhinitis, nasobronchial allergies etc., have been reported from different parts of the world during the last 20-25 years also high incidences of positive reactions to algal organisms in house dust sensitive patients have been reported by aerophycologists^[9,50,59,60]. Ishida^[61], in his very recent clinical study, has reported that the major chronic disease, gastric ulcer in Thailand, is caused via change in the morphology of six host species of the algal host *Chlorococcum* in association with five genera *Anabaena*, *Cylindrospermum*, *Mojavia*-*Nostoc* and *Oscillatoria*-*Phormidium*-*Lyngbya* complex.

It is noteworthy that the changing weather in the Jharia coalfields in the months of February-March-early April, and September-November coincides with the frequent occurrence of asthma, bronchial rhinitis and common cold and flu. Hence, it could be well possible that the allergenic algal form prevalent in the atmosphere of the coalmining and coal-based industries' areas of coalfields in general and Jharia coalfields in particular are responsible for causing allergic reactions prevalent during the months of the year, which are commonly but erroneously, attributed to *wet conditions* and *change in weather*. Therefore, it needs to be seriously and systematically investigated in respect of all the aspects, including clinical studies.

It, thus, manifests itself clearly that the health and the physiological processes of human beings can and often do, get adversely affected by the impact of airborne algal particulates through their inhalation, contact, dissemination and deposition (via consumption of algal food and food supplements using the alga *Chlorella*) in the human systems/organs, which, in recent years, has been amply demonstrated through several clinical investigations too.

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All the 13 algal forms, identified and characterized in the present study, are inhalant types, while some are both inhalants and contactants, and some even ingestants. Of these, all the seven Cyanophyceae members (*Chlorococcum*, *Goeocapsa*, *Anabaena*, *Oscillatoria*, *Lyngbya* Phormidium, *Nostoc*) are potentially allergenic, while manifestations allergenicity by some Chlorophyceae members (*Oedogonium*, *Spirogyra*, *Chlorella* and *Navicula*) have also come to light in recent years. Although different types of respiratory, skin, and other diseases are prevalent in coal-mining and coal-based industries' areas of Jharia coalfields, yet, in the absence of any details, and systematic clinical studies, it is very difficult to attribute the disease to any particular allergen. It should always be borne in mind that the atmosphere of coalfields is comprised of both abiotic (RSPM, SPM, SO_x, NO_x) and biotic (pollen grains, fungal spores, algal filaments, dust mites) air pollutants in association with fine dust particles, and each component plays its role in causing various types of allergic disorders and ailments and it is rather difficult to pinpoint as to by which particular allergen the disease has been caused. It is now fairly well recognized that a detailed knowledge and characterization of these allergens and more particularly identification of the metabolite(s) from the suspected allergen (in this case the identified algae) will provide a useful tool in the treatment and/or preventing the effects of specific aeroallergens in different individuals sensitive to that particular type of allergen. Clinical investigations on this line have been initiated and the results of such studies will be the subject matter of our future communication.

CONCLUSIONS

The present investigation has revealed the prevalence, in the atmosphere of Jharia Coalfields., of 13 species of algae, belonging to class *Chlorophyceae* and *Cyanophyceae*, viz., *Anabaena*, *Chlorella*, *Chlorococcum*, *Fragilaria*, *Gloeocapsa*, *Lyngbya*, *Navicula*, *Nostoc*, *Oedogonium*, *Oscillatoria*, *phormidium*, *Plectonema* and *Spirogyra*, some of which are potential allergens, responsible for causing health hazards manifested by different respiratory, skin, and other allergic disorders in humans.. The algal load

(population) is subject to seasonal variation. Save for the month of April and May, the prevalence of these algal species was found throughout all other months of the year, albeit in their varying concentrations, the maximum concentration being during the rainy season (July-October, even in November also). All these algae are mostly inhalant types, while some are contactants (*Anabaena*, *Fragilaria*, *Lyngbya*, *Phormidium* and ingestant *Chlorella*) too. Almost all the diatoms exhibit allergenicity, with potential for causing allergic health hazards (like hay fever, nasal blockage, bronchial asthma, allergic rhinitis, eczema, persistent dermatitis and other skin diseases). In humans living in and around coal-mining and coal-based industries' areas (Cement factory, Fertilizer factory, Coal waheries, Coke-making Plants) of Jharia coalfields Systematic identification of the specific metabolite(s) from the suspected allergic disease-causing algae and their correlation with the disease through clinical investigations are highly desirable.

ACKNOWLEDGEMENTS

The authors heartily thank Dr. Amalendu Sinha, Director, CIMFR, Dhanbad for providing facilities for this work and permission to publish this paper. Their gratitude flows strong to all the supporting staff of the Biotechnology Section/Environmental Management Division, CIMFR-Digwadih Campus, Dhanbad, for their ungrudging help in carrying out the experimental/analytical work, whenever required. Authors express their sincerest appreciation to Shri Satish Kumar, Lecturer, Botany Department, BSS Mahilaa College, Dhanbad for his valuable suggestions, critical comments, and assistance in the characterization of some algae.

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