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Cyanobacterial Crust in Hong Kong and Comments on Future Research

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The existence of cyanobacterial crust in Hong Kong under both experimental and natural conditions was noted. Thirty-three cyanobacterial species were identified from the soil surface at the site of an erosion experiment. Eleven species were unicellular and 22 filamentous. Fourteen were non-heterocystous and 8 heterocystous cyanobacterial species. None of the species were obligate terrestrial species. Species abundance was measured for one year, and clear seasonal and aseasonal groups of species were established. Macro-environmental data were reported, including soil characteristics, rainfall, rainfall variability and pH, both soil and surface runoff. While the crusts appeared to be fairly stable, except at typhoon intensities, further work is needed to establish degrees of temporal and spatial variation. Non-crust forming cyanobacteria grew on the soil surface under grass and fern. We discuss the future research outlining the potential uses of cyanobacteria in substrate remediation both as an aid to aggregate stability on cut slopes and as a means of accelerating natural plant succession on cut and cement plastered slopes.

Key Words: algal crusts, bioremediation, cyanobacteria, Hong Kong, humid tropics, soil biology

INTRODUCTION

A major review of the systematics and ecology of soil algae makes no mention of cyanobacterial crusts in humid tropical environments though cyanobacteria have long been known to be an important component of pioneer communities world-wide (Metting 1981). The success of these ancient organisms in colonizing bare surfaces stems from their autotrophic characteristics, in particular the ability of many species to fix atmospheric nitrogen (Allison et al. 1937). Scattered information is, however, available on cyanobacterial species on moist soils from various parts of the world (Ghose 1923; Geitler 1932; Frémy 1933; Singh 1940; Booth 1941; Prasad 1949; Fritsch 1957; Desikachary 1959; Johnson 1962, 1974).

Microflora and their exudates are known to have positive effects upon soil aggregate stability (Watson and Stojanovic 1965; Aspiras et al. 1971; Moavad et al. 1976; Gehring et al. 1994). Cyanobacteria form erosion-resistant crusts in arid and semi-arid environments (Watson and Stojanovic 1965; Gayel and Shtina 1974; South and

Whittick 1987; West 1990; Elsas et al. 1997). Most of these studies, however, refer to extratropical regions. In Hong Kong a recent study of aggregate stability on periodically-burnt hill slopes by Ternan and Neller (1999) makes no mention of such a role despite the presence of cyanobacteria on soil surfaces. In 1998 a marked drop in the rate of erosion on a sloping plot kept bare of higher plants over the preceding five years was observed at Wong Chuk Yuen, Hong Kong, and it was observed that cyanobacterial crusts had formed (Hill et al. 1999). Cyanobacteria are a major component of intertidal zones on Hong Kong's rocky shores (Nagarkar 1998a, 1998b; Nagarkar and Williams 1999). This is the first observation of their existence in terrestrial environments in Hong Kong. After crusts were recognized at our erosion experiment sites it was quickly observed that they were widespread on unvegetated soil surfaces in many parts of Hong Kong, especially on badlands, which comprise about 2% surface area of the region.

At our experimental site, Wong Chuk Yuen, Hong Kong, both on erosion plots and upon the surface of tracks through the mixed scrub, fern and grass land, crusts have formed in patches. They vary in size from a few square centimeters to many square meters. The crusts are clearly more extensive where organic matter,

mainly roots, is largely absent. Similar extensive crusts have been noted in badland areas in the territory. The crusts are 2-3 mm thick, sometimes reaching 5 mm, readily detachable from the soil surface when dry. They consist of 4 elements: various species of unicellular and filamentous cyanobacteria, mucilage, other organic matter and sand, silt and clay particles. When dry the crusts crack into irregular polygons, 2-4 cm in diameter, and the edges sometimes rise one or two millimeter above the center so that the polygons become concave lens- or meniscus-shaped. The extent to which they are naturally be detached from the soil surface has not been established though visual inspection following several major typhoons at a badland site has given clear evidence of pitting of the crust. Pits, 2-4 mm in depth and 2-3 cm in diameter were clearly visible on gentler slopes. No such pitting had been observed in the previous six years' observations (Hill per. obs.).

How widespread cyanobacterial crusts are in humid tropical environments is unknown. They certainly exist near pathways such as in nature reserves and parks, in areas of badland and probably in inactive quarries and sand-pits (Hill per. obs.). They may also exist on heaps of mine-waste. It seems likely that on substrates of sufficient moisture cyanobacterial crusts are eventually colonized by macroalgae, mosses and higher plants. But on substrates of insufficient moisture they seem to remain more or less stable for decades, as in Hong Kong's badlands.

In the Southeast Asian region generally, works on soil algae are limited, particularly on soil cyanobacteria (but see Johnson 1962, 1974). Metting (1981) contains 975 references, only 14 referring to Southeast Asia, with one to crusts. No information is available on soil cyanobacteria from Hong Kong. This paper presents the very first species checklist of soil cyanobacteria from Hong Kong and raises sets of questions concerning the future directions of study.

MATERIALS AND METHODS

A survey was conducted on soil erosion studies sites and surrounding areas located on hill slopes at Wong Chuk Yuen (114° 6'E, 22° 26'N), Hong Kong (Fig. 1). Every two months between November 1998 to September 1999, cyanobacteria attached to the soil surface were collected using a single-sided razor blade. Loosely attached and dried cyanobacterial crusts were removed by hand. All the samples were transferred into
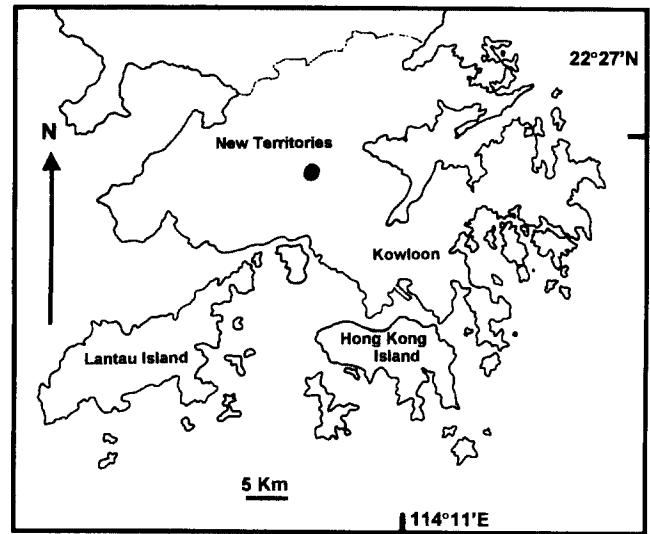

Fig. 1. Location of the survey area at Wong Chuk Yuen, New Territories, Hong Kong. (● sampling site)

plastic vials. In the laboratory, samples were thoroughly washed with distilled water. Washed samples were further centrifuged (3000 rpm for 5 min) to remove attached sediment particles and the pellet was blotted on blotting paper. Sediment-free wet samples were observed under a light microscope (Olympus BX 60). Cyanobacterial species were identified on the basis of their morphological characteristics following Geitler (1932), Frémy (1933) and Desikachary (1959).

Climatic conditions

Climatic conditions of Hong Kong are distinctly seasonal. The hot and wet season runs from May to September and is usually referred to as summer with mean air temperatures range between 25-30°C, and maximum up to 36°C. More than 80% of annual rainfall is recorded during summer with highly variable yearly fluctuations. The cool and dry season is winter (November-March) with mean air temperatures range between 15-18°C, and minimum below 10°C.

Soil characteristics

At survey site the soil was a stony sandy clay loam derived from volcanic materials of Jurassic age (sand 62%, silt 12%, clay 26%). Soil pH was reported as 4.7 at 0-20 cm depth with surface run-off ranging from 3.8 to 6.0 with the average of 4.8 on the plots on which the cyanobacteria are found (Guan 1993). Soil nutrient properties vary according to the depth. At 0-15 cm depth, total nitrogen was recorded at 0.13%, total phosphorus, 0.06% and available potassium, 53 μg/g. At 15-30 cm

Table 1. Check-list of thirty-three species of cyanobacteria recorded from soil surface at Wong Chuk Yuen, Hong Kong, showing seasonal occurrence (presence/absence) from November 1998 to September 1999. (* Unicellular species; † Non-heterocystous filamentous species and ‡ heterocystous filamentous species)

Species	NOV 1998	JAN 1999	MAR	MAY	JULY	SEP
‡*Anabaena anomala* Fritsch	+	+	+	+	+	+
‡*Anabaena spiroides* Klebahn	+	+	+	+	+	+
*Aphanothece conferta Richter	+	+	+	+	+	+
*Aphanothece saxicola Näg.	+	+	+	+	+	+
*Chroococcus macrococcus (Kütz.) Rabenh.	+	+	+	+	+	+
*Chroococcus minutus (Kütz.) Näg.	+	+	+	+	+	+
*Chroococcus montanus Hansgirg	+	+	+	+	+	+
*Chroococcus schizodermaticus West	+	+	+	+	+	+
*Chroococcus tenax (Kirchn.) Hieron.	+	+	+	+	+	+
*Chroococcus turgidus (Kütz.) Näg.	+	+	+	+	+	+
*Gloeocapsa aeruginosa (Carm.) Kütz.	+	+	+	+	+	+
*Gloeocapsa quaternata (Breb.) Kütz.	+	+	+	+	+	+
†*Lyngbya aestuarii* Liebm. *ex* Gomont	+	+	+			
†*Lyngbya mesotricha* Skuja	+	+	+			
†*Microcoleus chthonoplastes* Thuret *ex* Gomont	+	+	+			
‡*Nostoc muscorum* Ag. *ex* Born. *et* Flah.	+	+	+	+	+	+
†*Oscillatoria chlorina* Kütz. *ex* Gomont	+	+	+			
†*Oscillatoria limosa* Ag. *ex* Gomont	+	+	+			
†*Oscillatoria okeni* Ag. *ex* Gomont	+	+	+			
†*Oscillatoria princeps* Vaucher *ex* Gomont	+	+	+			
†*Oscillatoria sancta* Kütz. (Gomont)	+	+	+			
†*Oscillatoria subtilissima* Kütz.	+	+	+			
†*Oscillatoria tenuis* Ag. *ex* Gomont	+	+	+			
†*Phormidium corium* (Ag.) Gomont	+	+	+			
†*Phormidium tenue* (Menegh.) Gomont	+	+	+			
†*Schizothrix telephoroides* (Mont.) Gomont	+	+	+			
‡*Scytonema hofmanni* Ag. *ex* Born. *et* Flah.	+	+	+	+	+	+
‡*Scytonema javanicum* (Kütz.) Born. *ex* Born. *et* Flah.	+	+	+	+	+	+
†*Spirulina labyrinthiformis* (Menegh.) Gomont	+	+	+			
†*Symploca elegans* Kütz. *ex* Gomont	+	+	+	+	+	+
*Synechococcus aeruginesus Näg.	+	+	+	+	+	+
‡*Tolypothrix arenophila* West *et* West	+	+	+	+	+	+
‡*Tolypothrix fragilis* (Gard.) Geitler	+	+	+	+	+	+

depth total nitrogen were 0.09%, total phosphorus was 0.5% and available potassium was 25 µg/g (Guan 1993).

RESULTS

Thirty-three species of cyanobacteria were identified from soil surface at Wong Chuk Yuen, Hong Kong. Eleven were unicellular and the remaining 22 were filamentous. Of the 22 filamentous species, 14 were non-heterocystous species and 8 were nitrogen-fixing heterocystous species (Table 1). Unicellular species mainly belong to the genera *Aphanothece*, *Chroococcus* and *Gloeocapsa* whereas the filamentous species mainly belong to the

Lyngbya, *Oscillatoria* and *Phormidium* genera. Nitrogen-fixing heterocystous species mainly belong to the genera *Anabaena*, *Nostoc*, *Scytonema* and *Tolypothrix* (Table 1). Most of the species (~ 80%) were rare and only few individual cells were encountered during identification. Among dominant species, *Microcoleus chthonoplastes*, *Phormidium tenue*, *Scytonema hofmanni* and *Anabaena anomala* were most frequently distributed.

Filamentous species belonging to *Lyngbya*, *Microcoleus*, *Oscillatoria*, *Phormidium*, *Schizothrix* and *Spirulina* were observed only during winter 1998-99 (Table 1). Nitrogen-fixing heterocystous filamentous cyanobacteria and unicellular cyanobacteria, however, were recorded both

during winter and summer 1998-99. During the winter *Phormidium tenue*, *P. corium*, *Microcoleus chthonoplastes*, *Oscillatoria tenuis*, *O. subtilissima*, *Lyngbya aestuarii* were abundant. *Microcoleus chthonoplastes*, however, was most abundant of all. During summer *Scytonema hofmanni*, *Nostoc muscorum*, *Anabaena anomala*, *Gloeocapsa aeruginosa*, *G. quaternata*, *Synechococcus aeruginesus*, *Chroococcus turgidus* and *C. minutus* were abundant species and *Scytonema hofmanni* was most abundant cyanobacterium as compared to the other species. During late summer and early winter (September-November) *Tolypothrix fragilis* and Tolypothrix arenophila were most abundant species. *Aphanothece saxicola* and *C. turgidus* were abundant during late winter and early summer (April-March).

DISCUSSION

A variety of morphological forms of soil cyanobacteria comprise the 33 species recorded in the present study. All the species are new records for Hong Kong soil though many species recorded in the present study have previously been described from the Hong Kong marine environment (Nagarkar 1998a, 1998b, 1999).

Information for cyanobacteria species diversity from terrestrial soil is scarce world-wide, especially from tropical locations. However, the diversity of Hong Kong cyanobacteria so far recorded is low as compared to other tropical locations. Many species recorded in the present study have been described in floristic surveys of soil cyanobacteria from various parts of the world. All the 33 species have been reported from Indian soils (Ghose 1923; Singh 1940; Prasad 1949; Desikachary 1959) and Sri Lanka (Fritsch 1957). In Singapore and Malaysia, soil cyanobacteria species richness is relatively high but only 13 Hong Kong species were recorded from Singapore (Johnson 1974) and four species from Malaysia (Johnson 1962). The species recorded from Hong Kong are, therefore, widely distributed amongst various tropical locations.

None of the species recorded in the present study can be considered as obligate terrestrial soil cyanobacteria since all these species have been recorded from wide variety of habitats other than terrestrial soil. Species of *Oscillatoria*, *Lyngbya*, *Phormidium*, *Spirulina*, *Chroococcus*, *Gloeocapsa*, *Anabaena* and *Nostoc* have been recorded from freshwater and marine environments (Geitler 1932; Desikachary 1959; Thajuddin and Subramanian 1992, 1994; Nagarkar and Williams 1999).

Cyanobacterial species richness showed temporal variation, being least in the hot summer and highest in the cool winter. Species of *Lyngbya*, *Phormidium*, *Oscillatoria*, *Schizothrix*, *Spirulina* and *Microcoleus* were considered as winter species (Table 1). Unicellular cyanobacteria such as *Chroococcus* and *Gloeocapsa* and heterocystous cyanobacteria such as *Nostoc*, *Anabaena*, *Scytonema* and *Tolypothrix* were considered as aseasonal species since they were recorded throughout the year.

For ecologists it is important to identify cyanobacteria *in situ* on soil surface. Although cyanobacteria are visually conspicuous on soil surface, due to limited variety in visual, morphological, appearance (*e.g.*, colour), they often give the impression of low species diversity. High species richness of cyanobacteria from Hong Kong and other parts of the world suggests that cyanobacteria are an important biological component of soil surface and cannot be ignored in future studies.

Future research

Since some Hong Kong soils are covered with cyanobacterial crust throughout the year, it is worthwhile listing related sets of questions concerning the future directions of study. First are the questions of spatial and temporal variability of cyanobacterial crusts and their components. While this study suggests that they are widespread on bare soil surfaces, systematic survey will be required to establish how far this is so and to establish the nature of relationships with the substrate. On rocky shores in Hong Kong cyanobacteria exhibit a distinct seasonal pattern, flourishing under the cooler conditions of winter and declining in summer. Observations thus far suggests a similar pattern may exist on land. On the shores the impact of seasonal factors is not uniform with respect to species (Nagarkar and Williams 1999). The same is clearly so at our site but it remains to be established for other locations.

Second are questions relating to the immediate environment in which cyanobacteria grow (Fogg *et al.* 1973). The limited thickness of the crusts would suggest that light is major constraining factor. However, in other locations cyanobacteria have been found at considerable depth (Johnson 1962). Hydrogen ion concentration (pH) is another factor. Fogg *et al.* (1973) claim that 'cyanophyceae prefer neutral or alkaline conditions'. But in Hong Kong and tropical areas generally, soils are acidic even where formed upon alkaline parent materials and this may have some effect on the species composition of their communities (Johnson 1962). Our data sug-

gest that cyanobacteria flourish in rather acidic conditions. Some cyanobacteria flourish in the absence of organic matter (Zimmerman 1993).

A third set of questions relates to climatic factors. Some species of cyanobacteria are capable of surviving extreme temperatures and desiccation (Fogg et al. 1973). However, little is known of the interaction of these organisms with the physical environment and local microclimatic conditions except where they grow on rocky shores (Nagarkar 1998a, 1998b; Nagarkar and Williams 1999). Full-sun temperature conditions at a depth of 2 mm under bare soil have been reported at Singapore to range from 23°C at night to 51°C during the daily heat peak (Hill 1979). More extreme conditions are likely to be found in Hong Kong and adjoining regions. Rainfall is likely to have a major effect upon cyanobacterial biomass. Shtina (1992) noted that a day following irrigation the biomass of cyanobacteria increased fourfold. Cyanobacterial species abundance, however, declines in the Hong Kong littoral environment during the wet season (Nagarkar and Williams 1999). Dew may play a significant role in the supply of moisture to cyanobacteria on hill-slopes. Several weeks of no rain, with strong sun, did not result in obvious signs of desiccation of the crusts.

A fourth set of questions relates to crust stability and thus to erosion. A considerable amount of work has been done on this issue in temperate, arid and semi-arid regions, especially in North America, the former Soviet Union and Australia (Bond and Harris 1964; Watson and Stojanovic 1965; Aspiras et al. 1971; Moavad et al. 1976; Zimmerman 1993; Gehring et al. 1994; Malope et al. 1987; Degens et al. 1996). Cyanobacteria play a clear role in enhancing soil aggregate stability first by the intermeshing of branching filaments, characteristic of a number of species such as Scytonema, and second by the mucilage that many produce. Field investigations might include measurement of the capacity of crusted and uncrusted soil to absorb water, using a ring infiltrometer. Though that approach does not directly measure aggregate stability, it gives an indication of the soil's ability to absorb water, to reach field capacity and to generate runoff (Booth 1941). Many standard laboratory tests involve rather severe treatment of the aggregates such as wet sieving and may not be appropriate. An alternative method in Hong Kong is to grow crusts in cups on known soil substrates and then to test for various rainfall regimes in a rain-chamber, comparing crusted and uncrusted soils.

Allied to erosion is the question of water repellence, investigated largely in the US and Australia where 'fairy rings' on lawns and pasture prove difficult to irrigate. Wallis and Horne (1992) showed that repellence could be induced in soils colonized by some species of Aspergillus and Penicillium. Organic coatings associated with these moulds appeared to be responsible (See also Giovannini et al. 1983; Hallett and Young 1999). Cyanobacteria also produce 'organic coating' in the form of polysaccharide-rich mucilage. This may be water-repellent. Such repellence would lead to more total runoff and thus concentration of flow. Repellence might therefore decrease the mobilization of sediment by rain-splash and increase overland flow where crust is present.

Further questions concern cyanobacteria living on the soil surface under grass and fern. They appear to be much less abundant than in the open and do not necessarily form a distinct crust. The specifics of their existence are totally unknown. Some may be nitrogen-fixing. Reddy and Giddens (1975) noted the presence of nitrogen-fixing cyanobacteria in a sub-tropical soil under fescue-grass cover in Georgia, USA. The amounts of both nitrogen and carbon in the crust increased in step with the age of the grass sod. There are also associations between specific higher plants and the particular cyanobacteria growing under them. Such is certainly the case for Boletus sp. fungi and pines, for example.

This leads to a final issue of remediation. Cyanobacteria not only bind soil surfaces, but also they accumulate primary organic matter and enrich the soil with nitrogen. Gayel and Shtina (1974) showed the degree to which this is so for sands of the northern Caspian. Shubert and Starks (1985) pointed to the great utility of algae in colonizing and stabilizing mine spoil, while Shtina (1992) discussed ways of regulating soil algae, including the inoculation of soil with live algae and their metabolites. In tropical regions the biological remediation of bare slopes has typically involved planting trees, often exotics, and much less commonly cover-crops or erosion-control hedges (see Chen et al. 1998; Hill and Peart 1999). In Hong Kong, tree planting has been the rule on remoter sites with grass hydroseeding employed on sites accessible to the lorry-mounted mixers and pumps. These methods are costly, and many cut slopes are stabilized using a cement coating, which is effective but aesthetically deplorable (Hong Kong Government Geotechnical Office unpub. report).

An approach using inoculation of the soil surface with desirable species of cyanobacteria is worth investigation.

Zimmerman (1993) reviewed the inoculation of soil surfaces with various species of *Chlamydomonas*. If sufficient inoculum was applied to the soil surface, high rates of reproduction, 2-3 doublings per day, were achieved. Biomass increased at $3.8 \ g \cdot m^{-2} \cdot day^{-1}$ resulting in large increases in soil aggregate stability. Inoculation of cement-coated slopes to initiate higher-plant colonization for aesthetic purposes is also desirable. Whether cyanobacterial inoculation would have economic advantages over hydroseeding is another matter but, assuming that inoculated slopes could readily be colonized by higher plants, one advantage would be that such plants would likely be natives rather than exotics. Further investigations of these questions are justified on both scientific and practical grounds.

ACKNOWLEDGEMENTS

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