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Cyanobacterial pigments analysis extracted from different sources of District Allahabad

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Abstract
The pigment variation is specific features among microalgae. The paper aim to analyze cyanobacterial extracts of different sources of Allahabad, Uttar Pradesh. The main object here is the importance of the blue green algae especially because of the pigments present in this class of algae. It is characterized by their specific Phycobilin pigments, also other pigments such as Chlorophylls and Carotenoides are found. Pigments are present in all living matter and provide attractive colours and play basic roles in the development of organisms. This paper presents the information about the natural pigments of cyanobacteria and how they can be extracted and identified using different procedures and spectrophotometry. It emphasizes that the principal algal pigments are Phycobilins, Chlorophylls and Carotenoids.

Keywords: Cyanobacterial pigments, cyanobacterial extracts, blue green algae, Phycobilins

Introduction
Cyanobacteria are the photosynthetic organisms which possess the ability to synthesize chlorophyll and a number of valuable compounds such as phycocyanins and carotenoides (used as antioxidants). Pigments are chemical compounds that absorb light in the wavelength range of the visible. Produced colour is due to a molecule-specific structure (chromophore) and it is this structure that captures the energy due to which the excitation of an electron from an external orbital to a higher orbital is produced. As its result the non-absorbed energy is reflected or refracted to our eyes and captured, and then generated neural impulses are transmitted to the brain where they are interpreted as a colour (Vargas, Jimenez and Lopez, 2000) [9]. Pigments can be studied by dividing them into 3 classes- natural, synthetic, inorganic.
Cyanobacteria contain chlorophyll, which gives the green colour, but almost all species can form the blue pigment, "phycocyanin", under some conditions and some also form red pigment, phycoerythrin. Edwards (2003) [13], also confirmed that photosynthetic *Cyanobacteria* have chlorophyll and carotenoids in addition to the accessory pigments named "Phycobilins"**.** The algal samples were collected from six different aquatic and natural environments. These samples were used to obtain unialgal cultures by plating and streaking and by serial dilution. Also the growth of the cultures was maintained in BG- 11 medium under laboratory conditions. *Anabaena*, *Nostoc*, *Microcystis*, *Phormidium* were the algae that were identified with the help of the identification keys given by Desikachary (1959) [2] and further used in the extraction of pigments. Solvents like methanol, ethanol and acetone were used as extraction solvents but methanol showed the most suitable result in extracting chlorophyll. The evaluation of the pigment contents in the blue green algae is essential to indirectly check the measure of culture growth and also as a parameter to check the trophic levels of waters. Additional economic uses of natural pigments are, such as, use of phycobiliprotein as an additive in foods, cosmetic products and medical diagnostic reagents (Seo, *et al*., 2013) [13].

Materials and Methods
The present study has been carried out at the Department of Biological Sciences, Sam Higgin bottom Institute of Agriculture Technology and Sciences, Allahabad to analyze the pigment extracted from different sources. Six aquatic sampling sites were identified for the collection of water samples. These aquatic bodies are located around Allahabad district of Uttar Pradesh. The experimental site is situated between the North latitude 28°52' and at the longitude 81o 55'E with an altitude of 98 m msl. The climate, in general, is temperate characterized by hot summers with maximum temperature 47°C during May and June and least being i.e. 1°C in

January. On an average the area receives an annual rainfall of 850 to 1000 mm. Most of which is concentrated during monsoon season. It is located in the southeastern part of Uttar Pradesh and has tropical to subtropical climate with extremes of summer and winter. Most of the sampling sites are located in the S.H.I.A.T.S. (Sam Higginbottom Institute of Agriculture Technology and Sciences) campus. They are as:

River water (Yamuna)	Allahabad city
Saraswati Ghat	Allahabad city
Forestry department	SHIATS campus
Horticulture department (field)	SHIATS campus
Canteen	SHIATS campus
G. Hostel campus	SHIATS campus

The extraction techniques of cell components usually make use of chemical, mechanical and/or enzymatic procedures. In this work only the chemical and mechanical procedures were used, alone or simultaneously, with the aim of maximizing the extraction process efficiency. Water samples were collected from above mentioned locations during March and September months from the above listed sites. The samples were brought in the laboratory for further analysis and were grown and maintained in BG-11 medium. The collected water samples were used for the isolation of pure cyanobacterial strains and later for the extraction of pigments.

Preparation of unialgal cultures: The environmental samples were used to isolate single algae by serial dilution and plating and streaking methods. Out of several samples 12 pure cultures were obtained out of which 5 samples were found to be the blue greens which could be identified.

Maintenance of cultures: The unialgal cultures were maintained in the laboratory conditions in the culture room. The unialgal cultures were kept at 16/8 light and dark period and 28±.2 º C temperature, then they were identified according to keys given in Desikachary (1959) [2] and Geitler (1932) [2]. 2 ml of cultures samples were taken for the extraction process of pigments. The pigments extracted from the collected algae were - Chlorophyll, Carotenoides and the most important, the Phycobilins (C/R-Phycocyanin). The extraction of chlorophylls and carotenes were done using the three solvents- methanol, acetone and ethanol. Emperical correlations for total carotenoids evaluation by spectrophotometry are less common in the literature (M. Henriques, A. Silva and J. Rocha, 2007) [4].

Firstly, 0.05 – 0.5 g of thallus was taken out and weighed, secondly it was transfered to mortar and ground in 5 mL 0.1M phosphate buffer with a pH of 6.8, with acid-washed sand. Then it was centrifuged for 10 min at 1,000 x g. After this, the mixture was transfered to 25 mL volumetric flask and increased up to volume. Then the phycobilin concentration was determined using the correlations mentioned by Evans and Kursar and Alberte (1983) [7]. The concentration in millilitres is calculated according to the above mentioned correlations, and then the concentration of these pigments was also calculated in per gram of the algae according to the relation given by Maria Rio A. Naguit and Wilson L. Tisera, 2009 [15]. Algae were estimated for different pigment content such as chlorophyll, carotenoid, phycobilins. There are several methods to extract the pigments from these algae but maximum are based upon spectrophotometrical analysis with the following steps:

a) Separation of algal cells from the supernatant
b) Extraction of pigments by the help of an organic solvent
c) Spectrophotometric determination of the pigments in the extract.

The Concentration of Pigments per Gram of the Algae,

$$\frac{\text{Concentration in mg/L} \quad X \quad \text{Volume of solvent in mL}}{\text{Weight of Algal thallus in grams mg}} \times \frac{1000\ \mu g}{mg}$$

(Maria Rio A. Naguit and Wilson L. Tisera, 2009) [15]

Results

Table 1: Concentration of Pigments (Chlorophyll and Carotenoides (in µg / mL)) using Methanol, Acetone and Ethanol as solvents:

Algae	Methanol			Acetone			Ethanol
Anabaena	10.0725	8.8159	3.6966	13.4067	4.06372	7.43358	14.38542
Microcystis	12.4899	11.3212	4.2516	7.91281	9.55851	8.68546	13.65526
Nostoc	8.9712	1.8122	4.2255	9.48514	11.51046	8.56894	10.25608
Phormidium	10.9588	8.1880	2.8751	10.24731	11.54392	7.8732	8.13366
Anabaena 2	9.2129	7.6404	3.6621	10.24731	8.02808	5.71748	10.52996

Table 2: Concentration of Phycocyanins (µg/mL) according to the correlations givenBy Evans (1988) & Kursar and Alberte –

	Evans		Kursar & Alberte
Algae	**C/R-Phycocyanin**	**R- Phycoerythrin**	**Allophycocyanin**
Anabaena	0.044	0.0047	108.18
Microcystis	0.012	0.0058	107.61
Nostoc	0.030	0.00334	112.33
Phormidium	0.019	0.00321	94.89
Anabaena 2	0.013	0.00487	107.95

Table 3: The Concentration of Pigments Per Gram Of The Algae (Maria Rio A. Naguit and Wilson L. Tisera, 2009) [15]:-

Algae	Methanol			Acetone			Ethanol
	Chlorophyll		Carotn.	Chlorophyll			Chlorophyll
Anabaena	28771.5	251714.3	10542.8	38285.7	40600	21228.6	41085.7
Microcystis	124800	113200	42500	79100	95500	86800	136500
Nostoc	35880	7240	42200	37920	46040	34240	41000
Phormidium	24333.3	181777.7	63777.7	227555.5	256444.4	174888.9	180666.7
Anabaena 2	460500	382000	183000	512000	401000	526000	526000

Table 4: Concentration of Phycobilins

Algae	R-Phycoerythrin	C/R-Phycocyanin	Allophycocyanin
Anabaena	79.2	740	1803000
Microcystis	0.005808	607.5	1803000
Nostoc	41.75	376.8	1404125
Phormidium	40	237.5	1186125
Anabaena2	40	108.3	899583.3

Discussion

The environmental samples containing mixed algae were purified to obtain unialgal cultures.

The algae found in abundance were Anabaena, Nostoc, Microcystis. These algae were identified with the help of the identification keys given by Desikachary (1959) [2].

The levels of phycocyanin, allophycocyanin and phycoerythrin in total phycobiliprotein content in cyanobacteria vary not only with species but are also influenced by environmental factors. Cyanobacterial species with high content of phycobiliproteins can be considered as their prospective source for commercial use. The filamentous cyanobacteria, particularly nitrogen-fixing heterocystous species, are regarded as attractive organisms for the production of phycobiliproteins and other important chemicals (Borowitzka, 1988; Moreno *et al*., 1995) [16]. Although the focus of the above experiments is the importance of pigments so the pigments extracted from the type of algae also is of great importance. Ecological studies of BGA in submerged soils are limited by problems in methodology primarily in estimating algal biomasses quantitatively. Under natural conditions, BGA grow preferentially in environments that are neutral to alkaline.

The findings of present investigation show a positive correlation between pH and occurrence of BGA. Besides this other physico-chemical properties which affect the amount of pigment concentration in algae are presence of nitrogen, phosphorus, heavy metals and electrical conductivity of the water body. Cyanobacterial distribution was also found heterogeneous and limited in distribution and still not well known as no systematic analysis has correlated their presence or absence with environmental factors.

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