EFFECT OF COPPER SULPHATE ON THE BIOCHEMICAL PARAMETERS IN DIFFERENT TISSUES OF *LABEO ROHITA*

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Abstract

Aquatic ecosystems are very sensitive to heavy metal pollutants and the gradual increase in the level of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. The continuous exposure of metals can lead to hematological and biochemical disorders. In the developing countries, there was a great increase in the establishment of industries. Heavy metals are regarded as serious pollutants of the aquatic ecosystem because of their environmental persistence and their ability to be concentrated by aquatic organisms. The concentrations of certain heavy metals are extremely toxic to fish life in fresh water and it is suggested that heavy metals produce blood cell destruction. The concentrations of certain heavy metals are extremely toxic to fish life in fresh water and it is suggested that heavy metals produce blood cell destruction. Heavy metals produce and acute hematology crisis in aquatic organisms and pollutants generally caused rapid changes in blood parameters of fishes. Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. This graceful Indo-Gangetic riverine species is the natural inhabitant of the riverine system of India.

Key : Toxicity - Heavy metals – Copper sulphate – *Labeo rohita* – Freshwater fish.

Introduction

Environmental pollution not only causes a decrease in water quality, but it subsequently affects all living organisms in that system. Therefore, it is necessary to not only identify and manage these pollution sources, but also to maintain their effects on the health of aquatic environment (Aruna *et al.,* 1987). Heavy metal pollution is a major environmental problem facing the modern world (Perrier *et* al., 1972). The global heavy metal pollution is increasing in the environment due to increasing of human activities. Moreover, it is gaining in importance day by day due to its obvious impact on human health through the food chain (Ruperelia, et al.,1992). The danger of heavy metals is aggravated by their almost indefinite persistence in the environment because they cannot be destroyed biologically but are only transformed from oxidation state organic complex to another. In addition, they are highly toxic for both higher organisms and microorganisms (Garbisu and Alkorta, 2001). Heavy metals produce and acute haematology crisis in aquatic organisms (Sampath *et al*., 1998; Nanda, 1997;Saravanan and Harikrishnan 1999) have reported that stressors and pollutants generally caused rapid changes in blood parameters of fishes. Protein is the most abundant macromolecule in animals constituting over half of their dry weight. They regulate and integrate numerous physiological and metabolic processes in the body through hormones, enzymes and nucleo proteins; Palanisamy (2002). Copper is one of the heavy metals and is more toxic even in low concentration; Copper is widely used in industries viz., Mining, Combustion of fossil fuel Coal, gas) fertilizer and pesticide.etc., Copper and its compounds reach the aquatic environment from the above sources via. Surface run off and sediment transport from the waste materials. The effects of sub lethal concentration of copper on the different biochemical parameters like blood glucose, blood protein, blood albumin, blood cholesterol in the fresh water fish *Labeo rohita* have been studied. It is the most popular food fish. Its flesh is delicious and rich in protein content.

MATERIAL AND METHODS

COLLECTION AND MAINTENANCE OF THE EXPERIMENTAL ANIMAL

The fresh water fish Labeo rohita were collected from the fish farm located in Pinnalur, Cuddalure District, (20 km). The fingerlings were brought to the laboratory and transformed to the rectangular fiber glass tanks (100x175cm) of 500 liters capacity containing chlorine free aerated well water. Fingerlings were acclimatized for a maximum period of 15 days in the laboratory conditions at room temperature before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternate days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack. The fingerlings each measuring 4.5 to 6 mm in length and weighing 4 to 6mm were used for the experimental studies. The toxicant sample, copper was used for the present experimental studies. Different concentrations of the salt solution at mg/1 were prepared by dissolving the salt in chlorine free well water. To estimate the protein content in the chosen tissue was estimated by the method of Lowry et al., (1957); the total free amino acid content of tissues were estimated by the method of Moore and Stain (1954). For the estimation of glycogen the residue in the tube was homogenized in 5ml of 5%. TCA (5g of TCA and 100mg of silver sulphate in 100ml distilled water).To estimate the glucose the 10 mg of supernatant was powdered; charcoal was added and the methanol was allowed to evaporate by placing the tube in warm water bath for 30 minutes. The mixture was made up to 5ml by adding the required quantity of 10% TCA. The mixture was centrifuged and to 1 ml of supernatant 3ml of concentrated sulphuric acid was added carefully. Finally the collected data obtained from the quantitative study were expressed as the mean ± S.E. The mean values were calculated from 6 individual observations. P-values were calculated by the two tailed students‘t’ test.

RESULT AND DISCUSSION

Labeo rohita fingerlings exhibit abnormal behavioural changes when exposed to different concentrations of copper sulphate. Sudden heavy stress is laid on fish and the fish often tried to leap out of the toxic medium in order to avoid the toxic environment. In the present study, when *Labeo rohita* exposed to 120 hours sublethal concentration of Copper, the protein content in the gill tissue were decreased.

Protein

In the present study, when *Labeo rohita* exposed to 120 hours sub lethal concentration of Copper, the protein content in the gill tissue were decreased. The percent decrease of protein content in the treated gills were -11.52, -18.30, -24.28, -35.53 and -53.94 for 24, 48, 72, 96 and 120 hours respectively (Table.1). Fishes treated with sub lethal concentration of copper sulphate exhibits a marked decrease in the liver protein. The percent change over control values for all the five periods of sub lethal concentration are -4.17, -8.83, -6.02, -15.89 and -23.51 at 24, 48, 72, 96 and 120 hours respectively (Table.1). The observed values were highly significant at 5% level. The protein content of kidney decreased at 24, 48, 72, 96 and 120 hours. The percent change over control values for all the five periods of sublethal concentration are -6.97, -13.89, -29.83, -28.08 and -40.59 respectively (Table.1). When compared with control at treated the observed values are highly significant at 5% level. When *Labeo rohita* exposed to sublethal concentration of copper, a gradual decrease in the protein content of muscle is evident at all periods of exposure. The percent decrease over control for all the five periods of sublethal concentration are -5.88, -11.82, -19.94, 26.74 and -30.22 for 24, 48, 72, 96 and 120 hours respectively . When compared with control at treated the observed values are highly significant at 1% level.

Amino acid

The free amino acid levels of gill tissue exhibited changes from the control levels. When the *Labeo rohita* are exposed to sublethal concentration of copper, shows a increase at all hours of exposure. The percent changes over the control are +4.66, +13.16, +7.99, +25.74 and +30.90 at 24, 48, 72, 96 and 120 hours respectively (Table.2). When compared with control at treated the observed values are highly significant at 1% level. The liver tissue also showed a similar trend in the amino acid content. When *Labeo rohita* are exposed to sublethal concentration of copper. The patterns of changes are similar to liver amino acid content. The percent increase over control are +2.27, +13.81, +19.87, +9.83 and +42.10 for 24, 48, 72, 96 and 120 hours respectively (Table.2). The observed values were highly significant at 5% level. The *Labeo rohita* are exposed to sublethal concentration of copper, a gradual increase in the amino acid content of kidney tissue is evident at all periods of exposure. The percent increase over control for all the

five periods of sublethal concentration are +7.15, +17.52, +43.09, +29.05 and +42.36 for 24, 48, 72, 96 and 120 hours respectively (Table.2). The observed values were highly significant at 5% level. When compared with control at treated the observed values are highly significant at 5% level. The amino acid content of muscle tissue increased at 24, 48, 72, 96 and 120 hours. The observed changes over control are +0.83, +16.51, +13.36, +18.10 and +32.43 respectively (Table.2). The observed values were highly significant at 5% level. The observed values were highly significant at 5% level.

Glucose

In the present study, the *Labeo rohita* are exposed to 120 hours sublethal concentration of copper, the glucose content in the gill tissue were increased. The percent increase of glucose content in the treated gills tissue were +5.68, +10.03, +24.21, +14.23 and +27.78 for 24, 48, 72, 96 and 120 hours respectively (Table.3; Fig.3). The observed values were highly significant at 5% level. Fishes treated with sublethal concentration of copper exhibits a increase in the liver tissue glucose level at 24, 48, 72, 96 and 120 hours. The percent change over control values for all the five periods of sublethal concentration are +6.07, +20.97, +27.90, +32.47 and +46.30 respectively (Table.3). The observed values were highly significant at 5% level. The glucose content of kidney tissue increased at 24, 48, 72, 96 and 120 hours. The observed percent changes over control are +6.07, +15.90, +27.75, +0.43 and +51.26 respectively (Table.3). When compared with control at treated the observed values are highly significant at 5% level. The glucose levels of muscle tissue exhibited remarkable changes from the control levels. When the *Labeo rohita* are exposed to sublethal concentration of copper, shows a increase at all the hours of exposure. The percent changes over the control are +9.05, +14.52, +21.94, +26.48 and +34.67 for 24, 48, 72, 96 and 120 hours respectively (Table.3). The observed values were highly significant at 5% level.

Glycogen

The glycogen levels of gill tissue exhibited remarkable changes from the control levels. When the *Labeo rohita* are exposed to sublethal concentration of copper, shows a decrease at all hours of exposure. The percent changes over the control are -3.12, -8.28, -12.41, -18.20 and -25.42 for 24, 48, 72, 96 and 120 hours respectively (Table.4). The observed values were highly significant at 5% level. The *Labeo rohita* are exposed to sublethal concentration of copper, a gradual decrease in the glycogen content of liver tissue is evident at all periods of exposure. The percent decrease over control for all the five periods of sublethal concentration are -6.12, -11.29, -2.12, -23.60 and -26.65 for 24, 48, 72, 96 and 120 hours respectively (Table.4). When compared with control at treated the observed values are highly significant at 5% level. The observed values were highly significant at 5% level. The kidney tissue also showed a similar trend in the glycogen content when *Labeo rohita* are exposed to sublethal concentration of copper. The patterns of changes are similar to liver glycogen content. The percent decrease over control are -1.36, -7.00, -15.95, -18.70 and -29.20 for 24, 48, 72, 96 and 120 hours respectively (Table.4). The glycogen content of muscle decreased at 24, 48, 72, 96 and 120 hours. The percent changes over control observed are -6.66, -11.27, -21.19, -28.43 and -30.09 respectively (Table.4). When compared with control at treated the observed values are highly significant at 5% level. In the present experimental work, the effect of sublethal concentration of copper sulphate on the level of protein and amino acid content in different tissues of *Labeo rohita* has been studied (Table 2 & 3,) *Labeo rohita* exposed to sublethal concentration of copper shows an enhanced level of protein content at all period in the gill tissues. Gill is the first organ to face any foreign molecule that is carried through the blood circulation. In the gill epithelium there is an induction of metal binding proteins such as metallothinein which could arrest further migration of the toxic stress (Spry and Wood, 1989).

In the present investigation the level of protein concentration shows a decrease in the gill, liver, kidney and muscle during the period (24, 46, 72, 96 and 120 hours) of exposure (Table 2). Saha and Bandyopadyay (1999) have noticed the fall in the total protein level of muscle, liver and kidney after exposure to heavy metals in *Channa punctatus*. A marked fall in protein content under the stress of pollution may be due to altered enzyme activities (Vincent et al., 1996; Mallareddy, 1987). In the gill epithelium there is an induction of metal binding proteins such as metallothinein which could arrest further migration of the toxic stress (Spry and Wood, 1989) Roesijedi et al., (1982) have also observed enhanced level of protein in the gill tissue of muscle exposed to copper. Liver being the first target organ to face many foreign molecule that is increased through portal circulation is susceptible to more damage (Janyantha Rao 1984). Usually liver is the detoxifying organ (Huttarer et al., 1969) and it possesses all the machinery for detoxification of many molecules.

Tissues	Control	Hours of Exposure				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Gill %	115.46 ± 4.12	102.15*± 2.04 -11.52	94.32**± 2.82 -18.30	87.42**± 2.74 -24.28	74.43**± 3.01 -35.53	53.17**± 1.62 -53.94
Liver %	170.42 ± 3.42	163.31*± 4.34 -4.17	155.36*± 3.42 -8.83	160.15*± 3.43 -6.02	143.34**± 2.94 -15.89	130.34**± 3.72 -23.51
Kidney %	86.42± 3.15	80.39*± 2.42 -6.97	74.48*± 2.36 -13.81	60.64**± 1.94 -29.83	62.15**± 1.89 -28.08	51.34**± 1.64 -40.59
Muscle %	135.34±5.49	127.38± 3.75 -5.88	119.33*± 2.35 -11.82	108.34**± 3.15 -19.94	99.15**± 1.95 -26.74	94.43**± 2.46 -30.22

Table-1: Variations of protein content in a fresh water fish *Labeo rohita exposed* to 120 hours sublethal concentration of copper sulphate

(-) indicates the percent decrease over control Mean± SE (Mean of five individual observations) +significant at 1% level ** Significant at 1% and 5% level

(Values expressed in µg/gm wet wt. of the tissues)

Table-2: Variations of amino acid content in a fresh water fish *Labeo rohita* exposed to120 hour's sublethal concentration of copper sulphate

Tissues	Control	Hours of Exposure				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hr
Gill %change over	214.14± 9.14	224.14± 5.34 +4.66	242.34± 9.34 +13.16	231.25± 8.24 +7.99	269.28**± 5.46 +25.74	280.32**± 9 +30.90
Liver % change over	145.34± 9.18	148.64± 6.92 +2.27	165.42± 6.34 +13.81	174.22*± 6.24 +19.87	159.64± 5.70 +9.83	84.15**± 4. +42.10
Kidney %change over	98.13± 5.02	105.15± 3.42 +7.15	115.33± 7.48 +17.52	140.42**±8.14 +43.09	126.64*± 7.39 +29.05	139.70*± 9. +42.36
Muscle % change over	120.32±4.15	125.48± 7.34 +0.83	140.19± 8.64 +16.51	136.40± 8.19 +13.36	142.10*± 7.34 +18.10	159.34*± 4. +32.43

(-) indicates the percent decrease over control, Mean± SE (Mean of five individual observations)

+significant at 1% level** Significant at 1% and 5% level

(Values expressed in µg/gm wet wt. of the tissues)

Table-3: Variations of glucose content in a fresh water fish *Labeo rohita* exposed to120 hour's sublethal concentration of copper sulphate

(-) indicates the percent decrease over control, Mean± SE (Mean of five individual observations)

Tissues	Control	Hours of Exposure				
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**
Gill %	140.34± 9.30	148.32± 6.32 +5.68	154.43± 7.34 +10.03	174.32± 10.34 +24.21	163.64± 9.08 +14.23	179.34*± 10.38 +27.78
Liver %	105.34± 6.35	112.15± 7.39 +6.07	127.43± 9.15 +20.97	134.74± 10.42 +27.90	140.15*± 10.76 +32.47	154.18*± 11.34 +46.30
Kidney %	82.34± 5.34	87.34± 2.36 +6.07	95.44± 2.69 +15.90	105.19± 8.42 +27.75	118.12**± 6.36 +0.43	124.55**± 8.19 +51.26
Muscle	110.15±4.42	120.12**± 8.34 +9.05	126.15± 9.24 +14.52	134.32± 9.18 +21.94	139.32*± 8.15 +26.48	148.34*± 9.36 +34.67

+significant at 1% level** Significant at 1% and 5% level

(Values expressed in µg/gm wet wt. of the tissues)

Table-4: Variations of glycogen content in a fresh water fish *Labeo rohita* exposed to120 hour's sublethal concentration of copper sulphate

Tissues	Control	Hours of Exposure				
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**
Gill % change over	98.34± 5.48	95.27*± 4.32 -3.12	90.19*± 3.31 -8.28	86.13*± 4.34 -12.41	80.44*± 2.69 -18.20	73.34**± 2.43 -25.42
Liver % change over	130.12± 7.42	122.15*± 4.18 -6.12	115.42*± 4.42 -11.29	102.52*± 3.36 -2.12	99.41**± 4.34 -23.60	95.44**± 2.78 -26.65
Kidney % change over	75.42± 4.36	74.39± 3.36 -1.36	70.14± 2.19 -7.00	63.64± 2.76 -15.95	61.31*± 3.24 -18.70	53.39**± 2.65 -29.20
Muscle % change over	90.34±6.03	84.32*± 4.18 -6.66	80.15*± 3.19 -11.27	71.19*± 4.08 -21.19	70.34**± 3.42 -28.43	63.15**± 3.76 -30.09

(-) indicates the percent decrease over control, Mean± SE (Mean of five individual observations)

+significant at 1% level** Significant at 1% and 5% level

(Values expressed in µg/gm wet wt. of the tissues)

Finally, The free blood glucose content shows a phenomenal increase throughout the experimental periods it is due to liver impairment to utilize glucose for glycogenolysis where as in the blood protein, blood albumin and blood cholesterol content were highly decreased. The hypo activity caused by copper sulphate toxicity may leads to the utilization of this quantity, shrinkage of the nuclei and vacuolization of albumin. Copper sulphate damages the liver and the proposition of esterified cholesterol decreases. Hyper cholestremia due to impairment of liver and inhibition of enzymes which converts cholesterol into bile.

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References

1. Alkorta, I. and Garbisu, C. (2001) Phytoremediation of Organic Contaminants in Soils. Bioresource Technology, 79, 273-276.

2. Aruna, D. and Gopal, V. 1987. Effect of sublethal level of mercury on haematological parameters, Indian J. Environ. Hlth., 2952-56.

3. Nanda, D.R., and Mishra, B.B., 1997. Reclamination with cyanobacteria Toxic IJPBA, Jul - Aug, 2013, Vol. 4, Issue, 4 R. Bakiyaraj et al. / Effect of Heavy Metal Copper on the Marine Cyanobacterium Phormidium tenue (Mengh.) Gomont 786 © 2010, IJPBA. All Rights Reserved. effect of mercury contaminated waste soil on biochemical variables. Cytobios. 92(370-371), 203-208.

4. Pazhanisamy, K. 2002. Studies on the impact of Arsenic on a fresh water fish, Labeo rohita (Hamilton). Annamalai university.

5. Perrier, H., Perrier, I. Gudefin, A. Gras, 1972. Adrenaline induced hyper cholesterotemia in the rainbow rout Salmo gaivdneri Rich., a separate study in male and female trout and the effect of adrenergic blocking agents, Camp. Biochem. Physiol. 43A: 341-347.

6. Ruperelia, S.G., Verma, Y. and Meta, N.S. and Rawti, V.M. 1992. Cadmium accumulation and biochemical alteration in the liver of freshwater fish. Sarotherodon mossambicus (Peters), J. Ecotoxicol. Environ. 2: 129-136.

7. Hutterer, F., F.M. Wengraf, A. Schaffner and H. Poper 1969 “Hepatocellular adaptation and injury” Structural and biochemical changes following dieldrin and methyl butter yellow. Lab. Investi., 20: 445-459.

8. Jayantha Rao, K., c. Madhu and V.S.R. Murthy 1983 Histopathology of malathion of gills of a freshwater teleost, Tilapia mossambica J. Environ. Biol, 4 (1) : 9-13.

9. Sampath, K., James, R., and Akbar, K.m., 1998. Effect of copper and zinc on blood parameters and prediction of their recovery in Oreochromis mossambius (Posces : cichlidae). Indian J. Fish, 45,129-139.

10. Salt.D.E., Blaylock.M., Kumar Nanda.P.B.A., Dushenkov.V., Ensley.B.D., Chet.I., Raskin.I., (1995), Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants, BioTechnology, 13(5), pp 468–474.

11. Saravanan, J.S. and R. Harikrishan 1999. Effect of sublethal concentrations of copper and endosulfan on haematological parameters of the freshwater fish, Sarotherodon mossambicus (Trewaves) J. Ecobiol., 11, 13-18.

12. Lowery, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1957. Protein measurement with the Folin-phenol reagent. J. Bio. Chem. 193, 269-275.

13. MOORE, S., and STEIN, W. H. (1954).-Procedures for the chromatographic determination of amino acids on four per cent cross-linked sulfonated resins. J. Rial. Chem. 211: 893-906.

14. Spry, D.J., and **Wood, C.M**. (1989) A kinetic method for the measurement of zinc influx in the rainbow trout and the effects of waterborne calcium. *J. Exp. Biol*. 142 pp: 425-446.

15. Saha JC, Dikshit AK, Bandyopadhyay M, Saha KC. 1999; A review of arsenic poisoning and its effects on human health. Crit Rev Env Sci Technol. 29(3):281–313