CHAPTER – 8.

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The sensitivity of selected physiological and biochemical studies were made on free and immobilized cultures of diazotrophic cyanobacterium *Nostoc calcicola* Breb, exposed to copper. The results obtained are summarized as:-

(1). Cu-uptake pattern in both free and immobilized cells was typically biphasic, comprising of (a) rapid binding of Cu on the cell wall followed by (b) the subsequent metabolism-dependent uptake.

Cu uptake in free cells at 60μM Cu$^{2+}$ with a metal buildup of 96.89 n mol Cu mg$^{-1}$ protein min$^{-1}$; in comparison, immobilized cells showed 3-fold (300.82 n mol Cu mg$^{-1}$ protein) at 60μM Cu$^{2+}$.

(2). Free cells exposed to dark (72 h), showed an abrupt decline in Cu uptake (7.16 n mol Cu mg$^{-1}$ protein) amounting to more than 9-fold difference from the light-grown control cells (96.89 n mol mg$^{-1}$ protein). Such dark cells when supplied with exogenous ATP (10μM), showed enhanced Cu uptake by a factor of 10. The incomplete recovery in such a case could be attributed to the short supply of reductants in cells exposed to dark. The dark-incubated immobilized cells on the other hand, reflected only a 1.34 fold lowering in Cu uptake (200.15 n mol Cu mg$^{-1}$ protein). Such comparisons clearly indicate that immobilized cells have sufficient energy in reserve even if subjected to
non-photosynthetic conditions (72 h). The exogenously supplied ATP (10μM) to “dark” beads also; could not raise the Cu uptake to the level of “light” beads.

(3). Immobilized cells were characterized by a faster rate of phosphate uptake than the free cells. Cu inhibited phosphate uptake depending on the concentration as well as exposure time. Immobilization was non-competitive. Immobilization was associated with decrease in vivo activity of Ca\textsuperscript{2+}-dependent ATPase compared to a sharp decline approaching in case of Mg\textsuperscript{2+}-dependent ATPase. Lower Cu concentrations in contrast, were stimulatory to the activity of both the enzymes at least up to 1 h. The in vivo and in vitro activities of both the enzymes show similar pattern of Cu-inhibition, thereby ruling out any in vivo factor involved in regulating Cu toxicity to these enzymes in cyanobacteria.

(4). The higher nitrogenase activity in immobilized cells over the free cells has been attributed to (a) enhancement of heterocyst frequency, (b) creation of anaerobic condition, and (c) increase in the level of photosynthetically generated ATP. Likewise, the immobilized cells were also characterized by higher GS activity in comparison to free cells.

(5). \(^{14}\text{CO}_2\)-uptake in immobilized cells was less sensitive to Cu compared to free living cells. 1 h Cu exposure of cells under both the state of cells was significant to know about the pattern of \(^{14}\text{CO}_2\)-uptake. The immobilized cells saturated for Cu uptake, could carry on \(^{14}\text{CO}_2\)-uptake more efficiently than the free cells.
Summary

(6). Characterization of Cu-resistant (Cu') strain revealed its ability to grow at 70µM Cu. The 5µM Cu is lethal concentration for Cu-sensitive (Cu⁸) strains. The overall pattern indicated the acquisition of metal tolerance in the former. In Cu uptake comparisons, the Cu-resistant (Cu') strain showed only 26% less cellular metal buildup (72.15 n mol Cu mg⁻¹ protein) at 1 h compared to Cu⁸ (96.89 n mol Cu mg⁻¹ protein). Such a marginal reduction of Cu uptake in Cu-resistant (Cu') strain could not be taken as the sole mechanism of Cu tolerance as the same strain also showed significant Cu-efflux. Cu-efflux suggested being one of the mechanisms to develop heavy metal tolerance. In a time-course follow-up, Cu-resistant (Cu') strain showed almost 80% metal efflux (57.12 n mol Cu mg⁻¹ protein) over the same period.

(7). Cu efflux in the Cu-resistant (Cu') strain depended to a major extent, on the light generated ATP as the “dark” cells brought about 95% inhibition. The exogenous supply of ATP to such cells could restore efflux by only 30% compared to no improvement in “light” cells thus suggesting that photosynthetically generated energy in the latter case was optimum to drive Cu-efflux. Cu-efflux in the Cu-resistant (Cu') strain also initiated only for 100-fold lower Cu concentration in the external medium (0.6µM Cu) and not before. The close and constant proximity between the Cu efflux rate for 1000-fold diluted sets and those of Cu free medium in the first 1 h suggested that higher dilution or the lower amounts of Cu (i.e.; 0.06µM in the present case) offered regulations for the efflux event.