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EFFECT OF DIETARY *SPIRULINA PLATENSIS* ON METAL DISTRIBUTION IN *LABEO ROHITA* EXPOSED TO SUBLETHAL CONCENTRATION OF MERCURIC CHLORIDE.

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ABSTRACT
Pollution of aquatic environment by heavy metals is an extremely imperative and serious problem. The environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems. The accumulation of heavy metals in freshwater ecosystem has been a major concern. Fish being one of the main aquatic organisms in the food chain may often accumulate large amounts of certain metal above the levels in the aquatic environment. *Spirulina platensis* is a cyanobacterium, used in many countries as nutritional supplement for human and animal consumption, labeled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols, minerals and vitamins. *S. platensis* is well known for its protective effect. Keeping this in mind the property of S.P., the study was aimed to elucidate its protective effects against the elimination of mercuric chloride from the carp, *Labeo rohita*. In the present study, experiments were designed to investigate the impact of dietary *Spirulina platensis* supplementation on the metal distribution in the gills, liver and muscle tissues in carp, *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 result showed that, the gill tissue elicited the highest mercuric chloride accumulation followed by the liver and muscle. Mercuric chloride accumulation was high in T1 groups and it gradually decreased as the *Spirulina platensis* level increased in T2 to T5 groups. The maximum reduction of mercuric chloride accumulation in tissues occurred with the supplementation of 6% (T4) *Spirulina* diets followed by 10% (T5) and 2% (T2) *Spirulina* diet respectively.

KEYWORDS: Bioaccumulation, *Labeo rohita*, Merculric chloride, Protective effect, *Spirulina platensis*,

INTRODUCTION

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.,* 2007). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.,* 2004). Fish have the ability to accumulate heavy metals in their tissues by absorption along gill surface and kidney, liver and gut tract wall to higher levels than environmental concentration (Annabi *et al.,* 2013). Accumulation of heavy metals by organisms may be passive or selective; and differences in accumulation of heavy metals by organisms could be as a result of differences in assimilation, egestion or both (Egila and Daniel, 2011). Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake (Jarup L. 2003). 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 21 days period of time (Shelke, 2015).

Spirulina provides protection against mercuric chloride-induced oxidative stress and alteration of antioxidant defence mechanism in the liver. These activities were largely related to phycocyanin, an active protein of *Spirulina* (Romay *et al.,* 1998). 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 21 days period of time (Shelke, 2015). *Spirulina platensis* (SP), a nutritionally enriched filamentous cyanobacterium, possesses diverse biological and nutritional significance having bio-modulatory and immunomodulatory functions. (Khan *et al., 2005*). Phycocyanin (Pc) is a biliprotein of the blue-green alga. This protein contains a tetrapyrrole phycocyanobilin, which is responsible for antioxidant properties of Pc (Bhat *et al.,* 2001). It has been reported that Pc has significant antioxidant and radical scavenging properties, offering protection against oxidative stress (Lissi *et al.,* 2000). Antioxidants can reduce arsenic toxicity through chelating it and scavenging free radicals (Rana *et al.,* 2007). 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

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chloride toxicity in *Labeo rohita* (Shelke, 2016). In the present study, experiments were designed to investigate the protective effect of dietary *Spirulina platensis* metal accumulation in carp, *Labeo rohita* against the sublethal toxicity of mercuric chloride.

MATERIALS AND METHODS

The live major carp, *Labeo rohita* were obtained from a Girna river near Chalisgaon city. 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 ± 1Ċ, 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 Merk India Ltd. (Mumbai, India) and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration. Probit analysis 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 patensis* 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. (Hardy, 1980). In addition to the control diet, five diets (0, 2, 4, 6 & 10 %) were prepared with different *Spirulina patensis* levels. The experimental diets were by adding the appropriate level of *Spirulina patensis* with chosen intergradient to boiled water, mixed well and steam cooked form15-20 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 acclimatization 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. Triplicates were maintained for each group.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina patensis* free diet. Test animals belonging to 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were exposed to 0.12 ppm of mercuric chloride.
Group-II: individuals was fed with *Spirulina patensis* free diet, however 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were fed with 2, 4, 6 and 10% *Spirulina patensis* 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 epoxy coated cement tank. (Capacity: 100L) containing 100L water. The water was not changed during the experiment but was aerated for 14 h to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, PH, salinity and hardness of water were estimated during non- aeration period. Two series of experiment were conducted in the present study.

Exp. I: Like the first series of experiment, a parallel experiment was conducted simultaneously for 7, 14 & 21 days to study the impact of dietary *Spirulina patensis* on selected parameters and metal accumulation in *Labeo. rohita.* Test animals fed ad libitum with chosen experimental diets to respective exposures twice a day at 07:00 and 18:00 hrs. for 1 h each. Test animals were starved for 24 h prior to the conclusion of the experiment for the estimation of metal accumulation. Faecal matter was randomly collected by using feeding trays and dried in hot air oven at 60Ċ to estimate the mercuric chloride content.

Mercuric content in liver, muscle, gills, faces and water were estimated at the end of the experiment on days 21. Three replicates of samples (except water) from each tissue from each group were digested in a water bath at 100 Ċ with a mixture of concentrated nitric acid and perchloric acid in the ratio 1:2 until the formation of a white residue. The cooled residue was dissolved completely by adding 1N HCL and made up to 25 ml with distilled water. (FAO, 1975). The mercuric chloride concentration in water was estimated following the method of APHA. (2005). The solution was filtered through cotton wool and the filtrate was subjected to metal analysis in atomic absorption spectrophotometry. The instrument was calibrated using standards prepared from mercuric chloride. The bioconcentration factor (BF) was determined as (tc_{21}–tc_{20}) mercury concentration in water, where tc_{21} = tissue concentration on day 21 and tc_{20} = tissue concentration of days 0.

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RESULTS AND DISCUSSION

Table 1: Effect of dietary *Spirulina platenis* level on metal distribution in tissues (µg $HgCl_2$/g wet tissue), faces (µg $HgCl_2$/g dry matter), and water (µg $HgCl_2$/l) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

Component	Diet (*Spirulina* content)					
	Control	0%	2%	4%	6%	10%
Gill	ND	0.454 ±0.007	0.391 ±0.004	0.269 ±0.002	0.242 ±0.004	0.225 ±0.003
Liver	ND	0.380 ±0.004	0.268 ±0.018	0.216 ±0.002	0.195 ±0.018	0.186 ±0.003
Muscle	ND	0.123 ±0.001	0.087 ±0.002	0.066 ±0.003	0.060 ±0.001	0.057 ±0.001
Faces	ND	0.025 ±0.001	0.065 ±0.004	0.980 ±0.012	1.060 ±0.002	1.070 ±0.006
Water	ND	0.112 ±0.003	0.093 ±0.002	0.090 ±0.001	0.088 ±0.006	0.086 ±0.009
BF	ND	26.67 ±0.003	7.12 ±0.033	0.41 ±0.001	0.33 ±0.030	0.30 ±0.005

BF: Bioconcentration factor ND: Not detected.
i) Each value are mean ± S.D. of three estimations.

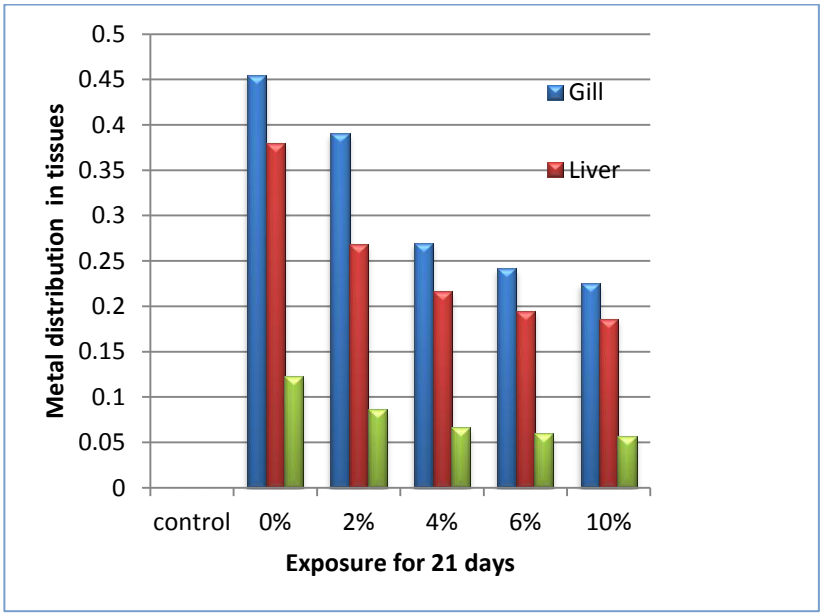

Figure 1: Effect of dietary *Spirulina platenis* level on metal distribution in tissues (µg $HgCl_2$/g wet tissue), faces (µg $HgCl_2$/g dry matter), and water (µg $HgCl_2$/l) in *Labeo rohita* exposed to sublethal concentration of mercuric chloride for 21 days.

In the present study, the bioaccumulation of mercuric in the gills liver and muscle tissues is evaluated in the fish, *Labeo rohita* exposed to sub-lethal concentration of mercuric chloride for 7^{th}, 14^{th} and 21^{st} days. The elimination of accumulated mercuric chloride through faces increased with increasing the dietary level of *Spirulina* in the diet (Table-1and Figure-1). Mercuric chloride elimination through faces in T1 groups was 0.025 µg dry matters as against 1.060 and 1.070 µg dry matters in T4 and T5 groups respectively. A positive correlation coefficient was obtained for the relationship between the supplementation of dietary *Spirulina* and elimination of mercuric chloride through faces.

Elimination of mercuric chloride through faces reduced the body burden of mercuric chloride which directly improved the food utilisation and haematological parameters. In the present study, the mercuric chloride concentration in liver tissue was found to be dose and time dependent. Similar findings were reported by (Karuppasamy, 1999), who has described the bioaccumulation as dose and time dependent in phenyl mercuric acetate exposed fish *Channa punctatus*.

In the present study, the muscle of *Labeo rohita* has showed a lower degree of mercuric chloride accumulation when compared to gill and liver tissues at sublethal concentration of mercuric chloride intoxication. The observations of the present study are in good agreement with (Maher *et al.,* 1999), who have suggested the least quantum of arsenic in the muscle of *Mugil cephalus*. Accumulation of heavy metals (As, Cd, Hg, and Zn) in muscle tissues of fishes have been well described by several investigators (Gupta and Sharma, 1994; Karuppasamy, 1999). (Kalay *et al.*, 1999), have stated the lower levels of lead in the muscle of treated fish, *Mullus barbatus*. In the field study it appears to accumulate less concentration of arsenic in the muscle and gill tissues than liver and kidney (Maher *et al.,* 1999). *Spirulina palatensis* reduced the accumulation of mercuric chloride in tissues in elimination of accumulated metal through faces, lessening the metal burden and its toxicity on fish. Spirulina contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. (Henrikson R, 1994) B-carotene of Spirulina maintains the mucous membrane firmely (Henrikson R, 1994) and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleasing and detoxifying phytonutrient against the toxic substances (Henrikson R, 1994).

The present study showed that *Spirulina* reduced mercuric chloride accumulation in tissues and increased mercuric chloride elimination through feces, lessening the metal burden and its toxicity to fish. The reduced growth rate in fish given a sublethal level of mercuric chloride was probably due to the tissue burden of mercuric chloride which, in turn, could have caused a reduction in feed intake, an increase in metabolic cost, or poor food conversion efficiency. *Spirulina palatensis* reduced the accumulation of mercuric chloride in tissues by elimination of accumulated metal through faces, lessening the metal burden and its toxicity on fish. Spirulina contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. Carotenoid of *Spirulina* maintains the mucous membrane firmly and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleansing and detoxifying phytonutrient against the toxic substances. It indicates that *Spirulina* has the ability to eliminate and detoxify the accumulated mercuric chloride.

CONCLUSION

Protective effect shown by *Spirulina platensis* on metal accumulation in *Labeo rohita* exposed to sublethal concentration Mercuric chloride. *Spirulina* contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. Carotenoid of Spirulina maintains the mucous membrane firmly and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleansing and detoxifying phytonutrient against the toxic substances.

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