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Amelioration of lead toxicity by cyanobacteria *Scytonema* and *Hapalosiphon*

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SUMMARY

In the present investigation a comparative study has been made with free and immobilized *Scytonema* sp. and *Hapalosiphon* sp. cells subjected to lead treatment. These Immobilized cells gave enhanced growth and nitrate reductase activity as compared to free cells without added lead (Control). In sublethal doses of lead, immobilized cell were able to over come the Pb toxicity to considerable levels. In fact, the growth and nitrate reductase activities were stimulated with added lead under immobilized conditions has compared to Pb treated free cells and also to investigate whether immobilization can help retain nitrate reductase activity if nitrate concentration as increased in the medium and also mitigate lead toxicity. Between both the cyanobacteria *Scytonema* sp. was more efficient to remove Pb toxicity. The observations of the present study clearly demonstrate the protective effect of immobilization in *Scytonema* sp. *and Hapalosiphon* sp. against Lead toxicity. With this aim the nitrate reductase activity was compared in free and immobilized cells of two heterocystous filamentous free-living cyanobacteria *i.e.* *Scytonema* sp. and *Hapalosiphon* sp., isolated from paddy fields and amelioration of lead metal toxicity.

Key words : Cyanobacteria, Lead, Immobilization, Nitrate reductase, Amelioration.

Industrialization and domestic activities have resulted in increased mobilization and deposition of heavy metals pollutants including Pb in natural habitats. Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust. Heavy metals such as lead ranked second, on the 2003 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority list for hazardous substance because it is a toxic widespread pollutant, which enters the environment by both natural and anthropogenic causes. It is one of the major heavy metals of the antiquity and has gained considerable importantance as a potent environmental pollutant. Lead itself does not break down, but lead compounds are changed by sunlight, air, and water. When released to the air from industry or burning of fossil fuels or waste, it stays in air about 10 days. Apart from the natural weathering processes, Pb contamination of the environment has resulted from mining and smelting activities, Pb containing paints, gasoline and explosives as well as from the disposal of municipal sewage sludge enriched in Pb. Lead sticks to soil particles. It stays a long time in both soil and water. High levels of lead are toxic to plants, animals and other microorganisms (ATSDR, 1993).

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In response to heavy metals, microorganisms have evolved various measures via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, and complexation and oxidation-reduction reactions. It has been proved that they are capable of adsorbing heavy metals (Chen, Hong and Pan, Shan-shan, 2005). Cyanobacteria are a group of photosynthetic, oxygen evolving prokaryotes, which have a capacity to fix atmospheric nitrogen, and their ubiquity on the planet and dominance in every conceivable ecosystem is a major example of their ecophysiological resilience and adaptability. The submerged conditions of rice field provide congenial habitat for cyanobacteria where they functionally act as the most efficient system providing biological fixed nitrogen to the crops. Numerous studies have shown that microorganisms especially the cyanobacteria can interact with heavy metals. They also possess several detoxification mechanisms to counteract metals stress which include biosorption, bioaccumulation, exclusion of heavy metals, change in metal binding proteins, trapping of metals in bodies containing sulphoquinovocyl diglyceride, and compartmentalization into polyphosphate bodies.

Cyanobacterial cultures can be turned into relatively homogenous preparations by immobilization and altering the condition of immobilization can control particle size. Although various method are available for the immobilization cell entrapment is probably most extensive used method. Immobilization techniques have been widely used in modern biotechnology. Natural immobilization of cyanobacteria may have far reaching implications both in undefined industrial setting and as nitrogen fixing environmental amendments. While there are many demonstrations that cyanobacterial cultures take up heavy metals no comparison of free cell and immobilized cells have been reported in this regard.

Inspired by such considerations the present work will be attempt to study the basic aspects of lead toxicity which is liberated into the water used for irrigation from factories related to paint industries, batteries, electroplating and also from immersion of idols in the water on *Scytonema* and *Hapalosiphon* species which are the strong nitrogen fixers of Indian paddy fields. Under natural habitats, *Scytonema* and *Hapalosiphon is* immobilized as mats in the rice fields and few if any studies have been conducted on naturally immobilized cyanobacterial cells.

MATERIALS AND METHODS

The rice field strains *Scytonema* and *Hapalosiphon* were isolated from rice fields of Sehore (M.P.) for comparative study. The isolation and identification of cultures were made with the aid of available taxonomic key Desikachari (1959) and Bergey's Manual, Bergey (2001). The cloning and purification was done with the aid of standard microbiological techniques and grown in BG11 medium and maintained at 27±°C, 2500± 200 lux light intensity and 14:10 hour light and dark rhythm.

Immobilization was done by the cell entrapment method using calcium alginate as described by Singh *et al*. (1989). The beads thus formed were washed with doubled distilled water and suspended in culture media containing different concentrations of metal solutions. Lead solutions with different concentrations were prepared by dissolving $PbCl_2$ in conc. HCl with double distilled water (1:99) and were filtered with 0.45 μ Millipore filter before adding to the medium.

Nitrate reductase activity of *Scytonema* and *Hapalosiphon* will be studied in free cells and immobilized cells and compared. The estimation of *in vivo* nitrate reductase activity was measured by the method of Camm and Stein (1974) and as slightly modified by Kumar and Kumar (1980). The activity is based on the total nitrite formed. The nitrite present in the sample was expressed in μg $NaNO_2$/ μg Chl *a* using a standard curve.

The uptake of lead was under-taken in an Atomic Absorption Spectrophotometer Model 2380 Perkin Elmer and expressed in mgl^{-1} by the method described by Singh *et al*. (1989). The result has been calculated by the difference observed in the concentration of metal comparing the initial value (mgl^{-1}) added in culture medium containing the cyanobacterium/immobilized beads and the value (mgl^{-1}) remaining in the medium after 8 day.

RESULTS AND DISCUSSION

Cell immobilization could protect the organism's growth against the toxicity of Pb at LC_{50} as compared to lethal concentrations in both *Scytonema* sp. and *Hapalosiphon* sp. Nitrate reductase activity of free and immobilized cells was studied in both *Scytonema* sp. and *Hapalosiphon* sp. (Fig. 1). The enzyme activity in free cells treated with lead was substantially inhibited but immobilized cells treated with 20ppm Pb was not affected by the metal treatment in both cyanobacteria. Control immobilized cells (without Pb) also had higher nitrate reductase activity than control free cells. Pb addition markedly decreased the enzyme activity in free cells but immobilized cells exposed to sub lethal concentrations of metal could overcome this decrease. Fig. 2 shows that both *Scytonema sp.* and *Hapalosiphon sp.* can tolerate increased concentration of nitrate in medium (25mM). Between both cyanobacteria *Scytonema sp* is more efficient to remove Pb toxicity compare to *Hapalosiphon sp.* After the eighth day of inoculation, immobilized control cultures of *Scytonema sp.* and *Hapalosiphon sp* showed 80% and 75% increase in growth over free control cells, respectively (Fig. 3).

The observations in the present study clearly indicate a protective effect of immobilization on *Scytonema* sp. *and Hapalosiphon* sp. against lead toxicity. The mechanism of toxicity of metals to cyanobacteria are not fully known but several heavy metals retard the flow of electrons in electron transfer reaction in mitochondria and chloroplast and thus can be expected to have a detrimental effect on respiration, photosynthesis and other processes

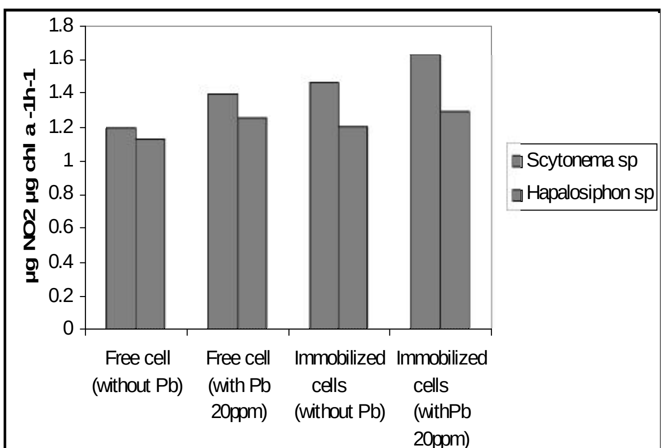

Fig.1 : A comparative table showing the effect of Pb on nitrate reductase activity of free and immobilized *Scytonema* sp. and *Hapalosiphon* cells at 72 Hrs.

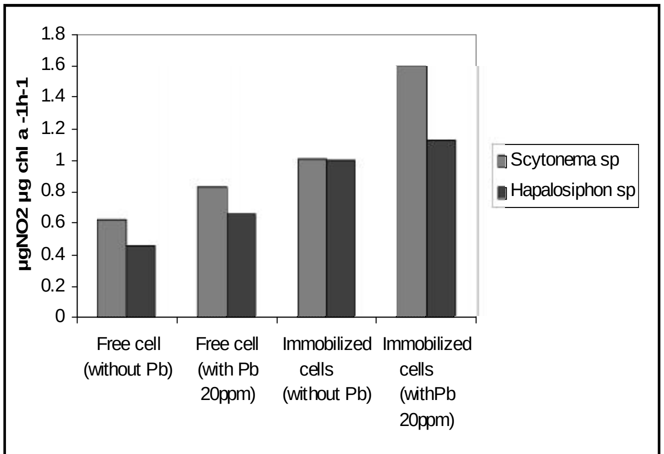

Fig.2 : Shows the effect of nitrate on nitrate reductase activity (25mM) of *Scytonema* sp and *Hapalosiphon* sp.

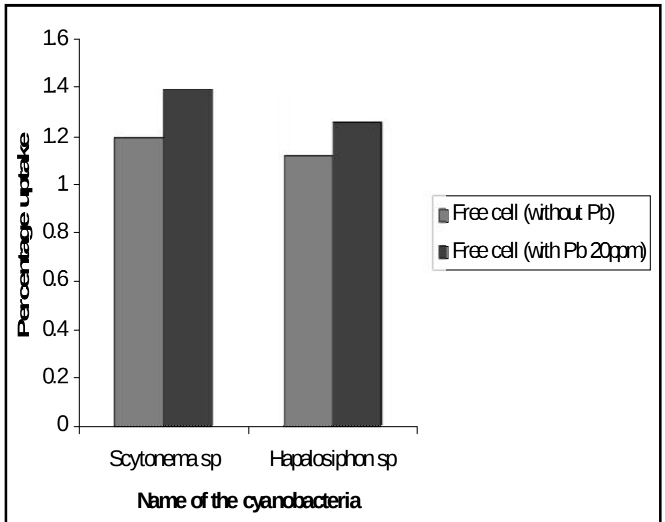

Fig. 3 : Shows the Uptake of Lead added to the medium as lethal doses after 8 days of growth of free cells and immobilized cells of *Scytonema* sp. and *Hapalosiphon* sp.

related to it. On the cyanobacterial cell surface proton-active carboxyl, phosphoryl, hydroxyl, and amine functional groups have been shown to deprotonate and bind metal ions to form metal-ligands surface complexes (Camm, and Stein, 1974, Yee *et al.*, 2004). However, the cell surface in cyanobacteria is not homogenous. In mucilage-producing cyanobacteria as found very often in the rice fields, it has been suggested that the binding of metals should take place through the polysaccharides.

Basically, immobilization increases nitrate reductase activity maybe due to change in the cellular behavior by directly modifying the intrinsic characteristics of the culture as beads by forming a monolayer on the surface of the culture medium thus increasing not only surface area but also increasing availability of other important environmental parameters like light and oxygen concentration. The increase in nitrate reductase activity in lead treated cells might be due to increase in surface area of thylakoids, thereby increasing the photosynthetic potential by active synthesis of thylakoidal protein and associated pigments (Phoenix *et al.*, 2002). This, on the other hand, produces the stimulatory effect on nitrate reductase because of increased reductant supply on which the enzyme is naturally dependent. The observed protection due to calcium alginate immobilization on Chlorophyll a content, nitrate reductase activity 20 ppm of lead could be due to the metal binding capacity of alginate. Alginate, which is a mixture of polyguluronic acid and polymannuronic acid, has abundant hydroxyl groups which bind the metal ions and prevent them from entering the cells in full concentration thereby protecting against the decrease of chlorophyll a content and enzyme activities as observed in free cells. Banerjee and Mishra (2002) have also shown the mitigating effect of immobilization on cyanobacteria subjected to heavy metal stress.

Immobilized cyanobacteria can tolerate heavy metal contamination and increased concentration of nitrate. It is pertinent to note that Pb addition has no effect on nitrate under immobilized condition. The uptake of lead was higher in immobilized condition in comparison to free cells, due to the presence of abundant hydroxyl groups in the alginate, which bind the metal ions.

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