

## DISTRIBUTION OF EPIZOIC CYANOBACTERIA ON MOLLUSCAN SPECIES OCCURRING AT A ROCKY SHORE (BULEJI), KARACHI, PAKISTAN

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### ABSTRACT

The present study is the part of investigation involving identification of cyanobacteria from coastal water of Karachi. As report on the distribution cyanobacterial epizoic in coastal area of Pakistan is uncommon. In this study sixty five species of cyanobacteria were identified from various marine molluscan species collected from Buleji, a coast of Karachi. Most of cyanobacteria were from the order Chroococcales and Nostocales and only a small number of species were found to belong to Chamaesiphonales and Pleurocapsales. The epizoic species consists of 26 unicellular and 39 filamentous non-heterocystous cyanobacterial species. Heterocystous forms were not found during these studies. Cyanobacterial species showed a variable capacity to grow in various type of media. Also, some cyanobacteria were observed specifically on particular molluscs species, while other were common on all species.

**Key-words:** Cyanobacteria, epizoic, molluscas, Buleji, Karachi.

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### INTRODUCTION

Symbiosis is an interrelationship between two organisms which range from simple parasitism to physiological interdependence of the species (Green, 1977). The symbiotic relationship on the degree of association is termed as commensalism, inequalism, mutualism and parasitism. In commensalism, one member achieves the benefits but does not cause harm its host. Inequalim is a relationship where one member lives, in the body (digestive tract) or in the home of other member without being parasite. Mutualistic symbiosis benefits both partners, whereas parasitism refers to an association where one species live in or upon another and take nutrition from the host, where host is affected adversely.

Though symbiosis is common among organisms in both terrestrial and aquatic habitats but well developed in the marine environment, such as tropical water (Li, 2009; Charpy *et al.*, 2012; Díez *et al.*, 2007). In marine environment symbiosis can vary from random, casual or facultative association through more obligatory groupings that benefits one or both member of to finally those which are parasites (O'Brien *et al.*, 2005). Marine cyanobacteria have been recorded in a variety of symbiotic association with marine plants and animals, for example, species of the genera: Protozoa, Porifera, Polychaeta, Cnidaria and Mollusca, as well as marine algae and other plants (Adams, 2000; Rai *et al.*, 2000, 2002; Adams *et al.*, 2006; Bergman *et al.*, 2007). Cyanobacteria may grow on the surface of these organisms (epizoic & epiphytic) and they infect and proliferate inter and intracellularly in these organisms (endosymbiotic).

In the marine environment, symbioses was observed between cyanobacteria and sponges, ascidians (sea squirts), echiuroid worms, diatoms, dinoflagellates and a protozoan (Carpenter, 2002). The interaction between cyanobacteria and algae is restricted to shallow, sub-tidal or intertidal benthic and pelagic zone of the ocean (Carpenter and Capone, 1992).

### CYANOBACTERIAL ASSOCIATION WITH MARINE FAUNA

The symbiotic association of epizoic cyanobacteria with sponges has long been known (Alex *et al.*, 2012; Usher, 2008; König *et al.*, 2006; Thajuddin and Subramanian 2005; Thacker and Starnes, 2003; Feldman, 1933) and *Oscillatoria* and *Aphanocapsa* species reportedly occur endosymbiotically (Caroppo *et al.*, 2012; Pagliara and Caroppo, 2010; Sara 1971; Vacelet, 1971; Berthhold *et al.*, 1982; Wilkinson, 1978). Cyanobacteria has also been reported associated with ascidians (Wegley *et al.*, 2007; Lafargue and Duclaux, 1979; Sybesma and Bak, 1979; Lewins, 1977), cnidarians (Charpy *et al.*, 2012; Costa *et al.*, 2004) copepode (O'Neil, 1998; O'Neil and Roman, 1992; Magalef, 1953), fish guts beaks and scales (Aleem, 1980; Tsuda *et al.*, 1972; Prescott, 1969), rotifers (Huber-Pestalozii and Naumann, 1929), Polyzoa (Gerdes, 2005; Desikachary, 1959; West and Annandale, 1911), sea urchin

(Mortensen and Rosenvinge, 1934), polychaete tubes and molluscan shells (Radea *et al.*, 2010; Pantazidou, 2006; Aleem, 1980; Park and Moor, 1935; Fritsch, 1945).

There are few studies on epizoic cyanobacteria from Pakistan and these are on freshwater fauna. Cyanobacterial species *Oscillatoria anguina*, *Phormidium fragile* and *P. tenue* were reported from the shell of turtle, *Trionyx gagiticus curier* (Anjum *et al.*, 1980). In another study the cyanobacterial species, *Lyngbea martensia*, *Microcystis aeruginosa*, *Oscillatoria formosa*, *O. princeps*, *Phormidium fragile* and *Anabaena* sp. were reported on shells of snails (*Lymnaea* sp. (Hussein and Anjum, 1982). No information is available on cyanobacterial association with marine organisms inhabiting the Pakistani coast. In the present study the biodiversity of cyanobacteria and its association with marine fauna has been explored.

## MATERIALS AND METHODS

The mollusks species belonging to the three classes Amphineura, Gastropoda and Bivalvia were collected in plastic bags separately from rocky shore. Lists of mollusk studied is given in Table 2. The loosely attached meiofauna and flora on each shells was washed with sterilized filtered sea water using soft brush. Each shell was then scraped by sterilized scalpel. Collected material was inoculated in three different media i. e. ASNIII, MN and Miquels media (Bano and Siddiqui, 2003). The samples were incubated in constant light at  $30 \pm 2^\circ\text{C}$ . Cultures were periodically observed for any visible growth of cyanobacteria. Actively growing material was taken with sterilized wire loop and smeared on glass slide and observed using a light microscope. Scraping from shells were also observed directly under a microscope. The taxonomic assessment was done according to Rippka *et al.*, (1979), Desikachary, (1959), Anagnostidis and Komarek, (1985, 1988) and Komarek and Anagnostidis, (1986, 1989).

## RESULTS

In the present study a survey of cyanobacterial flora association with 1 species of amphineura, 8 species of gastropods and 1 species of bivalve were carried out (Table 1 and 2). A total of 65 species of cyanobacteria were identified belonging to 21 genera, 4 orders and 7 families. All the epizoic species are new record for the coastal waters of Pakistan and among the 65 species of cyanobacteria, 26 were unicellular and 39 were non-heterocystous filamentous forms (Table 1). Among the four orders of Cyanobacteria associated with mollusc were order Nostocales, represented by 40 species of cyanobacteria followed by the order Chroococcales representing 17 species. In the order Pleurocapsales and Chaemaesiphonales the number of species were 2 and 7 respectively (Table 1 and 2). The association between cyanobacteria and molluscan species showed that the highest numbers of cyanobacterial (36 species) were present on the shell of *Xancus pyrum* and the lowest number (8 species) was recorded from *Pecten crasiocostatus*. (Table 1 and 2). The other gastropod species, *Nerita albicilla*, *Turbo coronatus*, *Cerithium carbonarium* and *C. hanelyi* showed the presence of 15, 14, 13 and 12 cyanobacterial species, respectively. There were certain species of mollusc, that is, *Cerithium rubus*, *Monodonta australis*, and *Pecten crasiocostatus* which showed no association with cyanobacterial species belonging to the orders Chroococcales, Pleurocapsales and Chaemaesiphonales, respectively (Table 1 and 2).

Cyanobacterial species attached to molluscan species were compared with the species observed from the other habitats of rocky shore (Bano and Siddiqui, 2003). The comparative data showed that that thirteen species were specifically associated with molluscan shells and were not found in other habitats of rocky shore.

Molluscan-species-specific association with cyanobacteria was also observed in the present study. It may be noted that out 65 epizoic species 33 species showed a molluscan species-specific association, that is, they grow on the shell of only one of the ten species of mollusc studied. Among the 33 species-specific, nearly 50% were recorded on the shell of *Xancus pyrum* (17 species) (Table 1 and Fig. 1). Similarly cyanobacteria species specificity was as follows for *Chiton oceanica*, (3 species), *Monodonta australis* (1 species), *Planaxis sulcatus* (5 species), *Nerita albicilla* (2 species), *Turbo coronatus* (2 species), *Cerithium rubus* (1 species) and *C. carbonarium* (2 species) (Table 1). *Pecten crasiocostatus* and *C. hanelyi* had no cyanobacterial species that is specific on their shells. The species-specific cyanobacteria were represented by both unicellular and filamentous forms. The maximum number of species-specific cyanobacteria were classified under genus *Phormidium* (9 species) and genus *Oscillatoria* (5 species). Three species each were represented in the genera *Planctothrix* and *Chroococcus*. The genus *Gloeocapsa* represented two such species, and only one species each was included in genera *Gloeothoece*, *Synechococcus*, *Aphanothece*, *Dermocarpa*, *Borzia*, *Pseudoanabaena*, *Lyngbea* and *Hormoscilla* (Table 1). Cyanobacterial species *Myxosarcina spectabilis*, *M. burmensis* and *Phormidium amplivaginatum* appeared to be associated with all molluscan species. There are some 15 species which commonly grow on four or more types of mollusk (Table 1).

Table1. Distribution of cyanobacterial species on ten different species of molluscs inhabiting a rocky shore near Karachi.

Cyanobacteria species	Medium										
		<i>Chiton oceanica</i>	<i>Xancus pyrum</i>	<i>Monodonta australis</i>	<i>Planaxis sulcatus</i>	<i>Nerita albicila</i>	<i>Turbo coronatus</i>	<i>Cerithium rubus</i>	<i>Cerithium hanelyi</i>	<i>Cerithium carbonarium</i>	<i>Pecten crasioscostatus</i>
<b>Ckroococcales</b>											
<i>Synechocystis pevalekii</i>	ASNIII, MN, MM	-	+	-	+	-	-	-	+	-	+
<i>S. aquatilis</i>	MN	-	-	-	-	-	-	-	+	-	+
<i>Gloeocapsa gelatinosa</i>	ASNIII, MM	+	+	-	-	-	-	-	-	-	-
<i>G. Compacta</i>	ASNIII	-	-	-	-	-	-	-	-	+	-
<i>G. cripidinum</i>	MN	-	-	-	-	-	+	-	-	-	-
<i>Chroococcus cohaerence</i>	MN, MM	-	+	+	-	-	-	-	-	-	-
<i>C. turgidus</i>	MM	-	+	-	-	-	-	-	-	-	-
<i>C. minutus</i>	ASNIII, MM	-	-	-	+	-	-	-	-	-	-
<i>C. montanus</i>	MN	-	-	-	-	+	-	-	-	-	-
<i>Synechococcus elongatus</i>	MN	-	+	-	-	-	-	-	-	-	-
<i>Gloeothece palea</i>	MM	-	+	-	-	-	-	-	-	-	-
<i>G.samoensis</i>	ASNIII, MM	-	-	+	-	-	+	-	-	+	-
<i>G. rhodochlymys</i>	ASNIII, MN, MM	-	-	-	+	+	+	-	+	+	-
<i>G.fusco-lutea</i>	MM	-	-	-	+	-	-	-	-	+	-
<i>Aphanocapsa littoralis</i>	ASNIII, MN	+	+	-	-	-	+	-	+	-	-
<i>Aphanthece microscopica</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>Merismopedia eleganse</i>	ASNIII, MN, MM	+	-	+	+	+	-	-	-	-	-
<b>Chamaesiphonales</b>											
<i>Chroococidiopsis indica</i>	ASNIII, MN	-	+	-	+	-	-	-	-	-	-
<i>Dermocarpa leibleiniae</i>	ASNIII, MN, MM	+	+	-	-	+	-	+	-	+	-
<i>D. olivacea</i>	ASNIII, MN, MM	-	-	-	-	+	-	-	-	-	+
<i>D.flahaultii</i>	ASNIII, MN	-	-	-	-	+	+	-	-	-	-
<i>D. parva</i>	MN	-	-	-	-	+	-	-	-	-	-
<i>D. clavata</i>	ASNIII, MM	-	-	-	-	+	-	+	-	-	-
<i>D.sphaerica</i>	ASNIII, MN, MM	-	-	-	-	-	+	+	+	-	+
<b>Pleurocapsales</b>											
<i>Myxosarcina spectabilis</i>	ASNIII, MN, MM	+	+	+	+	+	+	+	+	+	-
<i>M. burmensis</i>	ASNIII, MN, MM	+	+	+	+	+	+	+	+	-	-

## Nostocales

<i>Borzia tricularis</i>	MM	-	+	-	-	-	-	-	-	-	-
<i>Komvophoron anabaenoides</i>	ASNIII, MN	-	+	+	-	-	-	-	-	-	-
<i>K. schmidlei</i>	ASNIII, MN, MM	-	+	-	-	+	+	-	-	+	-
<i>K. minutum</i>	ASNIII, MN, MM	-	+	+	+	+	+	-	-	+	+
<i>K. Crassum</i>	ASNIII, MN	-	-	-	-	+	+	-	-	-	-
<i>Psuedoanabaena galeata</i>	ASNIII, MN, MM	-	-	+	+	-	-	+	+	+	+
<i>P. limnetica</i>	MM	-	-	-	-	-	+	-	-	-	-
<i>P. lonchoides</i>	ASNIII, MM	-	-	-	-	-	-	+	+	-	-
<i>Lyngbea preleganse</i>	MM	-	-	-	+	-	-	-	-	-	-
<i>Hormoscilla pringsheimii</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>Oscillatoria limnetica</i>	ASNIII, MN, MM	+	+	-	-	-	-	-	-	-	-
<i>O. tenuis</i>	ASNIII	+	-	-	-	-	-	-	-	-	-
<i>O.amphigranulata</i>	ASNIII, MN	+	-	-	-	-	-	-	-	-	-
<i>O. minnesotensis</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>O. annae</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>O.nitida</i>	MN	-	+	-	-	-	-	-	-	-	-
<i>O. pseudogaminata</i>	ASNIII, MM	-	+	+	+	-	-	-	-	+	-
<i>Spirulina labyrinthiformis</i>	ASNIII	+	+	-	-	-	-	-	-	-	-
<i>S. subsalsa</i>	ASNIII, MM	-	+	-	-	-	-	-	-	-	-
<i>S. major</i>	MM	-	+	-	-	-	-	-	-	-	-
<i>Tychonerhodonemama</i>	ASNIII	+	-	-	-	-	-	-	-	-	-
<i>Planktothrix mougeotii</i>	ASNIII	-	-	+	-	-	-	-	-	-	-
<i>P. agardhii</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>P. planctonica</i>	MM	-	-	-	+	-	-	-	-	-	-
<i>Phormidium purpurascence</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>p. tenue</i>	ASNIII, MN	-	+	-	-	-	-	-	-	-	-
<i>P. insigni</i>	ASNIII	-	+	-	-	-	-	-	-	-	-
<i>P. angustissimum</i>	ASNIII, MN, MM	-	+	-	-	+	-	-	+	-	-
<i>P. corium</i>	MN	-	+	-	-	-	-	-	-	-	-
<i>P.kuetzingianum</i>	ASNIII, MN, MM	-	+	-	-	-	-	+	+	+	-
<i>P. africanum</i>	MN	-	+	-	-	-	-	-	-	-	-
<i>P.amplivaginatam</i>	ASNIII, MN, MM	-	+	+	+	-	+	+	+	+	+
<i>P. fragile</i>	ASNIII, MM	-	+	-	+	+	-	-	-	-	+
<i>P. breve</i>	MM	-	-	-	+	-	-	-	-	-	-
<i>P. incrustatum</i>	MM	-	-	-	+	-	-	-	-	-	-
<i>P. retzii</i>	ASNIII, MM	-	+	-	+	-	-	-	-	-	-
<i>P.mucicola</i>	ASNIII, MN	-	-	-	-	-	+	+	-	-	-
<i>P. luteum</i>	MM	-	-	-	-	-	-	+	-	-	-
<i>P. Papyraceum</i>	MM	-	-	-	-	-	-	-	-	+	-

Table 2. Total number of species observed on surface of molluscan species. Species are grouped according to their taxonomic order.

Cyanobacterial orders	<i>Chiton oceanica</i>	<i>Xancus pyrum</i>	<i>Monodonta australis</i>	<i>Planaxis sulcatus</i>	<i>Nerita albicila</i>	<i>Turbo coronatus</i>	<i>Cerithium rubus</i>	<i>Cerithium hanelyi</i>	<i>Cerithium carbonarium</i>	<i>Pecten crasiocostatus</i>
<b>Chroococcales</b>	3	8	3	5	3	4	-	4	4	2
<b>Chamaesiphonales</b>	1	2	-	1	5	2	3	1	1	2
<b>Pleurocapsales</b>	2	2	2	2	2	2	2	2	1	-
<b>Nostocales</b>	5	24	6	10	5	6	6	5	7	4
<b>Total</b>	11	36	11	18	15	14	11	12	13	8

Table 3. Lists of mollusk species studied for the epizoic species of cyanobacteria.

Amphineura	Gastropoda	Bivalvia
<i>Chiton oceanica</i>	<i>Xancus pyrum</i> <i>Monodonta australis</i> <i>Planaxis sulcatus</i> <i>Nerita albicila</i> <i>Turbo coronatus</i> <i>Cerithium rubus</i> <i>C. hanelyi</i> <i>C. carbonarium</i>	<i>Pecten crasiocostatus</i>

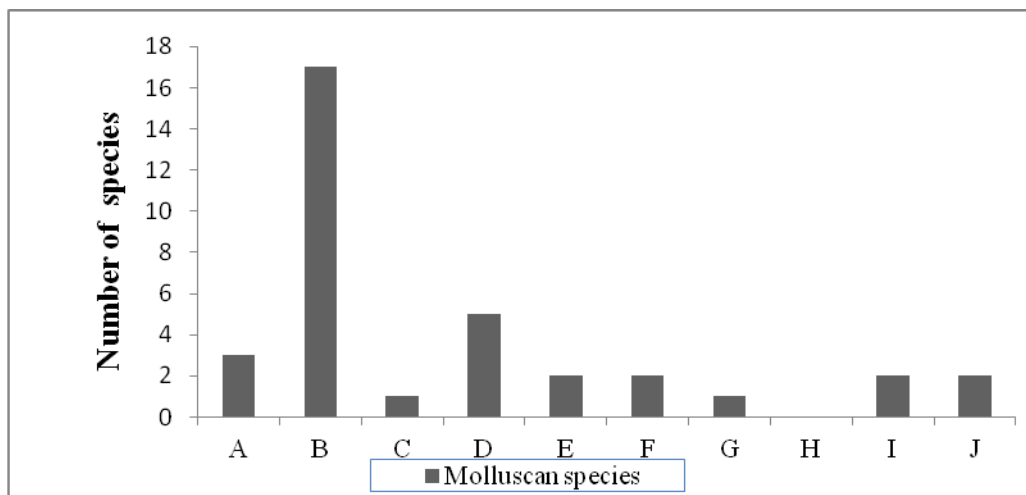


Fig. 1. Species-specific cyanobacteria associated with A-*Chiton oceanica*, B-*Xancus pyrum*, C-*Monodonta australis*, D-*Planaxis sulcatus*, E-*Nerita albicila*, F-*Turbo coronatus*, G-*Cerithium rubus*, H- *Cerithium hanelyi*, I-*Cerithium carbonarium*, J-*Pecten crasiocostatus*.

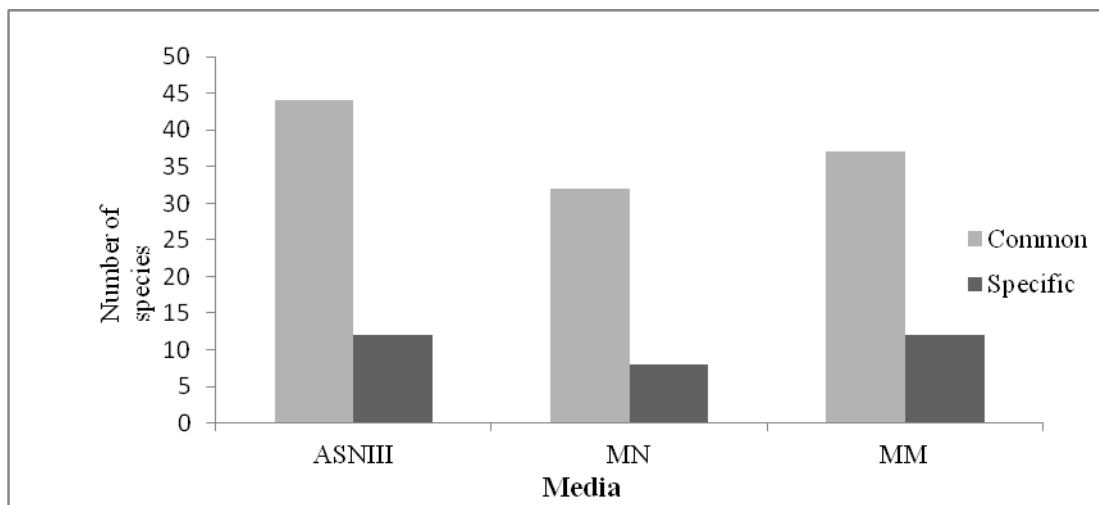


Fig. 2. Cyanobacterial species were common in all three media but some species showed up only in one specific medium.

For the growth of cyanobacteria species in culture three different types of media were used. Some species were more versatile as they appeared in all media and many showed up only in one or two types of media. The observation of the experiments were recorded (Table 1). The data indicates that the best media, in terms of number of species grown was ASNIII medium. A total of 44 species were able to grow in the medium. Whereas, MN and Miquel's media supported 32 and 37 species respectively. Some of the species showed up in all three media (15 species), and twelve species were observed exclusively in ASNIII, Miquel's media each. MN medium supports seven species that did not appear in the other two media (Fig.2). The species specifically growing in any particular media does not appear to be limited to one genera or particular orders. It rather an assorted assemblage of species suggested this to be an individual species characters.

## DISCUSSION

From the above result it is concluded that the molluscan shells are an excellent substratum for the attachment and growth of various cyanobacterial species. The species of mollusk selected were predominant at the intertidal zone of rocky coast (Buleji). Cyanobacterial growth were visible on most of the species by naked eye. Diverse species of cyanobacteria observed as epizoic, which have previously been reported as epiphytic, edaphic, epilithic and planctonic from India (Desikachary, 1959), and also as epizoic from freshwater habitats from Pakistan (Hussein and Anjum, 1982; Anjum *et al.*, 1980). The observed species have been reported from marine as well as freshwater habitats from different parts of the world (Aleem, 1980; Desikachary, 1959; Thajuddin and Subramanian, 1990, 1992, 1994; Santra and Pal, 1988; Shameel and Butt, 1984).

Large number of cyanobacterial species were associated with the shell of *Xancus pyrum* and other gastropod species, while less number with *Pecten crasiostatus* (Table 1 and 2). It is due to fact that *Xancus pyrum* mostly remain submerged in nutrient rich water in euphotic zone of the sea, similarly the other gastropod species are found in the intertidal zone and do not bury in the sand. This exposure to sunlight and nutrient favors the algal growth. Comparatively, the bivalve, *P. crasiocostatus* usually live buried in the sediment, where sufficient light may not be available and this hinders the growth of cyanobacteria on this shell.

*Myxosacina* species and *P. amplivaginatum* were attached on most of mollusk species. This could be due to species preferred the habitat.

The observed cyanobacterial species, such as *S. aquatilis*, *G. gelatinosa*, *C. minutus*, *C. turgidus*, *C. montanus*, *G. samoensis*, *A. microscopica*, *D. leibleiniae*, *D. parva*, *M. spectabilis*, *L. prelagans*, *O. tenuis*, *O. pseudogaminata*, *S. subsalsa*, *S. major*, *P. angustissimum*, *P. purpurascence*, *P. corium*, *P. retzii* and *P. tenue* are reported both as epilithic and edaphic forms from various types of soil and hard substrata in terrestrial and aquatic habitats, such as, river soil, cultivated and non-cultivated soil, mud mangrove swamps, beach rocks and stones surfaces (Desikachary, 1959; Aleem, 1980; Santra and Pal, 1988; Santra *et al.*, 1988; Maity *et al.*, 1987). *Phormidium fragile* and *P. tenue* have been reported as epizoic on freshwater turtle and snail shells (Hussein and Anjum, 1982; Anjum *et al.*, 1980). The comparative study of the organisms showed that above species preferred calcium rich or calcareous hard substrata for their settlement (Józef, *et al.*, 2015) In the marine environment calcareous shell of mollusks provide a

favorable habitat for attachments of cyanobacteria. Attachment of cyanobacteria and variability of species composition of associative cyanobacteria may simply be a function of variation in the habitat of gastropods as noted above. It seems true for many species growing on molluscan shells and also observed in other niches of Buleji coast. Also many of species reported here have been reported from marine and freshwater habitats from India (Desikachary, 1959; Aleem, 1980; Santra and Pal, 1988; Santra *et al.*, 1988; Maity *et al.*, 1987; Anand and Hooper, 1987; Thajuddin and Subramanian, 1990, 1992, 1994), and other parts of the world (Hussein and Khoja, 1993; Bauld, 1981; Hodgson and Abbot, 1992; Khoja, 1987).

The present study provide a basic information about epizoic cyanobacteria on various molluscan species inhabiting the rocky shore of Buleji and confirms the presence of many species reported from other parts of the world in the northern Arabian Sea bordering Pakistan. Epizoic cyanobacterial flora on organisms other than mollusca have not been studied nor the other coasts have been studied for the similar purpose in Paksitan. . Therefore, more research work need to be undertaken to get information about the diversity of epizoic cyanobacteria in the marine environment.

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