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Protective role of dietary *Spirulina platensis* on oxygen consumption in *Labeo rohita* exposed to sublethal concentration of mercuric chloride

Dr. AD Shelke and Dr. GP Wani

Abstract
The current study was designed to investigate the protective role of dietary *Spirulina platensis* on oxygen consumption of freshwater fish, *Labeo rohita*. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. The results showed that sublethal exposure of *Labeo rohita* fed with *Spirulina platensis* free diet (T1 groups) significantly decline in rate of oxygen consumption than those exposed to sublethal level of Mercuric chloride and fed *Spirulina platensis* supplementation diet (T2- T5 groups).

Keywords: Protective role, *Spirulina platensis*, oxygen consumption, mercuric chloride, *Labeo rohita*

1. Introduction
Mercury is persistent in the environment as it is an element that cannot be "broken down or degraded into harmless substances this means, that once mercury is released and brought into the circulation in the biosphere by human activity is does not disappear easily comparable to human lifetime [1].

During last few decades, pollution of aquatic environment by heavy metals is an extremely imperative and serious problem that has attracted the attention of researchers [2]. Mercury deposition into aquatic environments such as rivers, lakes, streams and estuaries is the major route of mercury transported into marine ecosystems. Mercury in the aquatic environment has been of great concern for scientists and environmentalists due to the methylation process by microorganisms and, possibly, by certain abiotic factors. As a result of this, the methylmercury produced, is more toxic and has the potential for bioconcentration and bioaccumulation via the aquatic food web [3]. The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the ecosystem, make these harmful chemicals to the aquatic ecosystem and consequently, to humans who depend on aquatic products as sources of food. Since heavy metals can accumulate in the tissues of aquatic organisms, these tissue concentrations of heavy metals can be of public health concern to both organisms and humans [4]. Heavy metals are known to act on gill physiology, resulting in a decrease in the oxygen consumption because of ion-regulatory and acid-base disturbances. Oxygen consumption, therefore, could serve as a biomarker in metal toxicity studies in fish and other aquatic animals [5]. The rate of oxygen consumption of *Amblypharyngodon mola* was decline, observed in subsequent acute and chronic exposure of mercuric chloride, arsenic trioxide and cadmium chloride. It can be due to onset of poisoning and gill damage, Formation of mucus film over the gill and on the body surface. This may reduce the efficiency of oxygen up take of fishes [6]. *Spirulina platensis* (sp) is used in many countries as nutritional supplement for human and animal consumption, labelled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols, minerals and vitamins [7]. *Spirulina platensis* is well known for its protective effect against heavy metal toxicity [8]. Dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the food utilization parameters like feed intake, consumption rate, weight gain, growth rate and feed conversion ratio (FCR) value significantly as the percent dose of *Spirulina platensis* was increased in a short period of time [9]. In the present study, experiments were designed to investigate the protective effect of dietary *Spirulina platensis* supplementation on the oxygen consumption in carp, *Labeo rohita* against the sublethal toxicity of mercuric chloride.

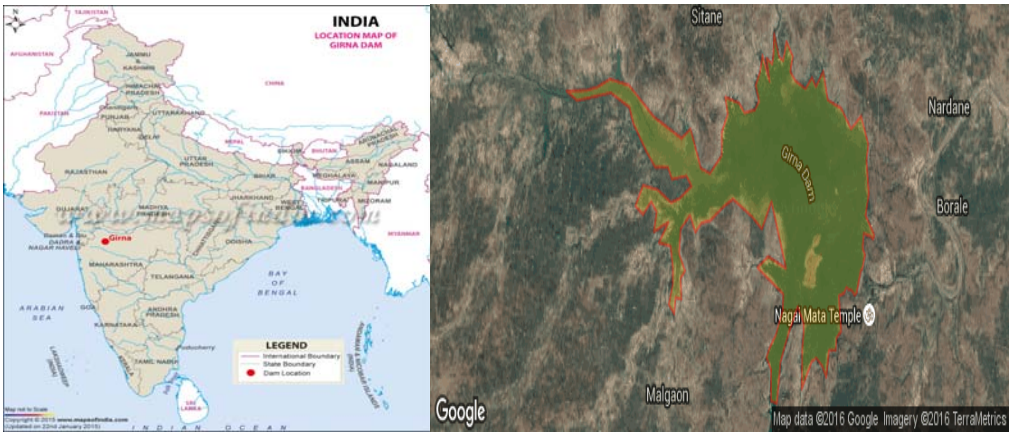
2. Materials and methods

The live major carp, *Labeo rohita* were obtained from a Girna river dams near Chalisgaon city, 20°29'16"N 74°39'41"E. They were acclimatised in laboratory condition for more than two weeks. The temperature, PH, Salinity and dissolved oxygen of the water were found to be 27 ± Ċ, 7.55 ± 0.1, 0.76 ± 0.09% and 7.20 ± 0.12 ml/ l respectively. During the acclimatisation, water was changed daily and fish were fed ad libitim with pelletised diet containing 35% protein. Acclimatised fish (1.30 ± 0.10g) were exposed to different concentrations (0, 0.03, 0.06, 0.09., 0.12, 0.15, 0.18 ppm) of mercuric chloride $HgCl_2$ obtained from Merck India Ltd. (Mumbai, India), dose concentration was selected on the basis of mercuric chloride toxicity study reported by [10] and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration. Probit analysis [11] was followed for the calculation of 96 hours $LC_{50.}$ Control group of fish was maintained in mercury free freshwater. Feed: In the present experiment, 35% protein diet was used as basal diet for *Spirulina platensis* supplementation. The intergradient of dried fish meal, ground oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportion by square method. [12]. In addition to the control diet, five diets (0, 2, 4, 6, and 10 %) were prepared with different *Spirulina platensis* levels. The experimental diets were by adding the appropriate level of *Spirulina platensis* with chosen intergradient to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2mm) were prepared with operated pelletizer and dried in sunlight. After drying diets were separated stored in refrigerator. Active and healthy fish (1.30 ± 0.10 g) were chosen from the acclimatisation tank and starved for 24 h prior to the commencement of experiment.

The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina platensis* free diet. Test animals belonging to 2nd, 3rd, 4th, 5th and 6th groups were exposed to 0.12 ppm of mercuric chloride.

Group-II: Individuals was fed with *Spirulina platensis* free diet, however 3rd, 4th, 5th and 6th groups were fed with 2, 4, 6 and 10% *Spirulina platensis* diets respectively. The experimental groups 1, 2, 3, 4, 5 and 6 are designated as C, E1, E2, E3, E4 and E5 respectively. The experiment was conducted in glass tank. (Capacity: 80L) Containing 80L water. The experiment was conducted for 7, 14, 21 days to study the impact of dietary *Spirulina platensis* on oxygen consumption in *Labeo rohita.* Fish were removed from each experimental group at the end of the 7, 14 and 21 days. Oxygen consumption of test fish was estimated [13]. Three samples were analysed and the data were subjected to Student's 't' test and correlation and regression analysis.



Location of Girna dam on Girna River near nandgaon, District Nasik, Maharashtra.

4. Results

In experimental groups T_1 with 0% *Spirulina platensis* the oxygen consumption was increased as compared to control for 7, 14 and 21 day was from -65.94, -69.40 and -74.49 respectively. *Labeo rohita* showed abrupt decrease in oxygen consumption in T_1 with 0% *Spirulina platensis* as compared with control but oxygen consumption gradually decreased when the dose of *Spirulina platensis* is increased from 2%, 4% and 6% in experimental groups T_2, T_3, T_4 & in experimental groups T_5 with 10% *Spirulina platensis* the oxygen consumption as compared to control for 7, 14 and 21 day was from -46.41, -49.99 and -54.50 respectively. Values are significant at *** = P<0.001, NS = Non-significant. (Table-1 and Figure-1). When the dose of dietary supplementation of *Spirulina platensis* was gradually increased in the *Labeo rohita, Spirulina platensis* shown protective role against the reduction in the mercuric chloride toxicity, because of that, the rate of oxygen consumption was gradually decreased.

Table 1: Effect of supplementation of dietary *Spirulina platensis* on oxygen consumption in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

Sr. No.	Treat.	% *S. p.*	Average oxygen consumption ± S.D. (ml. of O_2/gm of body weight./hr./lit.		
			7 days	14 days	21 days
1	Control	0%	0.1696 ±0.006324	0.1611 ±0.00707	0.1669 ±0.00624
2	T_1	0%	0.06787 ±0.00603*** -65.94	0.05938 ±0.00606*** -69.56	0.04666 ±0.006*** -74.49
3	T_2	2%	0.007211 ±0.00607*** -58.45	0.07211 ±0.00606*** -61.30	0.05938 ±0.00606*** -65.70
4	T_3	4%	0.08060 ±0.01202** -55.78	0.06787 ±0.006033*** -57.87	0.07211 ±0.00606*** -61.30
5	T_4	6%	0.009332 ±0.00609*** -53.17	0.08484 ±0.00647*** -56.51	0.07211 ±0.00607*** -58.45
6	T_5	10%	0.1068 ±0.001274^{NS} -46.41	0.09756 ±0.0607*** -49.99	0.08484 ±0.00609*** -54.50

i) Each value is a mean of three ± S.D.
ii) (+) or (-) signs indicate % variation over control.
iii) Values are significant at * = P<0.05, *** = *P*<0.001, NS = Non significant.

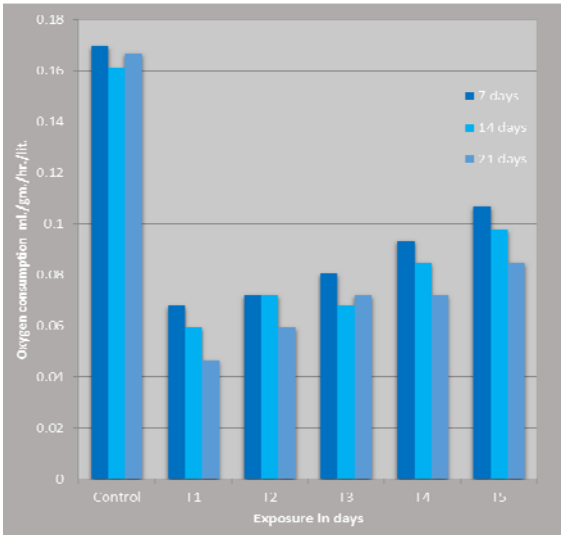


Fig 1: Variations in effect of supplementation of dietary *Spirulina platensis* on oxygen consumption in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

4. Discussion

Decrease in oxygen consumption may be due to interaction of toxic heavy metals with respiratory system of fish by asphyxiation, abnormality in function of gills and also inhibition of enzyme system. Interactions among the metals in aquatic media are capable of producing damage in gill tissues and ultimately caused death of fish [14].

Extensive damage in internal gill architecture, degenerative changes, swelling, fusion, atrophy and degeneration of secondary gill lamellae with bulging at tips. Reduction in the size of primary and secondary gill lamellae and necrosis of tissue were observed in freshwater fish, *Amblypharyngodon mola*, exposed to Mercuric chloride and Cadmium chloride [15].

Jeyaprakash and Chinnaswamy [16] have reported that most of the antioxidant capacities of *Spirulina* are due to presence phycocyanin [17]. Metal chelating potential of *Spirulina* was reported by Islam *et al.*, [18], who used *Spirulina* as a feed supplement against arsenic induced toxicities in ducks and reduction of tissue arsenic concentration from 80% to 20% was recorded. Gills are vital respiratory and osmoregulatory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area. Architectural changes in the gill morphology like formation of club shaped gill lamellae; fusion and necrosis of the gill lamellae and atrophy of central axis of the gill filament were observed in *Labeo rohita* under the toxic impact of chromium [19].

Cytotoxicity of test chemicals decreased when fish were fed with diet supplements, especially combination of *Spirulina* with tamarind since their RBC count were even higher (20-30%) than control fish reared only on standard diet [20].

The percentage of morphologically abnormal RBCs (poikilocytosis) where higher in the treatments (8- 31%), more particularly in Al^{+3} (18-31%), when compared with controls (3.0 -15%).

Majority of abnormal RBCs were beaked, as also in control. Such morphological aberrations may adversely affect oxygen carrying capacity of RBCs in the blood and so overall metabolism of the pollutant exposed organisms. Since magnitude of such aberrations decreased in the treated fish feeding on diet supplements, hence protective role of diet supplements in reducing cytotoxic effects [20].

Phycocyanin pigments of *Spirulina* stimulates erythropoietin (EPO) hormone production of hematopoiesis whereas C-phycocyanin and polysaccharide stimulate recovery of white blood cells and bone marrow cell counts [21].

The sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in RBC count but RBC count were increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the RBC count was increased from 1.64 to 2.08 (×106 mm^{-3}) as the percent dose of *Spirulina platensis* increased. Haemoglobin content was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was gradually increased in the mercuric chloride with *Spirulina platensis* diet from 2% to 10% and the haemoglobin content was increased from 5.80 to 7.00 g % as the percent dose of *Spirulina platensis* increased. In mercuric chloride exposed *Labeo rohita* and it may be due to the reduction of RBC count and HB content which reflected on tissue respiration.

The dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the haematological parameters like RBC count and haemoglobin content significantly as the percent dose of *Spirulina platensis* was increased in a short period of time [22].

5. Conclusion

In the present study the oxygen consumption were improved in mercuric chloride exposed *Labeo rohita* fed with *Spirulina platensis* supplementation diets as suggests the protective role of *Spirulina platensis* against mercuric chloride toxicity in

Labeo rohita has 14% phycocyanin pigment and it stimulates the erythropoiesis (EPO) hormone production for hematopoiesis. Phycocyanin pigment also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemical or radiation. This may be evidently reflected on overall oxygen consumption of animals exposed to mercuric chloride.

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