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Exploring the Impact of Epilithic Cyanobacteria on the Heritage Monuments of Kota, Rajasthan

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Abstract: ***Cyanobacteria also called Blue Green Algae are gram negative, oxygenic photosynthetic prokaryotes. They show the ability of efficient nutrient uptake mechanism. Ancient monuments and temples are more exposed to sunlight and moisture of the environment and are the easiest sites for microbial colonization. Microbes are able to colonize on the walls of historical monument by forming biofilms or patinas. Biofilms of microbial cells degrade the asthetic value of ancient buildings and require lots of time and investment to eliminate them. The primary colonizers of stone monuments are seen as photosynthetic microorganisms (algae and Cyanobacteria). Many of the species of Cyanobacteria are able to tolerate climate extremes and hence require more attention to be study. It has been seen that Cyanobacteria need only light and water to grow and therefore can grow endolithically (4). They can tolerate extreme climate conditions. Cyanobacteria are able to survive on exposed surfaces of ancient walls and are greatly involved in biodeterioration of monuments (2). This makes more concern about the study of Cyanobacteria as they are the agents of stone property degradation in future. Kota city is situated near the bank of river Chambal. Kota have semi-arid climate throughout the year. Garh Palace also known as City palace is a historic place since foundation laid in 1264 C.E by Prince Jait Singh of Bundi. It comprises number of architectural structures of ancient times. It has a huge museum which displays royal arms and ammunitions. Another ancient monument is Abheda Mahal situated near Chambal River, built by Ummed Maharaj. It is also rich in heritage with beautiful pond inside it. Total 12 species isolated from the walls of monuments generally belongs to genus Nostoc (1), of the genera Gloeocapsa (2), Gloeothece (2), Aphanothece (1), Chroococcoccus (2), Chroococcidiopsis (1), Tolypothrix (1) and Aulosira (1) of Cyanophyta. This study investigates the prevalence and impact of epilithic cyanobacteria on the walls of Garh Palace and Abheda Mahal in Kota, Rajasthan. Emphasizing the need for understanding cyanobacteria as role in biodeterioration, the study identifies various species colonizing these historical monuments and discusses their potential in degrading stone properties. Such insights are crucial for the preservation of heritage sites.***

Keywords: Biofilms, Endolithically, Biodeterioration, Monuments, Cyanobacteria, Photosynthetic

1. Introduction

Cyanobacteria are gram-negative bacteria. Fine types of Cyanobacteria have been identified as toxin producers, including two strains of Anabaena flosaquae, Aphanizomenon flosaquae, Microcyst awruginosa and Nodularia species. Cyanobacteria constitute a phylogenetically coherent group of evolutionarily ancient, morphologically diverse and ecologically important phototropic bacteria (8). They are identified by their ability to carry out oxygenic photosynthesis. It has generally been observed that Cyanobacteria synthesis chlorophyll as a major photosynthetic pigment and phycobiliproteins as light-harvesting pigments. Cyanobacteria are able to grow using CO_2 as the sole source of carbon, which they fix using primarily the reductive pentose phosphate pathways (5). Their chemoorganotrophic potential is restricted to the mobilization of reverse polymers during dark period. Cyanobacteria have left fossil remains as old as 2000-3500 million years and they are believed to be ultimately responsible for Oxygen of earth atmosphere. During their evolution they gave rise to the plastids of algae and higher plants. Many of the Cyanobacteria show the ability of performing photosynthesis as well as nitrogen fixation. They are found to be colonized all types of stone surfaces like limestone, marble, rocks etc. (1).

Today, Cyanobacteria make a significant contribution to the global primary production of the ocean and became locally dominant primary producers in many extreme environments. Their global biomass has been estimated to exceed 10^{15} gm. of wet biomass, most of which is accounted for by the marine unicellular genera Prochlorococcus and Synechococcus, the filamentous genera Trichodesmium as well as the terrestrial Microcoleus vaginotous and chroococcidiopdis sp. of barren land. As Cyanobacteria are able to survive on almost all types of habitats and on exposed surfaces of ancient walls, they are greatly involved in biodeterioration of monuments (6). This makes more concern about the study of Cyanobacteria as they the agents of stone property degradation in future.

Kota which was previously known as Kotah is situated near Chambal River. It has almost average rainfall of 660.6 mm. Garh Palace Kota (Figure 1.a) is a fort cum palace situated in the Kota district of Rajasthan. This place, also known by various other names like City Palace, Kota Garh, and Kota Fort, was founded in the year 1264 AD by Jait Singh after defeating the Chieftan Kotya Bhil of the local community. Located on the banks of River Chambal, this fort is blessed with numerous heritage collection of canons, paintings, armors, places, instruments, dresses, manuscripts, kiosks and buildings belongs to the times of Rajput rulers from 13th onwards. The scenic view of River Chambal from the top of the Fort is really scintillating.

Abheda Mahal (Figure 1.b) situated near the the Chambal river of Kota of Rajasthan. The palace was established by Ummed Singh JI Maharaj, the palace has a great history and

many different stories behind its establishment. The main building of Abheda Mahal is made up of yellowish color and has 3-storeys that mainly consist of long corridors, arched pillars, kiosks and small compartments or room. A lake named as the Abheda Jheel is situated on the backside of the Mahal that was plenty of lotuses grown in it. The lake was built by Raja Dheeraj of Kota in the year 1346. Crocodiles were kept as pets in the ponds on the backside of the Mahal and they were named too, during the reign of the kings. The walls of Mahal are decorated with the painting that depicts the rich culture, heritage, flora and fauna of the state at that time.

Figure 1 (a) Garh Palace
Source: https://www.tourmyindia.com/states/rajasthan/garh-palace-kota.html

Figure 1 (b) Abheda Mahal
Source: https://www.facebook.com/Kota.City.IND/photos/a.1315335245163758/3800073500023241/?type=3

2. Objectives

The main objectives of our work are to study the Cyanobacteria on the outer surfaces of historical monuments (Garh Palace and Abheda Mahal) of Kota, Rajasthan. The following objectives of research work are:

1) To study the different types of Cyanobacteria on the walls and surfaces of Garh Palace and Abheda Mahal.
2) To isolate the organisms, particularly Cyanobacteria, present on the selected monuments walls.
3) To identify the Cyanobacteria and its types by general microbiological laboratory processes.
4) To know and study about the Cyanobacteria present in the environment of Kota especially isolated from monuments.

3. Materials and Methodology

Materials

- Glasswares – Bottles, Test tubes, Conical flasks (150 mL), Glass rods, Measuring cylinder.
- Chemicals – BG-11 broth medium, Safranin, Glycerin.
- Equipment – Laminar air flow, Autoclave, Hot air oven, Light microscope, pH meter, Burner.
- Other Materials – Cotton, Aluminium foil, Test tube stand

Methods

Collection of sample:

- For collecting samples to isolate Cyanobacteria, firstly we visited the historical places of Kota City. Two places for the sample collection are: Garh Palace of Kota and Abhera Mahal.
- We went to both the places one by one. In the first round of sample collection we went to the Garh Palace. There we took a visit of entire palace. During the visit we took the sample from the wall of Garh Palace by simply scratching the walls gently with the help of spatula or blade. Then we collect that sample in a glass bottle filled with distilled water and covered by cap.
- In the second round of sample collection we went to Abheda Mahal where we took a visit of entire Mahal and collect the sample into the sample bottle and stored.

Preparation of Media:

- We prepared BG-11 media to grow Cyanobacteria from the sample.
- For prepare media we took prepared BG-11 broth and dissolved it into sterile distilled water.
- Then we heat the media on hot plate and steer it with clean glass rod.
- After media preparation we checked its pH and maintained it.

Preparation of sample:

We have collected the sample from Garh Palace and Abheda Mahal of Kota. The dilution of each sample done in sterile distilled water and prepared serial dilution upto 10^{-2} by the following steps –

- Take 1 mL of sample solution into 9 mL of dilution blank and label it as 10-1 as shown in Fig. 2 (a)(b).
- Mix the contents by rolling the tubes back and forth.
- Small volume of sample from the dilution tube inoculated in the conical flask of 150 mL containing BG-11 medium.

Inoculation of sample:

- Sample after dilution inoculated in the BG-11 medium Fig.2 (c)(d).
- BG-11 medium with inoculated flask placed in sunlight area for 7 days by covering them with cotton plug.

Staining:

For the staining of our growth we used simple staining technique including following steps:-

- Take 5 mL of growth suspension into a beaker.

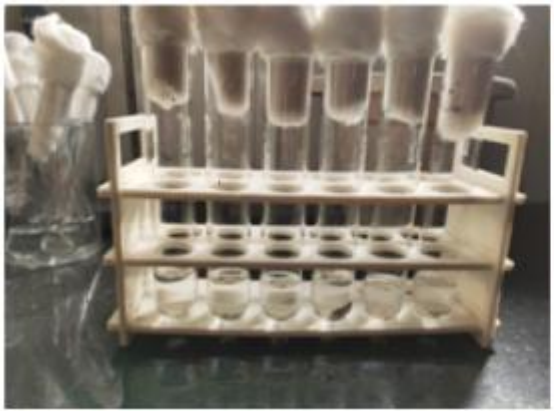
- Add 1-2 drops of safranin stain, shake well and leave it to stain.
- Take 1-2 drop of it onto a clean glass slide and 2 drops of glycerin then cover it by cover slip.
- Visualize the slide under light microscope.

4. Observation & Results

Most of the microbes found on the biofilms of rock surfaces are algae and Cyanobacteria. Cyanobacteria are generally dominant photosynthetic cells found on the stone monuments. A total of 12 species of Cyanobacteria in the sample of the wall of Garh palace and Abheda Mahal which belongs to the genus Nostoc(1), of the genera Gloeocapsa (2), Gloeothece (2), Aphanothece (1), Chroococococcus (2), Chroococcidiopsis (1), Tolypothrix (1) and Aulosira (2) was identified (Table 1, Fig. 3). It has been studied that rock-made historical monuments of Kota close to river Chambal mainly biodeteriorated by Cyanobacteria as moisture allows them to grow well. Dark patches on the external surfaces of monuments shows the biofilm load of Cyanobacteria (7). An attempt was made to study the biofilm of historical monuments of Kota as they are the heritage of our city.

Table 1: showing the species and genus presence (+) and absence (-) on the selected study area; Abheda Mahal and Garh Palace

S. No.	Genus/ species	Abheda Mahal	Garh Palace
1.	Aulosira		
	A. *terrestris*	+	+
	A. implexa	-	+
2.	Aphanothece		
	A. Bullosa	+	-
3.	Gloeothece		
	G. confluens	+	-
	G. palea	+	+
4.	Gloeocapsa		
	G. bisformis	+	+
	G. gigas	+	+
5.	Nostoc		
	N. pruniforme	+	-
6.	Chroococococcus		
	C. batavus	+	+
	C. hansgirgi	+	-
7.	Chroococcodiopsis		
	C.gigantea	-	+
8.	Tolypothrix		
	T. nodosa	+	+



(a) Serial Dilution of collected sample

(b) Test Tube of sample diluted

(c) Cyanobacterial growth on BG-11 of Garh Palace

(d) Cyanobacterial growth on BG-11 of Abheda Mahal

Figure 2: showing sample dilution and isolation of cyanobacterial cells on media BG-11

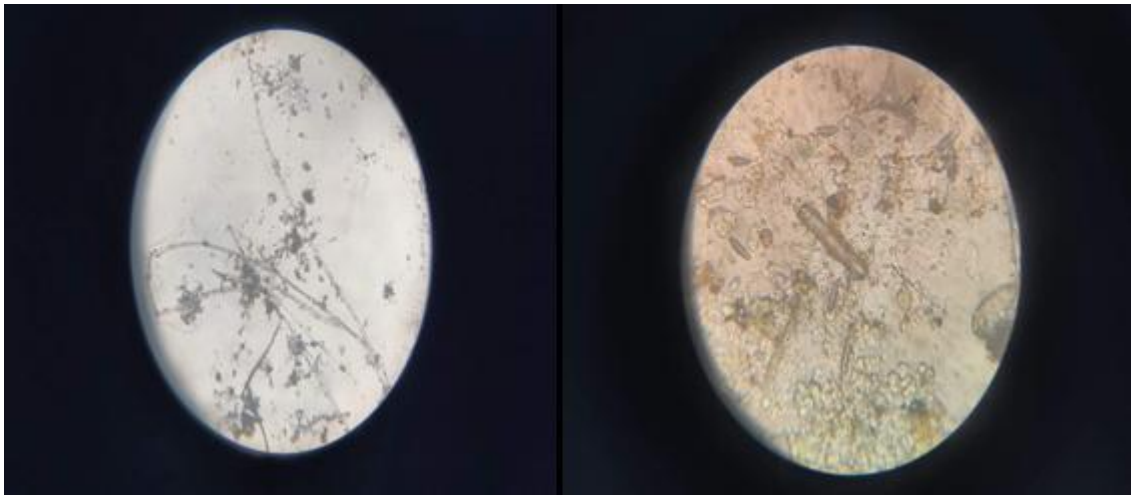


Figure 3: Microscopic visuals of various genus belonging to Cyanobacteria at 45X under light microscope.

5. Discussion and Conclusion

Several studies are going on for the protection of our cultural heritage. Studies primarily focus on the cause of deterioration of historical monuments and their remedial processes. A huge variety of organisms like Algae, Bacteria, Cyanobacteria etc. are colonizing on the walls of monuments and thereby degrading asthetic value of the historical monument (3). Several factors allow the growth of microbes on the interior as well as exterior walls of monuments including environmental factors, chemical nature of the stones and physical factors (9). In India generally monuments of ancient times were built near the bank of rivers. This promotes the growth and colonization of microbes on them. The present study deals with the study of microbes especially Cyanobacteria on the surfaces of monuments. So during our studies we are able to study various species of Cyanobacteria on Garh Palace and Abheda Mahal of Kota city. All the samples show growth of Cyanobacterial colonies on them. The purpose of this study is to analyze the variety and impact of epilithic cyanobacteria on the historical monuments of Kota, Rajasthan, emphasizing the need for conservation strategies to protect these heritage sites from biodeterioration. The study concludes that a diverse range of cyanobacteria species contributes to the biodeterioration of the Garh Palace and Abheda Mahal in Kota. This highlights the urgent need for conservation efforts to protect these historical monuments from microbial damage.

Author Contributions

Pooja Sharma designed and drafted the work. Neerja Shrivastava contributed in the final revision of the manuscript.

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Conflict of Interest

The authors declare that the research have no conflict of interest.

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