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REVIEW ARTICLE

Probable Role of Blue-green Algae in Maintaining Endemicity and Seasonality of Cholera in Bangladesh: a Hypothesis

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INTRODUCTION

Vibrio cholerae O1 has been isolated from aquatic environments in various parts of the world (1-14). However, little is known about its ecology, notably its interaction with the resident flora in an aquatic ecosystem. This gap in our knowledge is due to the fact that in the past, investigations of the biology of *V. cholerae* have been mainly based on the assumption that organisms causing cholera occurred only clinically in man. However, Colwell *et al.* (15) hypothesized that *V. cholerae* are autochthonous members of the bacterial flora of brackish water and estuarine systems. This hypothesis offered a new perspective on the ecology of this organism.

Marshall (16) suggested that the enhanced survival of microorganisms in aquatic environments is due to association with some non-living and living surfaces where they find the microenvironment favourable.

In the endemic areas of Bangladesh cholera epidemics occur twice a year. During epidemics, *V. cholerae* O1 are isolated from patients as well as from the surface water of pond, lake, river, etc. but disappears from the environment during the interepidemic seasons (17). The reservoirs or sites of survival and multiplication of *V. cholerae* during the interepidemic period are not known.

It is possible that *V. cholerae* O1 in the endemic environments in Bangladesh were not detected during interepidemic periods because the organism may hide in specially selected microhabitats not usually examined. Although this microenvironment has not been identified, we suggested earlier the possibility that the blue-green algae may provide such a microenvironment (18).

A good example of how reservoirs have gone undetected in Australia is shown by Rogers *et al.* (4). If there had not been an unusual drought, which resulted in the need for a supplementary water supply from a nearby river, the presence of *V. cholerae* O1 in Queensland rivers would have gone unnoticed. It was by accident that the rivers were found to be reservoirs of *V. cholerae* O1. The salinity of water in the rivers is low, in which *V. cholerae* O1 cannot survive long. Therefore, some sort of aquatic flora in this water system must have been supporting the survival of *V. cholerae* O1.

Islam *et al.* (19) demonstrated the long-term survival and multiplication of *V. cholerae* O1 inside the mucilaginous sheath of a blue-green alga *Anabaena variabilis*. We are not aware of any other studies which have shown such a long survival of *V. cholerae* O1 in fresh water in the laboratory. However, studies have demonstrated that *V. cholerae* O1 survive better with aquatic flora (14,20-30).

Our study, the first attempt to examine algae as possible reservoirs of cholera, clearly indicates that blue-green algae could act as a reservoir of *V. cholerae* O1 in the environment. Before this study, phycologists had observed the association of other Gram-negative bacilli and blue-green algae in the environment, and had tried to explain the various interdependent mechanisms of this co-existence (31). Although little information was provided on the identity of the Gram-negative bacteria, it is known that the most common bacteria isolated from blue-green algae include *Vibrio*, *Achromobacter*, *Aerobacter*, *Flavobacterium*, *Pseudomonas* and *Zoogloea* (32-34).

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Seasonality and endemicity of cholera in Bangladesh

The lower part of the Ganges Delta, thought to be the homeland of cholera, has a clear seasonal pattern of epidemics (30,35-38). The main cholera epidemic reaches its peak during the cooler months of the year, and a secondary smaller one occurs during the hot season. So far no satisfactory explanation has been given for this type of seasonal patterns of cholera.

The reason for maintaining endemicity probably lies in the mechanisms by which *V. cholerae* survives during interepidemic periods in these endemic areas.

Reservoirs of cholera

Many scientists at different times in various parts of the world have tried to identify reservoirs of cholera. The role of aquatic fauna as reservoirs of cholera has been discussed in detail (9,39-49). We will, therefore, concentrate here on aquatic flora, mainly blue-green algae as possible reservoir of cholera.

Blue-green algae as a possible reservoir

Islam *et al.* (50) studied the persistence of *V. cholerae* O1 in the mucilaginous sheath of a blue-green alga *Anabaena variabilis* in an artificial aquatic environment. They observed that *V. cholerae* O1 could survive in a culturable form in association with *A. variabilis* up to 120 h, but survived in water on which the alga was floating and in control water without alga more than 144 h (Fig. 1). Examination of the alga taken from the experimental flasks by phase contrast microscope after 10 days of this study showed that *V. cholerae* O1 entered into the mucilaginous sheath of *A. variabilis* (Fig. 2b). *V. cholerae* inside the mucilaginous sheath of *A. variabilis* became nonculturable but was found dividing by binary fission (Fig. 3a) and clustering around the heterocysts (Fig. 3b) which are the known sites for atmospheric nitrogen fixation. *V cholerae* O1 were detected inside the mucilaginous sheath up to 1.5 months using fluorescent antibody technique (Fig. 4b). This length of time is sufficient for *V. cholerae* O1 to persist during interepidemic periods. The viable but nonculturable state of *V. cholerae* O1 has also been demonstrated by others (50-55). It was also observed that *V. cholerae* O1 did not lose their ability to produce cholera toxin during survival in association with algae (57-59).

The production of mucinase by vibrios (60) may be a process by which plant and planktonic mucin are degraded in nature. This may be one of the factors which allow the association of *V. cholerae* O1 with mucilaginous blue-green alga *A. variabilis.*

Relevant information from studies on Gram-negative bacteria

Paerl and Gallucci (61) observed under the microscope that motile Gram-negative bacteria can easily discriminate heterocysts from vegetative cells. Bacteria interacting with the alga *A. oscillatorioides* filaments were observed to "bump" onto both vegetative and heterocyst cells but they did not adhere to either. When bacteria encountered heterocysts, flagellar rotation was often increased for 5 to 10 minutes. Then the bacteria attached to the heterocyst-vegetative cell junction; after this attachment, flagellar motion has stopped. Once attachment took place, both hosts and epiphytes started growing. It was observed that bacteria rarely penetrated cyanobacterial cell walls.

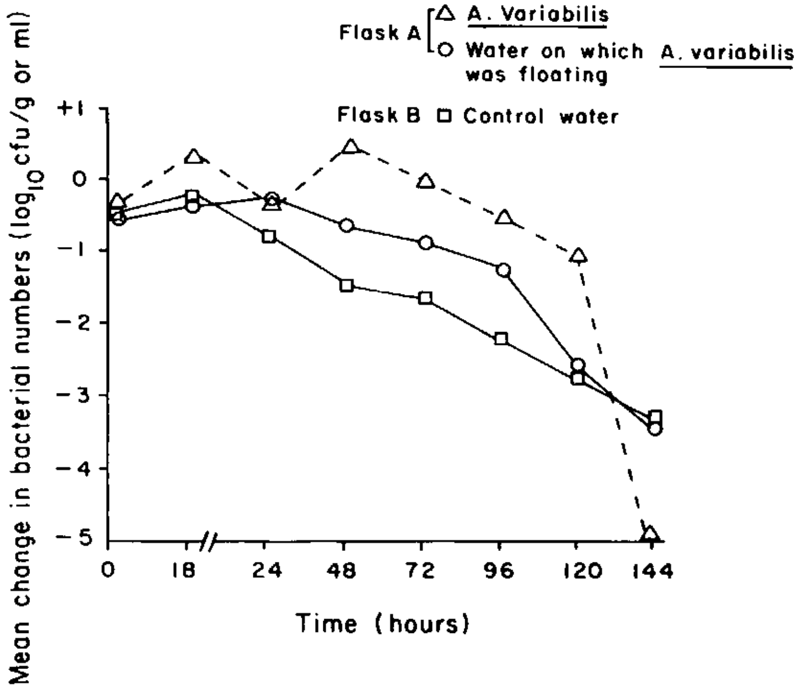


Fig. 1. Recovery of *V cholerae* O1 from *A. variabilis*, water on which *A. variabilis* was floating and control water of 0.05% salinity on TSA media. Flask A: *A. variabilis*, water on which *A. variabilis* was floating; Flask B: control water

Chemotaxis played a key role in the establishment and maintenance of cyanobacterial and bacterial association (61,62). Products of N_2 fixation, which were excreted at heterocyst-vegetative cell junctions were responsible for attracting diverse naturally occurring heterotrophic bacteria. Amino acids may be chemotactic agents since they are excreted by N_2-fixing *Anabaena.*

These studies with other bacteria demonstrated that heterotrophic bacterial and filamentous blue-green algal association may involve two processes. Firstly, the bacteria attach to the filament and then they utilize the algal metabolites as nutrients. Second, the attachment is initiated by chemotaxis and shows site and species specificity.

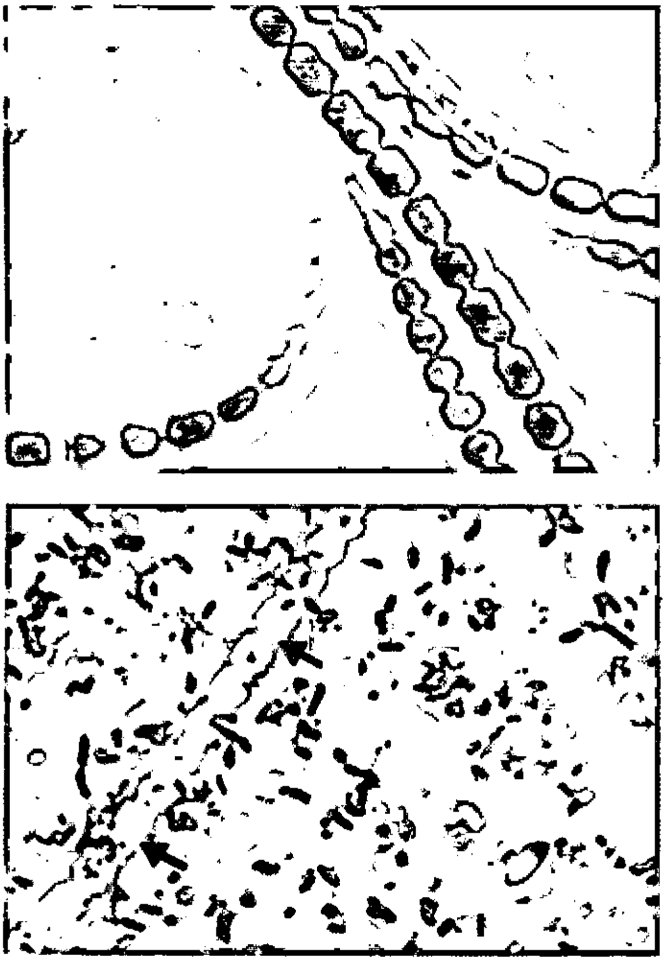


Fig. 2. (a) Phase-contrast microscopy showing *A. variabilis* not infected with *V. cholerae* O1. Original magnification X630. (b) Phase-contrast microscopy showing *V. cholerae* O1 inside mucilaginous sheath of *A. variabilis*. Arrows show a filament of *A. variabilis*. Original magnification X630

Field studies of *V. cholerae* O1 and blue-green algae

On the basis of all the above mentioned findings, field studies were carried out by Islam *et al.* (63) to detect *V. cholerae* O1 in blue-green algae from the aquatic environment of Bangladesh. They collected phytoplankton samples every 15 days between May 1988 and April 1989 from a pond in Dhaka city which is used for bathing, washing, swimming and occasionally drinking purposes. The phytoplanktons were mainly *Anabaena* sp., *Euglena* sp. and *Phacus* sp. *V. cholerae* O1 was detected by immunofluorescence in the mucilaginous sheath of *Anabaena* sp. in 16 out of 24 plankton samples (Fig. 5). *V. cholerae* O1 could be detected only in association with *Anabaena* sp. and not with other algae, e.g. *Euglena* sp. and *Phacus* sp. collected from the pond.

Fig. 3 (a) Phase-contrast microscopy showing multiplication of *V. cholerae* O1 inside mucilaginous sheath of *A. variabilis* O1. Arrow shows dividing cell of *V. cholerae* O1. Original magnification X630. (b) Phase-contrast microscopy showing aggregation of dividing cells of *V. cholerae* O1 on the detached heterocyts. A. Aggregated *V. cholerae* O1, H, heterocyst. Original magnification X630

This study clearly demonstrated an association between *V. cholerae* O1 and a blue-green alga *Anabaena* sp. in the natural aquatic environment in Bangladesh. These findings suggest that *V. cholerae* O1 may have a preference for association with blue-green algae, particularly *Anabaena* sp. or related species which have mucilaginous sheaths around them.

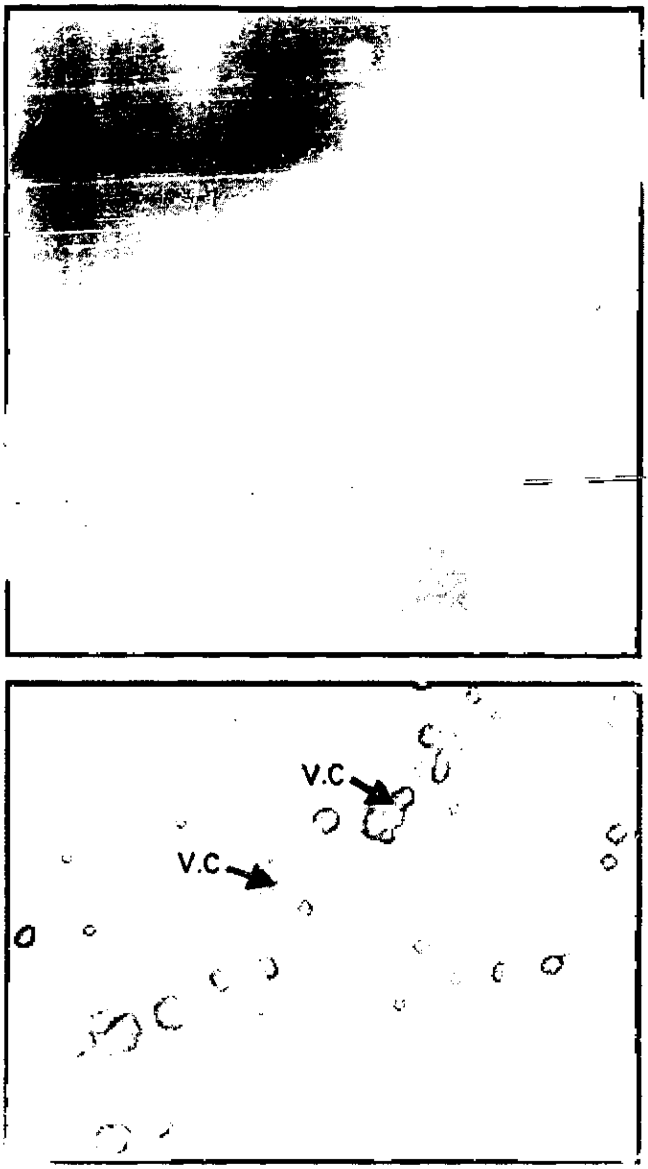


Fig. 4. (a) Fluorescent microscopy of *A. variabilis* not infected with *V. cholerae* O1. Original magnification X630. (b) Fluorescent microscopy of *A. variabilis* infected with *V. cholerae* O1. V.C., *Vibrio cholerae* O1. Original magnification X630

Extracellular products of blue green-algae

It is important to determine what are the main contributory factors that help *V. cholerae* O1 to survive so long inside the mucilaginous sheath of *Anabaena* spp. The first and foremost necessity for long term survival of *V. cholerae* is the availability of sufficient nutrients to maintain growth and reproduction. Therefore, it would be useful to establish what nutrients are produced by the blue-green algae could be used by *V. cholerae* O1.

Fogg (64) studied the nature of the organic nitrogenous products, found in filtrates from cultures of nitrogen fixing *Anabaena cylindrica*. He observed that the total amount of combined nitrogen liberated in extracellular form is relatively high in young cultures. To demonstrate the presence of amino acids, chromatographic analyses of the extracellular nitrogenous substances were carried out by Fogg with concentrated 12- and 28-day culture filtrates. It was observed that the filtrates contained various amino acids such as glutamic acid, alanine, serine, threonine, glycine, tyrosine, valine, leucine, phenylalanine and aspartic acid. Among these glutamic acid was found to be present in the highest quantity.

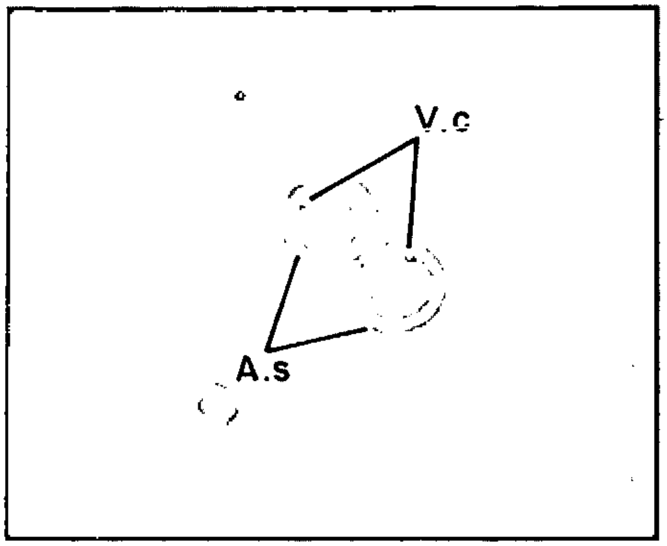
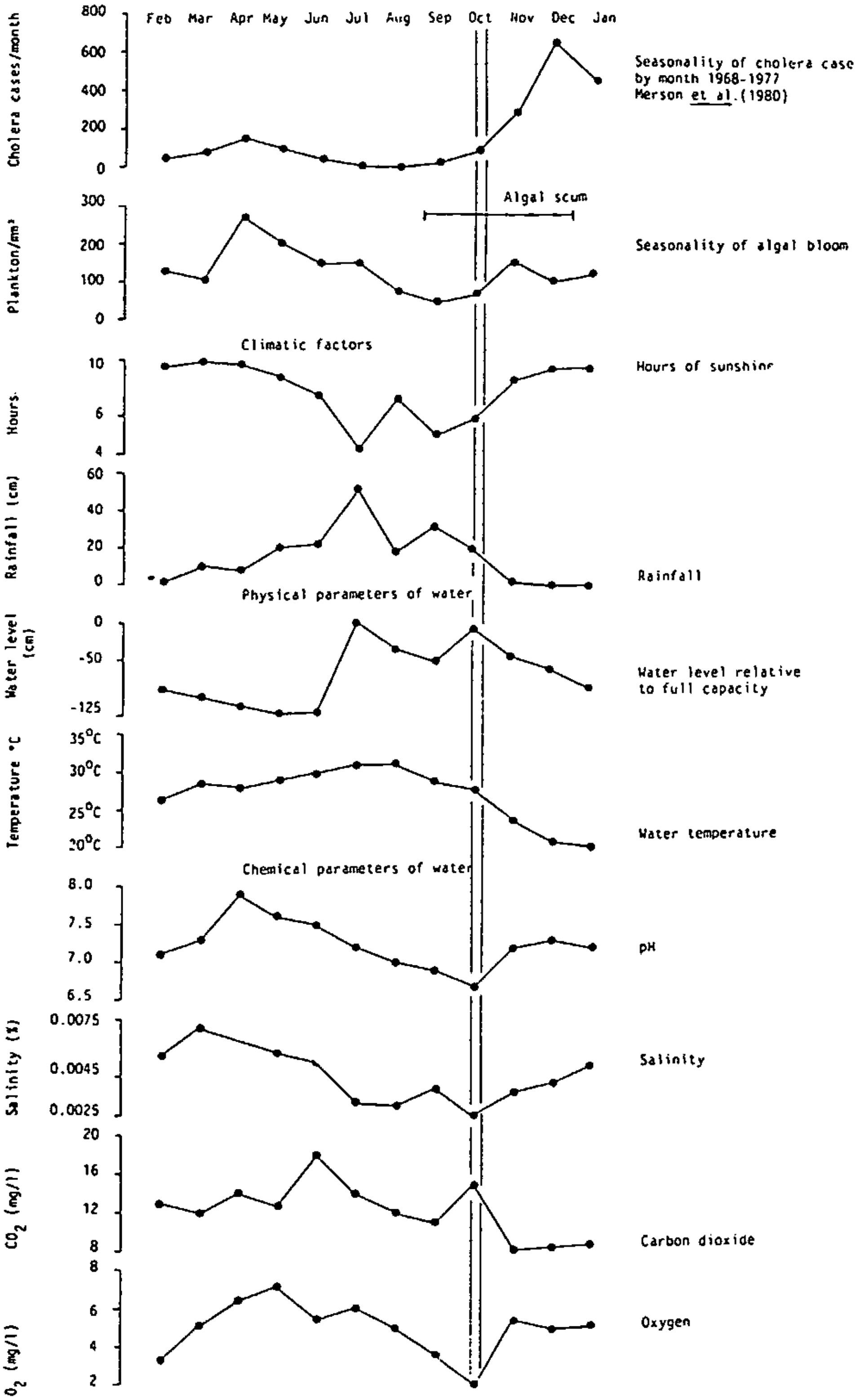


Fig. 5. *Anabaena* sp. (A.s.) showing associated non-culturable *Vibrio cholerae* O1 (V.C.); stained with fluorescein isothiocyanate coupled with specific monoclonal antibody and examined by epifluorescence (X400)

Besides organic nitrogen, the filtrates from *Anabaena* cultures have been found to contain appreciable quantities of pentose, e.g. arabinose, xylose, etc. The amount of pentose was found to increase as the cultures grew older but this increase was independent of the amount of extracellular combined nitrogen produced. It was also observed that the concentrated filtrates of *Anabaena* cultures also contain considerable amount of inorganic salts, e.g. of calcium, magnesium, etc.

Fogg and Pattnaik (65) studied the release of extracellular products from blue-green alga *westiellopsis prolifica* by using the nitrogen isotope ^{15}N as a tracer. They observed that the release of extracellular nitrogenous products was the highest at 35 °C, and that about 50% to 60% of the total extracellular combined nitrogen was of ammonia.

Jones *et al.* (66) examined the water soluble polysaccharides from the mucilage of a blue-green alga *Nostoc commune*. About 30% of the mucilage consisted of galacturonic and glucuronic acids, 10% of rhamnose, 25% of xylose and the remainder (35%) largely of galactose, glucose and an unknown sugar.



Source: Oppenhaemer et al. (1978)
(Salinity values obtained by dividing chloride values by 0.6)

Fig.6. Cholera seasonality, algal bloom formation, climatic factors and physicochemical parameters of water in ponds of an endemic area in Bangladesh

Bishop *et al.* (67) isolated a polysaccharide from *A. cylindrica* grown in a synthetic culture medium. Prolonged acid hydrolysis yielded glucose, xylose, glyconic acid, galactose, rhamnose and arabinose.

There is additional information (not cited here) on the release of different kinds of extracellular products by blue-green algae during their growth cycles (68-71). However, it would be useful to discuss which of these different kinds of extracellular products can be utilized by the associated *V. cholerae* O1?

Assimilation of extracellular products by bacteria

Among the carbohydrates produced by *Anabaena*, galactose and glucose were the dominant sugars, and among the polypeptides, serine was an important constituent (72). *V. cholerae* O1 can easily utilize glucose (73,74). Bacteria attracted to *Anabaena* showed strong chemotactic response to many amino acids and sugars (75). It was observed that, in particular, the amino acid serine had a consistent tendency to attract bacteria over a wide range of concentrations. Autoradiographic analysis of *Anabaena* and associated Gram-negative bacteria revealed the ability of attached bacteria to incorporate a range of amino acids and sugars excreted by *Anabaena*. The bacteria were found to have a chemotactic response to *Anabaena* mucilaginous sheath which indicated that their establishment in mucilaginous microhabitats was aided by attractive substances which were utilized for growth. Caldwell and Caldwell (33) demonstrated that bacteria associated with *Anabaena* utilized a low molecular weight component of the mucilage as a nutrient. Paerl (76) observed that some ^{14}C originally fixed as $^{14}CO_2$ by *Anabaena* through photosynthesis, was eventually utilized by epiphytic bacteria. He also observed that excretion products of *Anabaena* from heterocyst-vegetative cell junctions were readily utilized by epiphytic bacteria. Chrost and Brzeska (77) have also accumulated evidence that extracellular products of cultured *Anabaena* are directly utilized by bacterial microflora associated with this cyanobacterium.

All of these studies point to a similar conclusion that bacteria inside the mucilaginous sheath of blue-green algae utilize their extracellular products as nutrients. *V. cholerae* O1 may also act in a similar fashion, although the definitive experiments have not been done.

Mucilaginous covering of blue-green algae, a specialized microenvironment

The mucus of blue-green algae is a highly specialized microenvironment for bacteria. Paerl and Keller (78) investigated the abundance of Gram-negative bacteria inside blue-green algal mucilage and surrounding water columns. They observed 1000-5000 fold more motile rod-shaped Gram-negative bacteria attached to the heterocysts of both *Anabaena oscillatorioides* and *Aphanizomenon flos-aquae* than in water columns of eutrophic lakes supporting these cyanobacteria. Caldwell and Caldwell (33) isolated a *Zoogloea* sp. from *Anabaena flos-aquae*. By use of electron microscopy a density of 7.4×10^5 cells/ml of *Zoogloea* sp. was found in the planktonic macroenvironment while 2.6×10^{11} cells/ml were seen in the mucilage environment of *Anabaena flos-aquae*. The same bacterial species was isolated from blooms of *Anabaena flos-aquae* during two consecutive years. It was observed that the isolate required a pH greater than 8 for consistent growth. This bacterium could not grow alone on liquid media but could grow in liquid media only in presence of *Anabaena* extract. It was also observed that this bacterium could rapidly assimilate extracellular ^{14}C labelled organic matter produced by *Anabaena*. These observations revealed that the occurrence of the bacteria in cyanobacterial mucilage was not coincidental but reflected an obligatory bacterial requirement for the biological or physicochemical microenvironment of the mucilage. The association and long-term persistence of *V. cholerae* O1 with *Anabaena* sp. may indicate that a similar mechanism is being used.

Functional aspects of blue-green algal and bacterial association

One important functional aspect of blue-green algal and bacterial association is the exchange of carbon dioxide and oxygen. During algal bloom, the dissolved carbon dioxide conc. in water decreases following the rapidly rising demands due to photosynthesis, thus increasing the oxygen level steadily. This increase in oxygen hampers the photosynthesis and the nitrogen fixation of *Anabaena*. The oxidative breakdown of organic matter by heterotrophic bacteria leads to an increased carbon dioxide availability and lowered level of oxygen in the vicinity of the algal filaments. As a result, the photosynthesis and nitrogen fixing activities of *Anabaena* remain unaffected (31,78). Thus, it is now evident that in the *Anabaena* bacterial association, the algal host supplies the nutrients required for survival of associated bacteria; in return, the bacteria supply the carbon dioxide and reduce the oxygen level which helps photosynthesis and nitrogen fixation of the host *Anabaena*. Therefore, it has been observed that a perfect symbiotic association exists in nature between *Anabaena* and Gram-negative heterotrophic bacteria (79). A similar relationship may exist between the blue-green alga *Anabaena* sp. and *V. cholerae* O1 (19,80,81).

Distribution of blue-green algae in endemic areas of Bangladesh

There are reportedly 2500 different species of blue-green algae which are distributed in nature. From Bangladesh, about 307 different species have been reported (80,83-86). These algae have been collected from all kinds of water sources in Bangladesh (rivers, canals, ponds, ditches, drains, lakes, etc.). It is not known, however, which particular habitat containing blue-green algae may be important for vibrio survival. On the basis of our findings, and the evidence in the literature, it is proposed that two kinds of reservoirs may exist in the environment, both involving habitats which support the survival of *V. cholerae* O1 in association with blue-green algae. One we term "temporary reservoirs" and the other "permanent reservoirs."

Temporary reservoirs

We propose that the closed water systems including ponds, tanks, etc., can act as temporary reservoirs from where *V. cholerae* O1 can be transmitted seasonally into the local population due to seasonal multiplication in association with algal bloom.

Bangladesh is, geologically an alluvial plain, only a few feet above the sea level. The annual rainfall is about 85 inches during the monsoon. Moderate to heavy rainfall occurs during monsoon, and much of the land is flooded. People build houses on raised ground to protect them from inundation during the rainy seasons. Every house or group of houses stands on a flat raised ground which has been made by transferring earth by excavating the neighbouring area. The resulting depressions fill up with water during the monsoon and form ponds or tanks. Therefore, every house or a group of houses has a pond in the neighbourhood. These ponds play an important role in every day life of the inhabitants surrounding them. People bathe in them, and at the same time, wash their contaminated clothes in them; sometimes latrines are built on their banks and when a house is provided with a cesspool, it drains into them. Nearly entire refuse from houses either reaches the tank directly or is finally brought there when it rains; so the tanks are always heavily polluted and be rich in organic matter.

If we look back at the history of cholera in Bengal, the potential of village tanks as habitats for multiplication of vibrios in the environment was observed by Dr. Robert Koch (97) in 1884 when he isolated the comma bacillus from a tank in Calcutta during the 1883 epidemic. He wrote:

> "When they were first found, they were so abundant that their number could not have depended alone on the dejecta that had followed into the tank and on the wash water from cholera linen; an increase of them must have taken place".

Moreover, the infectious nature of village tanks has been described by various workers from time to time (87-91). They observed that the presence of *V. cholerae* non-O1 in village tanks is closely related to the incidence of cholera, being more frequent at the beginning of the cholera season.

Khan *et al.* (17) carried out an investigation to examine the role of surface water as reservoir of *V. cholerae* O1 and non-O1 in the environment. They collected water samples from 30 different surface water sources in and around Dhaka city during a period of 14 months. They found that pond waters yielded the highest isolation rate of *V. cholerae* non-O1 (31.7%), next highest from lakes (30.6%) and the lowest isolation was from river water (21.1%). The isolation rate was significantly higher from pond water than river water. This study again indicates the potential of ponds as sources of vibrios in endemic areas.

These ponds or tanks are also good habitats for blue-green algae. The algae grow in these ponds and seasonally produce blooms. However, these ponds are of different sizes; some are small and are subjected to drying every year, while others are larger and remain full round the year. Therefore, they can be divided into two categories:

1) Long-term temporary reservoirs (large ponds)
2) Short-term temporary reservoirs (small ponds)

Long-term temporary reservoirs

The large ponds may become dry only once every few years due to excessive drought, or consumption by users. In this kind of habitat, *V. cholerae* may survive for several years in association with blue-green algae and could theoretically cause epidemics every year in the surrounding population.

Short-term temporary reservoirs

These are small ponds which are filled up with water during the rainy season and then become dry during the summer. These small ponds and ditches are ideal habitats for different kinds of algae. As the water volume gradually decreases, the nutrient concentration increases and the algae multiply rapidly and form bloom. If *V. cholerae* O1 are introduced naturally into these water bodies, the organism may multiply during algal bloom. These small ponds can theoretically act as sources of epidemic only once a year, unless ponds are reinfected.

Permanent reservoirs

The open water systems such as canals, rivers, estuaries, which are not subjected to drying are considered to be potential permanent reservoirs. Blue-green algae are also common in these water systems in Bangladesh (92-94). Blue-green algal blooms also occur in these water systems (91). These habitats are not likely to be destroyed in the same way as temporary reservoirs. The importance of this kind of habitat in cholera epidemiology has been elucidated by various workers (95,96).

The open water systems as potential habitats of *V. cholerae* were first noticed by Koch (97) more than a century ago. In his historical address on cholera and its bacillus, he described the exact location of *V. cholerae* habitat in Bengal as:

> "The boundary between the inhabited and uninhabited part of the Delta is especially favourable; where the refuse from an exceptionally thickly populated country is floated down by the small streams, and mixes with the brackish water of the Sunderbons that flows backwards and forwards and is already saturated with putrified matter".

Moreover, the isolation of vibrios from different estuaries in the USA (6,15) and Bangladesh (98) is also consistent with our hypothesis.

Role of blue-green algae in the epidemiology of cholera in Bangladesh

Epidemiological investigations have failed to isolate *V. cholerae* O1 from the environment during non-cholera seasons (17,35,99). It may be possible that during this time, *V. cholerae* O1 remain attached to the blue-green algal mucilage and their numbers in water are below detectable levels as measured by the usual bacteriologic methods. The vibrios which survive in association with blue-green algae could again multiply when favourable growth conditions return.

V. cholerae have been isolated from various brackish water environments in the USA (15). Colwell *et al.* (6) hypothesize that *V. cholerae* is an autocthonous flora in brackish water environments. The salinity requirement of *V. cholerae* can be attenuated if this organism finds a highly specialized microenvironment inside the mucilaginous sheath of blue-green algae.

Similar modifications of salinity requirements were found in the case of *V. parahaemolyticus* in fresh water environments whenever they were in association with planktonic copepods (100). Therefore, on the basis of our findings and others, it may be speculated that the distribution of *V. cholerae* O1 is not only restricted to estuaries but can be extended to fresh water environments in association with blue-green algae.

Role of blue-green algae in the seasonality of cholera

One of the interesting characteristics of cholera epidemics is that the outbreaks of the disease in endemic areas take place simultaneously in widely separated villages (35,37). To explain this sudden appearance of cholera epidemics at multiple sites, Feachem (101) in a review of environmental aspects of cholera epidemiology hypothesized that some sort of triggering mechanisms in the environment operate to produce this increase of *V. cholerae* O1 in the environment.

The blooming of *V. cholerae* O1 in the environment may well be related to bloom formation of aquatic blue-green algae. Cockburn and Cassanos (89) carried out a study of the epidemiology of endemic cholera in the then East Pakistan (now Bangladesh). They investigated the relationship of the change of climatic conditions and the incidence of cholera. They systematically examined the physical, chemical and biological parameters of six ponds in an urban endemic area for 1 year. They observed that the seasonal variations of the disease coincided with fluctuations in the pH, salinities, etc. of the pond water. These fluctuations of pH were the result of the activities of blue-green algae in the water, raising the pH to a range which is advantageous for the replication of cholera vibrios over other intestinal organisms. They also observed that these ponds which served as the source of village water supply had great potential for spread of cholera.

Influence of different climatic and biophysicochemical parameters on algal bloom formation and the multiplication of vibrios

Algal blooms and cholera epidemics are known to occur at the same seasons of the year. It would be useful to see how the different biophysicochemical parameters in the environment change and are involved in the triggering mechanism of algal bloom formation and the multiplication of vibrios in the environment. The most intensive study was carried out by Oppenheimer *et al.* (90). The different parameters are summarized in Fig. 6. During winter, the sunshine continues for 8 to 9 hours every day, during which the rate of photosynthesis increases and the algae multiply. The higher number of algae use more dissolved CO_2 from the water for photosynthesis; as a result, the level of dissolved CO_2 goes down further than the previous months. The increased amount of dissolved O_2 in water may be due to excretion from the algae during the increased rate of photosynthesis by many of the algae. Due to decreased CO_2 content of water the pH rises. During this time of the year rainfall is very little.

Because of continuous surface evaporation and human use, the water volume is substantially decreased, thereby increasing the concentrations of dissolved organic nutrients. During winter, the temperature also decreases which favours blue-green algal growth. Therefore, increased nutrients, more sunshine and favourable temperatures altogether help blue-green algae multiply vigorously. After the peak of algal bloom, the vast amount of algae may die and disintegrate due to increased demand of food, and the *V. cholerae* O1 which had survived inside the mucilaginous sheath are released into a favourable biophysicochemical conditions for multiplication. For example, vibrios get huge quantities of nutrients, which are decomposition products of the mucilaginous sheath of blue-green algae (66,67), and the pH rises to an optimum level for growth. The salinity is also increased during algal bloom. Under all these favourable conditions, the rapid multiplication of vibrios takes place, and water becomes heavily contaminated with *V. cholerae* O1.

Finally, the inhabitants who live around the pond and use the water for various household and domestic purposes may become infected. Thus these ponds can act as potential sources of the spread of this disease, causing epidemics. The interactions of different parameters, algal bloom formation, and multiplication of *V. cholerae* O1 are shown diagrammatically in Fig. 7.

Similar phenomena have been reported by Kaneko and Colwell (40) in their studies of the annual cycle of *V. parahaemolyticus* in the Rhode river area of Chesapeake bay. They observed that *V. parahaemolyticus* attached to copepod surfaces were released into the water after the decline and decomposition of the zooplankton bloom during summer when the water temperature reached 19 °C to 20 °C.

Microbiotic cycles in the aquatic environment in relation to seasonality of cholera

All surface waters exhibit microbiotic cycles which are influenced by different physical, chemical, and biological factors.

The basic groups of organisms responsible for initiating microbiotic cycles in surface waters are the chlorophyll-bearing planktonic algae and bacteria.

Ventura *et al.* (102) studied the association between seasonal occurrence of *V. cholerae* non-O1 in the waste treatment lagoons and the seasonal cycle of El Tor cholera in Lima, Peru. They observed that the seasonal cycles of El Tor and *V. cholerae* non-O1 tend to coincide. Monitoring the *V. cholerae* non-O1 in sewage lagoons, they predicted the epidemic of El Tor cholera in Lima. That prediction was subsequently found to be correct, suggesting that bacteriologic surveillance of sewage lagoons for *V. cholerae* non-O1 may be useful in predicting *V. cholerae* O1 epidemics.

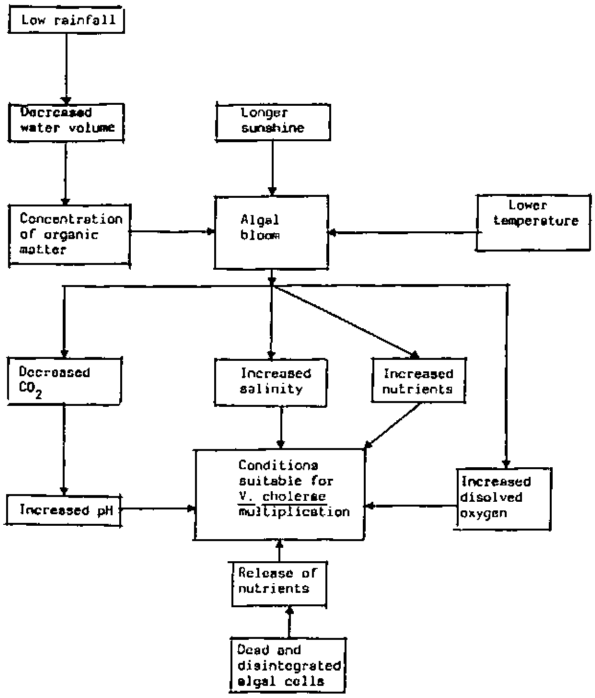


Fig.7. The relationship of different environmental parameters on algal bloom formation and multiplication of *V. cholerae*

Lesne *et al.* (103) carried out a study on temporal dynamics of *V. cholerae* non-O1 and pollution-indicator bacteria in experimental sewage stabilization ponds in arid mediterranean climate. They monitored this programme for 30 months at biweekly intervals. Various physical, chemical and biological environmental variables were also measured concurrently with the bacterial abundance in the monitoring programme. They observed a marked annual periodicity of abundance of *V. cholerae* non-O1 which decreases during the cold season and increases during the hot season. The temporal evolutions of *V. cholerae* non-O1 are negatively correlated with faecal contamination indicators (faecal coliforms and faecal *Streptococci*). However, the temporal evolution of *V. cholerae* non-O1 is highly correlated (Kendall rank correlation) with several environmental factors, e.g. temperature, pH and chlorophyll a concentration. This study suggests that not only do *V. cholerae* non-O1 not behave like faecal coliforms but that their behaviour is the inverse of the indicator organism.

The difference between faecal coliforms and *V. cholerae* non-O1 may be attributable to the symbiotic relation between *V. cholerae* and blue-green algae. A positive relationship between the amount of algal biomass and the number of *V. cholerae* non-O1 has been found. It is possible that the seasonal rise in algae in waste stabilization ponds increases the removal of faecal coliforms whilst promoting the survival of *V. cholerae*.

When a particular bloom of algae has occurred, the mass of nutrients and metabolites released from the algal cells are the main contributory factors which maintain the microbiotic cycle.

Silvey and Roach (104) carried out extensive counts on bacterial population and algae in lake water in Texas, USA during a period of 10 years from 1949 to 1959 which provided data showing population variations with time over 1-year period. It is significant that in every case, large increases in the number of Gram-negative bacilli always preceded an explosive growth of blue-green algae. Gram-negative bacilli showed a steady increase in number which began in April, reached a peak about late August to early September, followed by a rapid decline during September. Blue-green algal growth showed no increase, just minor fluctuations, until August when an explosive growth developed to reach a peak about 1-5 September and then declined along with the bacteria throughout the rest of September.

The microbiotic cycles in a water body are continuous processes, with regular time intervals. This kind of cycle may be occurring between blue-green algae and *V. cholerae* O1 in some water systems in endemic areas of Bangladesh, and thus the seasonality and endemicity of cholera is maintained.

CONCLUSION

Based on the above data, we hypothesize that *V. cholerae* O1 survive inside the nutrient-rich mucilaginous microenvironment of blue-green algae for long preiods of time in the environment. Blue-green algae are abundant in the aquatic environment of Bangladesh and as *V. cholerae* maintain a symbiotic relationship with these algae, the endemicity of cholera in Bangladesh has been maintained from time immemorial. Rapid multiplication of *V. cholerae* O1 takes place following the blue-green algal bloom formation. The presence of the organisms in the water column during epidemic cholera is due primarily to the release from the mucilage environment during reproduction and disintegration of algal cells during bloom formation. The seasonal multiplication of *V. cholerae* O1 following algal bloom formation plays an important role in maintaining the seasonality of cholera in endemic areas of Bangladesh. Studies are underway in our laboratory to test this hypothesis. The reservoirs of cholera have gone undetected for more than one hundred years since the discovery of Koch's comma bacillus in 1884, because the role of algae as possible reservoirs of *V. cholerae* O1 had never been examined.

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