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The toxicology of microcystins and its implication on human health.

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Abstract: *Eutrophication is a widely recognized problem of water quality deterioration. Discharge of urban, industrial and agricultural waste has increased the quantum of various chemicals that enter the receiving waters, and alters the physicochemical characteristic. The exploiding human population demanding more and more food, thereby pressing for more use of chemical fertilizers, and the residue of these chemicals reaches into fresh water reservoirs. The increasing nutrients results into prevalence of algal blooms in fresh waters, placing greater pressure on uses of water for both drinking and recreational purposes. Cyanobacterial blooms consisting Microcystis aeroginosa found in several parts of the world causes lethal poisoning to human, livestocks, wildlife and aquatic animals. Microcystin enters in the food chain through zooplankton, fish and aquatic animals. Microcystins toxins are naturally produced poisons stored in the cells of cyanobacterium M. aeroginosa. Human are affected with water related exposure of microcystins with range of symptoms including stomach cramps, vomiting, nausea, diarrhoea, fever, headache, muscle pain, flirtter of the mouth and liver damage. In addition, microcystins causes protein phosphatase inhibition and the cells suffers from oxidative stress. Thus, cyanotoxin phytotoxicity and zootoxicity strongly suggests a need for the surveillance of cyano-harmful algal bloom and the monitoring of water quality for drinking as well as for other purposes.*

Key Words: Allelopathy, Cyanobacterial blooms, Microcystin, Toxicity, Drinking water.

Introduction

Cyanobacteria (blue-green algae) are true bacteria of the class photobacteria and are amongst the oldest forms of life known and are widespread in aquatic environments, most notably in freshwater lakes and reservoirs. Cyanobacteria are important primary producers and play a crucial role in their ecosystems (Whitton and Potts, 2000). Cyanobacteria that have most relevance for man are the planktonic forms that float free in the water, particularly when they float to the surface to form a scum or "bloom". Cyanobacterial blooms are found abundantly in surface waters as a result of eutrophication and are responsible for production of different types of toxins in water bodies and causes health problems in humans and animals. The toxin producing cyanobacteria in water bodies have negative environmental impacts including reduced light and dissolved oxygen levels that may lead to fish kills, and may even cause shifts in plankton population (Jochimsen *et al.*, 1998). The health risk associated with ingestion of concentrated microcystins in animals tissue by bioaccumulation. *M. aeroginosa*, blooms impact through direct contact and ingestion that can cause skin and eye irritation, hay fever symptoms, dizziness, fatigue and stomach upset (Carmichael, 1995).

Cyanobacteria in different water have capacity for both aerobic and anaerobic photosynthesis and contains chlorophyll-a, carotene, xanthophylls, blue c-phycocyanin and red c-phycoerythrin. The last two pigments can only be found in cyanobacteria (Duy *et al.*, 2000). The photosynthetic apparatus of cyanobacteria is similar to algae. The lysis of a cyanobacterial bloom leads to the release of high amounts of blue-pigmented proteins, the so-called

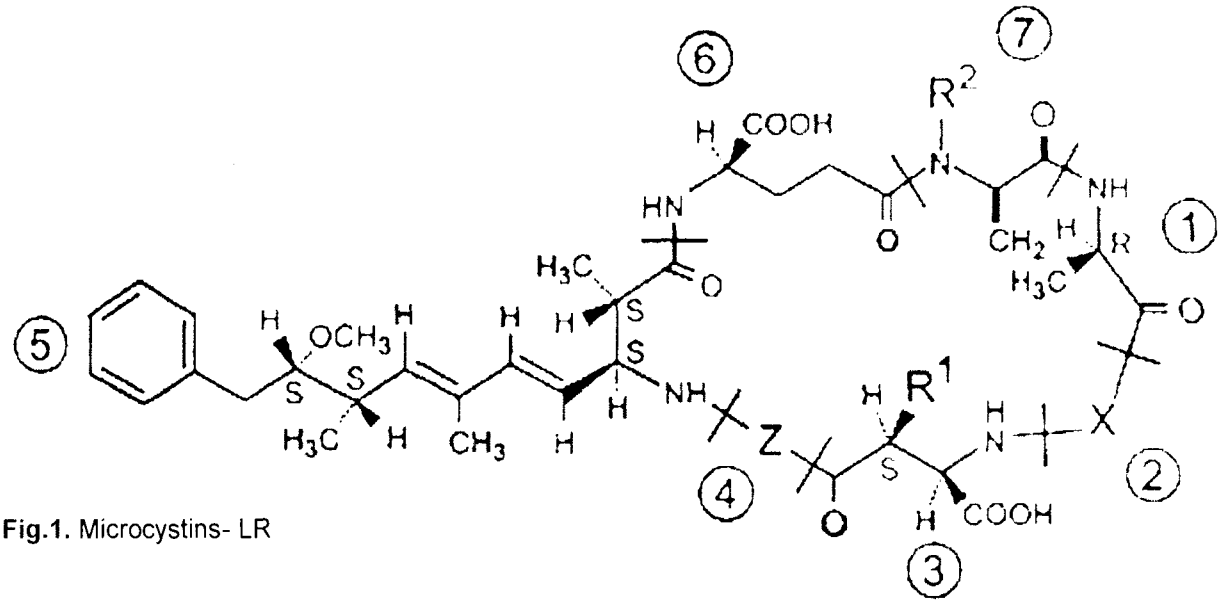

Fig.1. Microcystins- LR

phycobiliproteins. These blue pigments enable cyanobacteria to use a wider light spectrum than terrestrial plants and have provoked their early description as blue-green algae. Changes in cyanobacterial community composition, bloom dynamics and toxicity, in response to changing environmental factors including global warming are of great importance to our understanding of harmful cyanobacterial blooms.

Cyanobaterial Distribution and Water Quality: Cyanobacteria present in water in different part of world which affects water quality and health. Skulberg *et al.* (1993) studied more than 40 species belonging to *Nostoc* genus but toxigenic species includes *Nostoc linkia, Nostoc paludosum, Nostoc rivulare* and *Nostoc zetterestedítii*. Northcott *et al.* (1991) has characterized microcystin-LR from planktonic *Anabaena, Microcystis, Oscillatoria, Nostoc* and *Anabaenopsis species*, and from terrestrial *Hapalosiphon* genera. Dwivedi *et al.* (2005) studied the number of cyanobaterial species present in Keerat Sagar lake (11), Madan Sagar lake (9) from Bundelkhand zone of U.P., pond Indrajeet Khera (17), pond near SGPGI, Lucknow (10) pond in campus of National Botanical Research Institute, Lucknow (15). These ponds and lakes eutrophied by washing, bathing and sewage disposal supplemented with in flow of water through agricultural fields. Parikh *et al.* (2006) studied the water samples from the Vatva industrial area in Gujarat and found cyanobacterial species. Twelve species from 7 genera viz. *Chroococcus*, *Gloeothece*, *Gloeocapsa*, *Dermocarpa*, *Oscillatoria*, *Phormidium* and *Nostoc* were observed. Mitraki *et al.* (2004) studied phytoplankton population in other Greek lakes and observed that prolonged cyanobacterial blooms occur which last up to 8-9 months. Kudari *et al.* (2006) studied different lake and tanks at Dharwad and Haveri district in Karnataka and found fast progressions towards hypereutrophication due to anthropogenic activities, sewage and fertilizers used in agricultural fields appear to be the major causes of the eutrophication in these tanks. Gupta *et al.* (2006) reported that the high concentration of dissolved oxygen, NH_4-N and NO_3-N, that favours the growth of cyanobacterial community. Parikh *et al.* (2006) observed that higher cyanobacterial count with higher COD/BOD_5 ratio can be a very useful indicator.

Teubner and Dokulil (2002) reported that the 'balance of TN: TP: SRSi-ratios' attempts to

determine whether the seasonal succession of phytoplankton communities is influenced by or responds to nutrient stoichiometry. Because the molar average of TN: TP: SRSi = 20: 1: 17 for 118 measurements (7 lakes, 3 inflows, all seasons) was close to the marine optimum stoichiometric ratio, the ratio TN: TP: SRSi = 16: 1: 17 as a reference point for ecological stoichiometry. In lake it is reported that *Microcystis* were usually the dominant contributor to summer phytoplankton. The PO_4^{-3} concentration was also found to be relatively high similar to NO^-_3 however, unlike NO^-_3 the concentration of PO_4^{-3} remained more or less stable. Nitrate might be limiting the phytoplankton growth rather than phosphate. Stable PO_4^{-3} concentration and highly fluctuating NO^-_3 concentration resulted in steady decrease in N: P ratio from 3:1 in August (2002) to 2:1 in June, which further decreased to 1:1 in December (Mishra *et al.*, 2009). Briand *et al.* (2003) reported that abiotic factors such as high temperature, stratification and high phosphorous concentrations are enhancing their presence and persistence. Studies of Kagalou *et al.* (2003) in different lakes and observed that nitrogen/phosphorous concentrations ratio is less than the threshold of 10:1 which is considered as an indicator for strong nitrogen-limiting conditions, thus favouring the growth of N_2-fixing cyanobacteria. Kagalou *et al.* (2008) found that surface blooms of cyanobacterial dominant species *Microcystis sp.* and *Anabaena sp.* were recorded from early summer to late autumn. Increase in the concentration of Chlorophyll-a, and Chlorophyll-b to the river runoff at Netrovathi estuaries, west coast of India, during monsoon has been reported by Gowda *et al.* (2002).

Microcystins in water bodies: Distribution of microcystin is wide and found in Mediterranean lakes in Turkey (Albay *et al.*, 2003), in Portugal (Vasconcelos *et al.*, 1996), in lake Pamvotis (Greece) (Cook *et al.*, 2004), in temperate lakes of Canada (Kotak *et al.*, 1996), in Finish lakes (Lindholm *et al.*, 2003), in Brazil lagoons (Magalhaes *et al.*, 2001). Microcystins are considered endotoxins because the majority of the toxin is found within cell and released into water when the cell wall of the cyanobacterium is compromised in the presence of chemicals that inhibit new cell synthesis, enzymatic reactions or photosynthesis (Lam *et al.*, 1995). Matsunaga *et al.* (1999) reported that in September (1995) some 20 ducks died due to damage of liver and digestive tracks by bloom of *M. aeruginosa* at the site; Oo–ike pond, in Japan. Albay *et al.* (2003) collected 73 samples from Lake Sapanca at different depths (from the surface to 25 m) and cyanobacteria were found in 61 of the samples. Cyanobacterial populations mainly consisted of *Planktothrix rubescens*. The biomass (wet-wt) of this species varied from 16 µg l^{-1} to 544 µg l^{-1}. Willame *et al.* (2005), found highest microcystin concentration of 2231 µg g^{-1} in Belgium and Luxembourg Lakes. Gkelis *et al.* (2005) reported that bloom of *M. aeruginosa*, *Microcystis sp.* and *Anabaena* in lake Pamvotis during warm period producing the MC-LR and MC-RR.

Microcystins Structure : Microcystins are a family of cyclic polypeptides produced by different species of cyanobacteria (blue-green algae), which can form blooms in lakes and water reservoirs. Microcystin is a cyclic heptapeptide (Contain seven peptide-linked amino acids) with the general structure of cyclo-(D-alanine^1-X^2-D-MeAsp^3-Z^4-Adda^5-D-glutamate^6-Mdha^7) in which X and Z are variable L amino acids, D-MeAsp is D-erythro-β-Methylaspartic acid, and Mdha is N-methyldehydroalanine (Fig.1.). The amino acid Adda, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, is the most unusual structure in this group of cyanobacterial cyclic peptide toxin (Sivonen and Jones, 1999). Microcystin are monocyclic peptide are comparatively large natural product molecular weight 800–1100, contain seven amino acids, made up of five non protein amino acid and two protein amino acids. These two amino acids distinguish microcystins from one another. Using amino acid single letter code nomenclature, each microcystins designated a

name depending on the variable amino acid which completes their structure. The potent toxic microcystins-LR (MC-LR) contains amino acids Leucine (L) and Arginine (R) in variable position (Carmichael, 1988). Microcystins and nodularin producing cyanobacteria have gas vesicles. They are small and hollow air filled structures of cylindrical shape that provide buoyancy (Walsby, 1994). Gas vesicle enable the bacteria, after periods of water mixing, to float up from the deeper water layer back into the euphotic zone, where light for photosynthesis is provided, or reach deeper nutrient-rich layers by sinking. Therefore, these organisms have means to overcome spatial separation of nutrition and light. The ability to regulate their buoyancy is a major advantage over other phytoplankton species and may partly explain the enormous success of the toxin-producing species in the field. Despite these similarities individual toxin producing genera have different ecostrategies and inhabit different ecosystems (Dittmann and Wiegand, 2006).

Microcystin Toxicity : *M. aeroginosa* is toxic cyanobacteria present in water body contained the microcystin toxin gene (mcyA) explained by (Wilson *et al.,* 2005). Falconer and Humpage (2005) observed that cyanobacterial toxins by the WHO are the microcystins which caused acute liver injury and are active tumor promoters. The provisional guideline level of microcystin-LR for drinking water is 1 µg/l. Two unidentified microcystin variants were found in lake Taskisi surface samples at a concentration of 2.43 $\mu g l^{-1}$ microcystin–LR equivalent in the filtered cyanobacterial cell fraction (Albay *et al.,* 2003). The microscopic examination of the phytoplankton samples showed the dominance of the *Microcystis* genus in the cyanobacterial bloom. The highest microcystins concentration was observed in lake Oubeira, Eastern Algeria, (August 2001), at 29,163 µg/l. (Nasri *et al.,* 2004). An outbreak of acute liver failure occurred at a dialysis center in Caruaru, Brazil (8°17' S, 35°58' W), 134 km from Recife, the state capital of Pernambuco. At the clinic, 116 (89%) of 131 patients experienced visual disturbances, nausea, and vomiting after routine hemodialysis treatment on February 1996. Subsequently, 100 patients developed acute liver failure, and of these 76 died (Carmichael *et al.,* 2001). Microcystins and related polypeptides are potent hepatotoxins in fish, birds, and mammals. The consequence of an acute poisoning by these compounds is the rapid disorganization of the hepatic architecture (Falconer *et al.* 1981).

Leading to massive intrahepatic hemorrhage, often followed by death from hypovolemic shock or hepatic insufficiency (Carmichael, 1992). Matsushima *et al.* (1990) reported that microcystins penetrate with difficulty in the epithelial cells, which reflect tissue specificity, and their target cell is the hepatocyte. This cellular specificity and organotropism of microcystins is due to the selective transport system, the multispecific bile acid transport system, present only in hepatocytes. The work of Eriksson *et al.* (1990) clearly indicates that irrigation with water containing cyanotoxins can be a threat for both the quality and yield of crop plants. Also, the confirmed bio-accumulation of these toxins in plant tissues (Peuthert *et al.,* 2007; Crush *et al.,* 2008) constitutes a route for introduction of these toxins into the human food chain and ultimately a human health concern (Saqrane *et al.,* 2009). Kos *et al.* (1995) reported for the first time that microcystin inhibited the growth of mustard seedlings. The phytotoxic effects of cyanotoxins on terrestrial plants have increased, showing physiological and morphological alterations by cyanotoxins in a range of terrestrial plants (Pflugmacher *et al.,* 2007). Rinehart *et al.* (1994) by intraperitoneal (i.p.) mice bioassay ranged from 50 to >1,200 $\mu g\ kg^{-1}$ body weight. The survival time was estimated to be from 2-5 hours, and the calculated i.p. LD_{50} in mice ranged from 15 to 125 mg kg^{-1} body weight, liver damage with extensive haemorrhage necrosis and sinusoid capillary destruction, which showed the hepatotoxicity (Oudra *et al.,* 2009). Kagalou *et al.* (2008) observed that the accumulation of microcystins in fish tissue of *C. gibelio* and the target organ of microcystins is the liver. Zegura

et al. (2003) reported that the microcystin acts as hepatotoxins, when ingested by vertebrate they accumulate from the small intestine in the liver due to the active uptake by an unspecific organic anion transporter, known as bile acid carrier transport system. When the liver cell damage it includes loss of membrane integrity, apoptosis, cell blabbing, DNA fragmentation and strand break which may lead to death of the organisms by hemorrhagic shock.

Protein phosphatases are blocked if Adda interacts with the catalytic site of the enzyme (Goldberg *et al.,* 1995). Nishiwaki-Matsushima *et al.* (1991) noticed that microcystin accumulating organ are liver, and has tumor promoting capacity stimulating preneoplastic cells. Sekijima *et al.* (1999) noticed that diethylnitrosamine as tumor initiator; tumor-promoting activity of MC-LR was also demonstrated in rats after initiation by the natural mycotoxin aflatoxin B_1. Yatsunami *et al.* (1993) reported that hyperphosphorylation of retinoblastoma protein and p53 by okadaic acid, a tumor promoter. Ocadaic acid, a marine toxin produced by dinoflagellates of the species *Dinophysis* and *Prorocentrum,* acting via the same protein phosphatases inhibition mechanism. Halliwell and Gutteridge, (1984) observed that aerobic organisms generate reactive oxygen species (ROS) such as superoxide anion radical ($O_{2^{\bullet}}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) as a result of oxidative metabolism. •OH can initiate lipid peroxidation (LPO) in tissues, and to minimize the negative effects of ROS, fish, like other vertebrates, possess an antioxidant defence (AD) system, which utilizes enzymatic and non-enzymatic mechanisms. Some of the most important AD enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), while the nonenzymatic defenses include vitamins E, C and A, glutathione, and carotenes (Filho, 1996). Mitochondria are the sites where reactive oxygen species are mainly produced and red muscles are the most important source of mitochondria in endotherms. Therefore, this tissue is considered to be the main contributor to ROS generation in mammals and birds. However, in most fish red muscle makes up only a small proportion of tissues, and other tissues such as liver, kidney and gills (as the first tissue in contact with the xenobiotics in the water) are more important in this regard (Filho *et al.,* 2000). The reactive oxygen species (ROS) can be generated against the toxicant by the exposure of microcystin in both animal and plant cells (Botha *et al.,* 2004; Pflugmacher, 2004) and reacts with other cellular compounds such as lipids, proteins and DNA, which include superoxide, hydroxyl radical and radical of cellular organic compounds, causing damages such as peroxydation of lipid, protein and DNA. Zegura *et al,.* (2004) reported that DNA fragmentation started with oxidation of purines that seems not to undergo repair, but even provoked further DNA strand breaks, oxidative damage were prevented by ROS scavengers and partly diminished by hydroxyl radical scavenger. Oxidation of DNA furthermore resulted in the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (Maatouk *et al.,* 2004). The promotion of oxidative stress due to exposures to cyanobacterial toxins was shown in different plants from aquatic macrophytes (Mitrovic *et al.,* 2004) to higher terrestrial plants like *Brassica napus*, *Oryza sativa* and *Medicago sativa* (Chen *et al.,* 2004). Cyanobacteria are one of the most diverse groups of prokaryotes. Cyanobacterial blooms are found abundantly in surface water as a result of eutrophication and are responsible for production of different type of toxins in water bodies that causes health problem in humans and animals. There is urgent need of protection of the health, productivity and welfare of general population from the adverse effects of the cyanotoxins release by cyanobacteria. Changes in community structure of cyanobacteria, protection mechanisms, role of environmental factors, problems of eutrophication of fresh water bodies and toxic algal blooms needs immediate attention.

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