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Water Uptake and Loss by Antarctic Cyanobacterium *Nostoc commune*

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Abstract

Investigations were conducted in the Schirmacher Oasis, Antarctica, on water uptake by desiccated thalli of Nostoc commune, the dominant cyanobacterium occurring in the area. The results suggested that at the onset of austral summer (melting of snow/ice) in Antarctica, the metabolic activity in dehydrated thalli may be initiated after 5 h of water availability. On the other hand, cessation of metabolism may take place after 5 h of dehydration as a consequence of non-availability of water (formation of ice).

Indroduction

Resistance to water stress and desiccation is a feature of many cyanobacteria both of terrestrial and marine origin (Potts et al.,1983,Whitton and Potts, 1982). Little is known about physiological tolerance of cyanobacteria to water loss and uptake despite the potential significance of this trait in determining the distribution and activities of N_2-fixing form, and in the selection of strains for use in sustained agriculture for maximizing crop productivity. Desiccation has been postulated to play a major role in limiting N_2 fixation by cyanobacteria in natural habitats (Stewart, 1974).

The icy continent - Antarctica remains frozen round the year except during short austral summer. Survival and activities of cyanobacteria in these habitats are strongly dependent on physiological availability of water. During austral summer cyanobacterial communities absorb nutrients and water, and hence their metabolic activities are expressed at their optimal level As the temperature drops below freezing point water is transformed into ice, and due to high aridity it sublimes. The thalli of cyanobacteria gets dehydrated and remain in the form of dried flakes and possibly all metabolic activity ceases. Metabolic activities are reinitiated whenever the next cycle of summer water becomes available.

The present study was undertaken in the austral summer of 1991 -92 during eleventh Indian expedition to Antarctica to understand the time required for water uptake by dried thalli of *Nostoc commune* and initiation of metabolic activity in terms of changes in chlorophyll a pigment. Loss of water from fully hydrated colonies was also estimated. In order to confirm the field observation, similar experiments were conducted with laboratory grown cultures.

Material and Methods

(i) Cyanobacterial Mats: *Nostoc commune* used in the investigation was collected from Schirmacher Oasis, Antarctica as free living foliose mats, colonizing gravel and rocky substratum.

The mats were usually ensheathed in a thin mucilaginous matrix and were frequently flushed by melting snow.

For laboratory investigations, *N. commune* collected at Schirmacher Oasis was routinely grown in BG-11 medium (Rippkaet al.,1979) without combined inorganic nitrogen source. The cultures were maintained in 250 ml Erlenmeyer flask containing 100 ml liquid medium and illuminated with daylight fluorescent tabes (50 fE m^2S^{-1}). To simulate growth of cyanobaeterium under field conditions, the cyanobaeterium was inoculated on sterile quartz sand spread in petriplates and incubated as mentioned above. Liquid BG-11 medium was introduced periodically into the petridishes to a level to moisten the sand layer. The cyanobaeterium was allowed to form a thick mat on the surface of sand. Subsequently, the mats could be removed from the sand layer and cut into small pieces with the help of a cork borer.

(ii) Water exchange: Algal mats grown on sand in laboratory or occurring in field were harvested, removed free of the adhering sand particles and air dried. For water uptake experiments completely air dried pieces of algal mat (1 cm^2) were submerged in BG-11 medium and increase in weight was estimated by weighing the thalli at regular intervals of time. While weighing, residual water adhering to the mats was removed by placing the thalli between two sheets of blotting paper.

For water loss experiments, completely hydrated thalli were cut into pieces with the help of cork-borer, placed in open petridishes and exposed to air (temperature 20-25°C). The thalli were periodically weighed until constant weight was attained. For each experiment 45 pieces were used and the data presented in results are average of three independent experiments.

(iii) Measurement of Chlorophyll *a*: The thalli were placed in culture tube and 10 ml acetone (80%) was added. The tubes were plugged with rubber stopper and refrigerated (4°C) for 24 hours in dark. The superaatent was

removed and optical density (663 nm) was measured with the help of spectro-colorimeter (Bousch and Lomb). Quantitative estimation was made by using the equation of Myers and Kratz.(1955).

(i v) Statistical analyses: Linear regression analyses were performed using MSTAT (Ecosoft.Corp.USA) and Harvard Graphics.

Results and Discussion

The cyanobacterium *Nostoc commune* constitutes the dominant flora in Antarctic ecosystem and has been shown to fix atmospheric nitrogen (Fogg and Stewart, 1968). It is. not only the low temperature that regulates the terrestrial microbial survival and growth but also the restricted availability of water, associated osmotic stress and substratum instability. It has also teen demonstrated that N_2-fixation is more sensitive to water stress (Jones, 1977) and that desiccated thalli exhibit a series of integrated physiological responses following absorption of water (Scherer et al.,1984). Since photosynthesis precedes N_2-fixation after water absorption (Scherer et al.,1984), it was of interest to investigate changes in chlorophyll a following water uptake or loss in N. *commune*, a phenomenon which is operati ve at the beginning and end of austral summer in Antarctica.

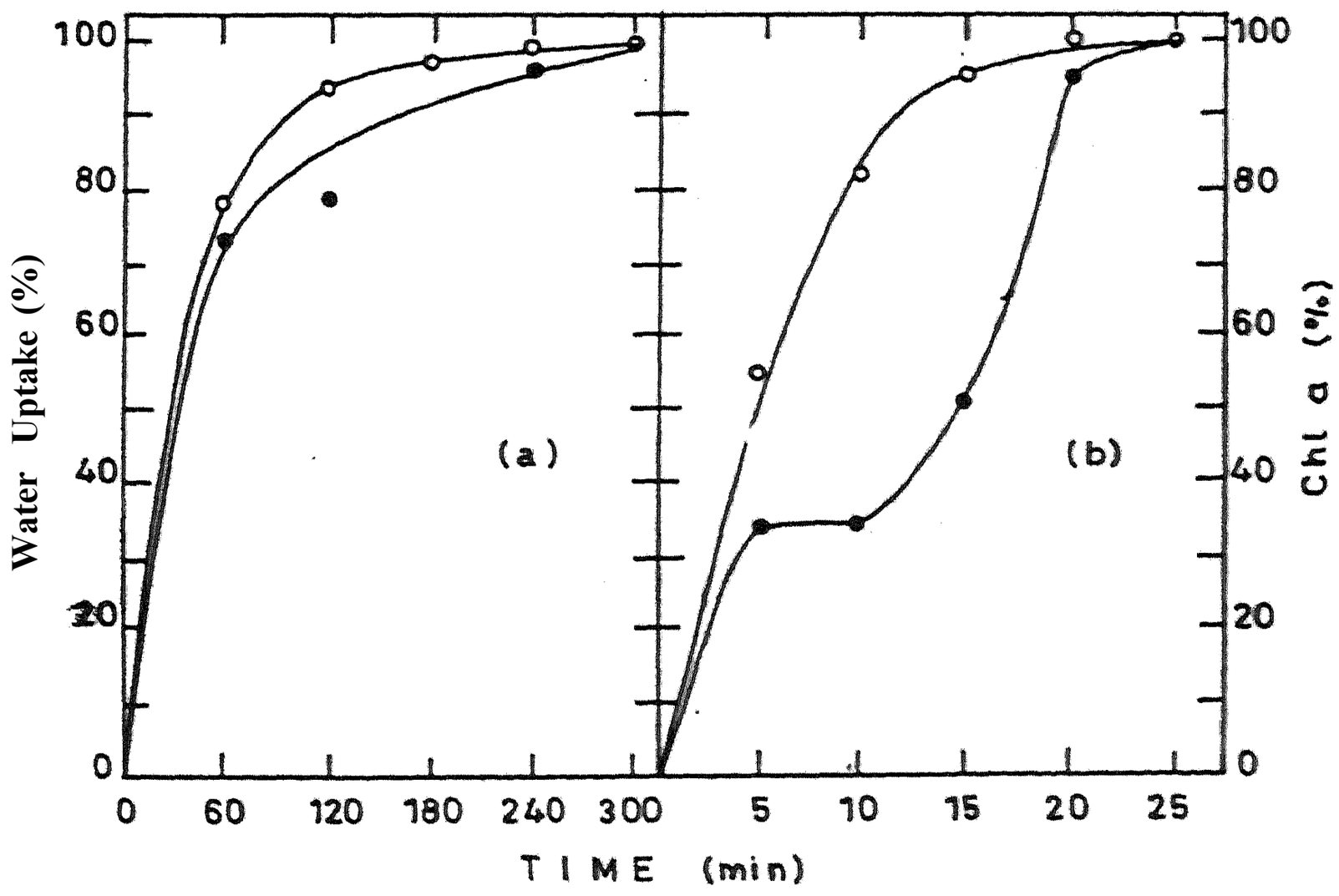

Fig 1: Water uptake by dried thalli of Nostoc commue (a) in field thalli and (b) in laboratory grown thalli. Water uptake (0—0).Chl a . (●—●)

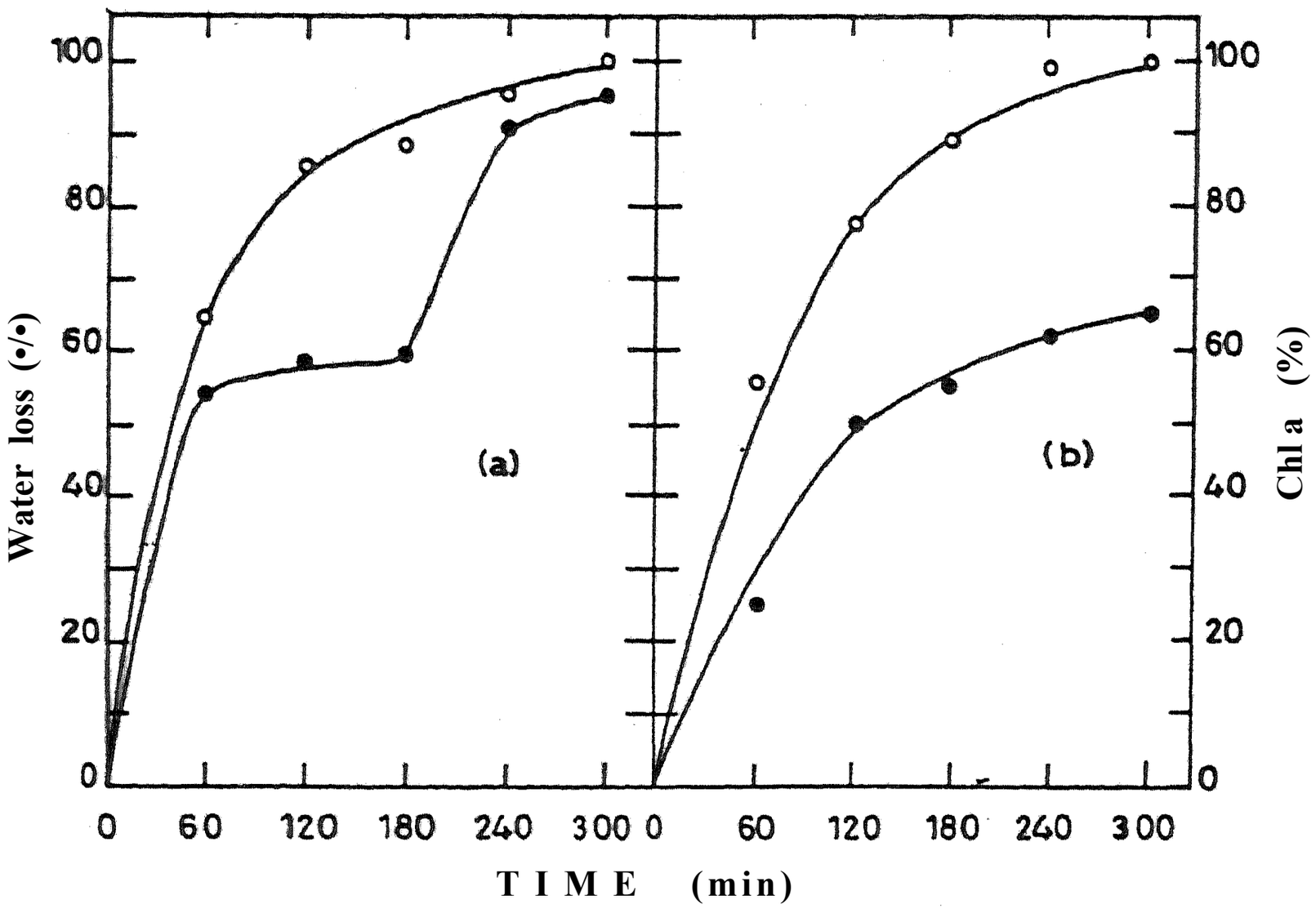

Fig 2 : Water loss by.fully hydrated thalli of Nostoc commune. (a) in field thalli and (b) in laboratory grown thalli Symbols as in Fig 1.

Investigation on water uptake by desiccated thalli of *N. commune* occurring in Schirmacher Oasis indicated that it took about 5 hours for the thalli to absorb water to a saturating level (Fig 1a). Regression analysis showed a positive correlation between increase in weight of thalli with the time of incubation (p) (Fig 3a). Alongwith the water uptake there was an increase in chlorophyll a content which was more rapid during first two hours of incubation, and declined subsequently (Fig la). The results indicate the possibility that once water is available following onset of austral.summer, the process of water absorption activates the thalli to induce chlorophyll a synthesis, the antenna pigment of photosynthesis.

An experiment similar to the above conducted with thalli grown under the laboratory conditions revealed that water uptake was much more rapid than the field specimens because it took only 25 minutes to regain water (Fig lb). Regression analysis showed a significant positive correlation between weight of thalli and incubation time (p) (Fig 3b). Although pattern of water uptake curve was essentially similar to those observed for field specimens, the chl a synthesis followed a biphasic pattern. During the first ten minutes of incubation there was no significant increase in chl a, however, there was a rapid increase during next ten minutes of incubation. The reason for such an apparent difference between the field and laboratory grown thalli is not yet clear but it

could be due to growth conditions of cyanobacterium, since under former condition Nostoc commune predominantly exists ie aseriate stage (Laz-aroff, 1973) while in the latter in filament and hormogonial stage only (unpublished observations).

Water loss from hydrated thalli is prevalent in Antarctica at the onset of winter season when free water turns into ice as a result of dramatic drop in

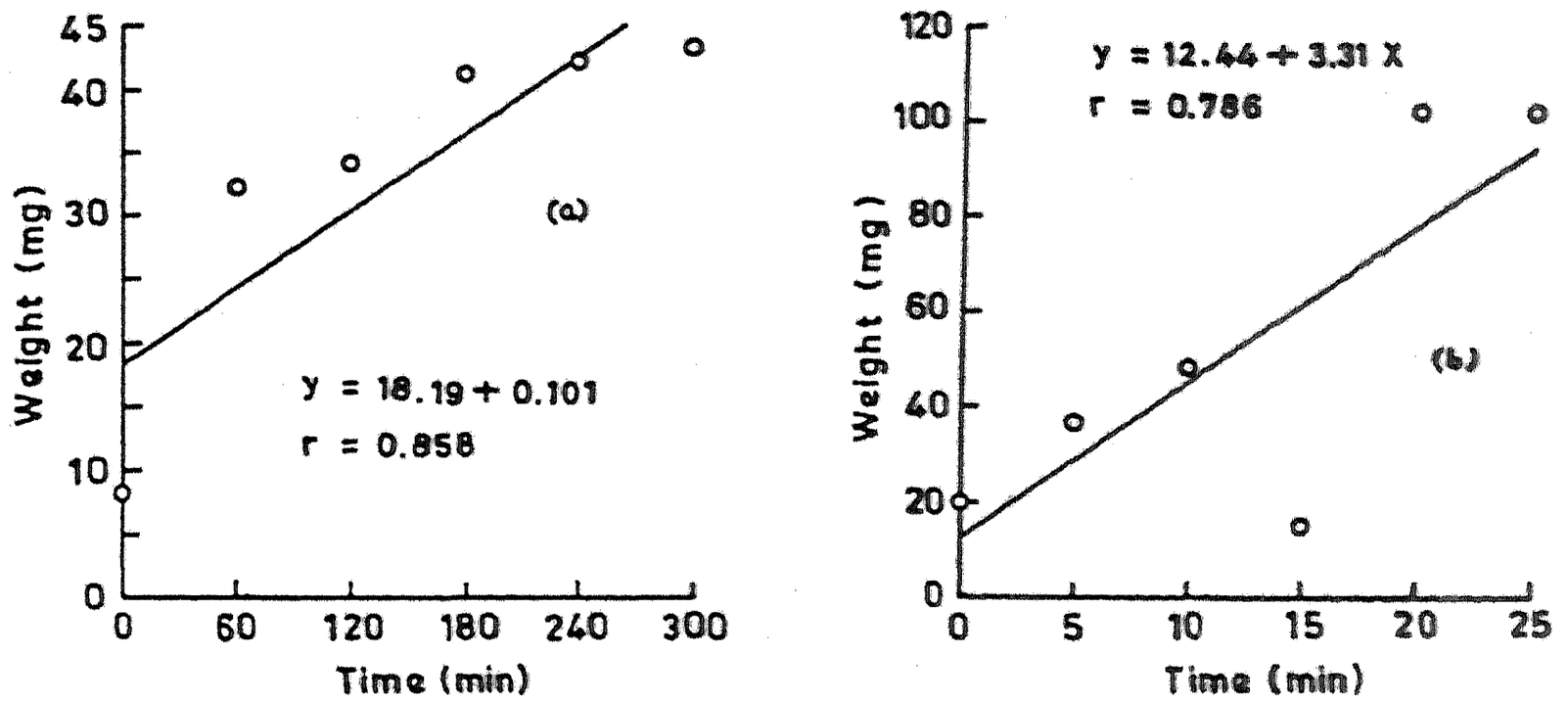

Fig 3 : Regression analyses between time and weight of water during uptake of water by fully dried thalli. (a) in field thalli and (b) in laboratory grown thalli.

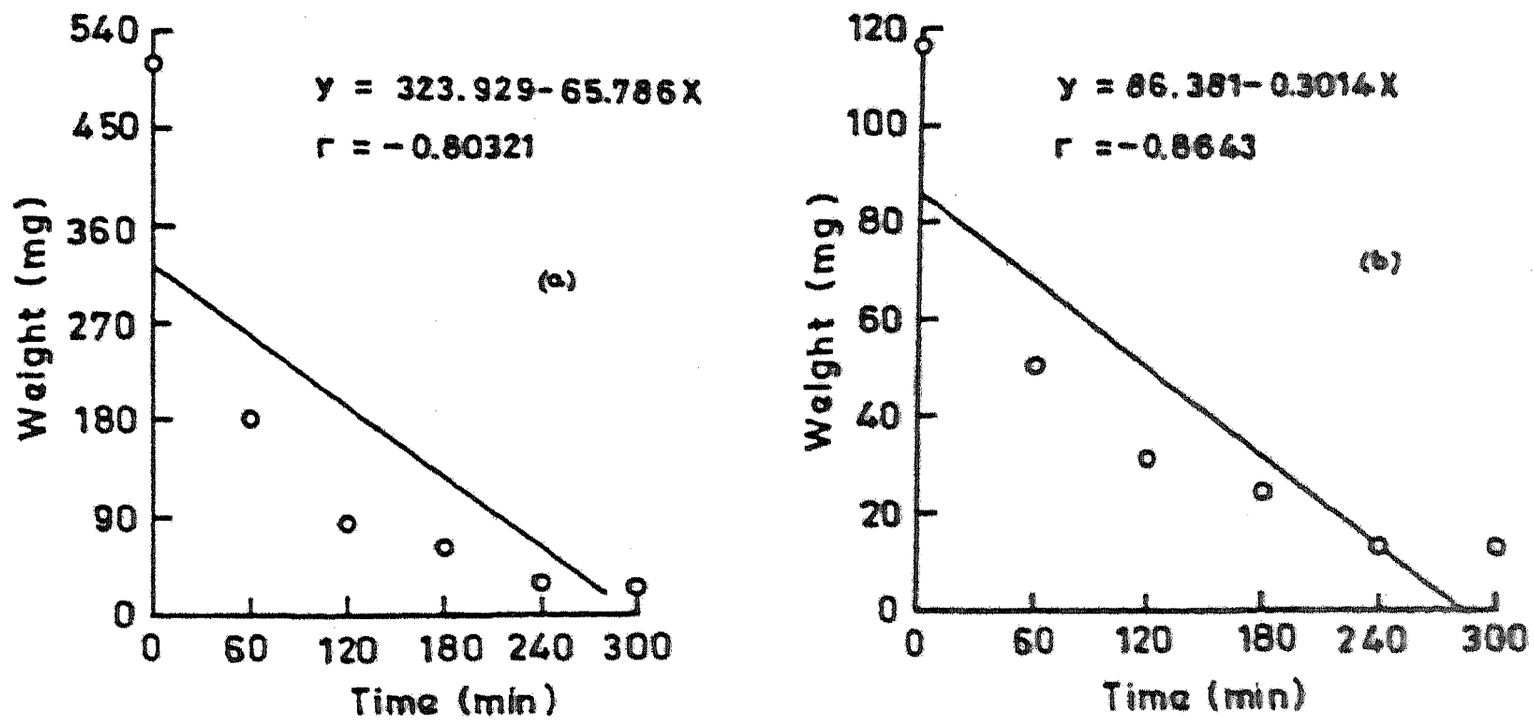

Fig 4 : Regression analyses between time and weight of water during loss of water by fully hydrated thalli (a) in field thalli and (b) in laboratory grown thalli.

temperature. This period is characterised by high aridity (relative humidity 40-45%). Consequently the ice also sublimes. Experiments conducted with fully hydrated thalli revealed that water loss was completed within a period of 6 hours (Fig 2a) and there was a significant negative correlation between the weight of thalli and period of incubation (p) (Fig 4a). However, loss of chl*a* followed a biphasic pattern. The initial phase lasted for 3 hours where only 60 % loss was evident. Subsequently 35% loss occurred during next three hours (Fig 2a). Results on water loss experiments conducted with laboratory grown thalli revealed that there was strong parallelism with those of field experiment. It took about 6 hours for complete dehydration of the thalli. Nevertheless, differences in chl *a* loss was apparent in the laboratory grown thalli where biphasic pattern was not evident. At the end of six hours of dehydration only 65% loss was observed (Fig 2b). In contrast the results obtained with field material (Fig 2a) showed that during Initial 65% dehydration of natural thalli there was a rapid loss of chl *a* (56%). When the thalli lost additional 20% water the chl *a* loss was resisted (5% loss only). Additional 20% water loss occurred during 120-300 minutes which was associated with 35% loss of chl a. In contrast, the loss of chl *a* in laboratory thalli occurred in only one Step and no loss occurred beyond 65% (Fig 2b). Regression analysis between weight of thalli and incubation time showed a significant negative correlation (p) (Fig 4b).

Although we do not know how far availability of water regulate the life cycle of *Nostoc commune* in nature,the observation that hydration of a dry thalli leads to a rapid development of chl *a* in the cells, indicates that it would require at least 5 hours of wetting before any metabolic activity may be initiated. Similar is the case when water is withdrawn from the wet thalli, i.e. it takes at least five hours after water withdrawal for metabolic activity to cease.

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References

Fogg,G.E.and Stewart,W.D.P.(1968): In-situ determination of biological nitrogen fixation in Antarctica. Br.Ant.Surv.Bull.,15,pp 39-46.

Jones,K.(l977): The effects of moisture on acetylene reduction *by* mats of blue-green algae in sub-tropical grassland. Ann.Bot.,41,pp 801 -806.

Lazaroff,N.(1973): Photomorphogenesis and Nostocacean development. In : (eds.N.G.Carr and B.A.Whitton) The Biology of Blue-Green Algae. *Univ.of* Califormia Press, Berkley,pp 279-319.

Myers,J.and Kratz,W.A.(1955): Relation between pigment content and photosynthetic characteristics in a blue-green algae. J.Gen.Physiol.,39,pp 11-22.

Potts,M, Ocampo-Friedman,R.,Bowman,M.A. and Tozun, B.(1983): *Chroococcus* S24 and *Chroococcus* N41 (Cyanobacteria): Morphological, biochemical and genetic charaterization and effect of water stress on ultrastructurc. Arch Microbiol, 135,pp 81-90.

Rippka,R.,Deruelles, J.,Waterbury, J.B., Herdman,M.and Stanier,R.Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J.Gen.Microbiol.,lll, ppl-61.

Scherer,S.,Ernst,A.,Chen,T.W.and Bogar, P.(1984): Re-wetting of drought resistant blue-green algae: Time course of water uptake and reappearance of respiration, photosynthesis and nitrogen fixation, Oecologia,62,pp 418-423.

Stewart,W.D.P.(1974): Blue-green algae. In: (edQuispel) The Biology of Nitrogen Fixation. North Holland PubL,Amsterdam,pp 202-237.

Whitton,B.A. and Potts,M.(1982): Marine littoral. In: (eds.N.G.Carr and B.A.Whitton) The Biology of Cyanobacteria. Blackwell Scientific Publ.London,pp 515-542.