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Impact of Physico-Chemical Variables of Test Media on Growth Performance of Metal Stressed Major Carps

Saima Naz,* Muhammad Javed, Amara Tahir and Hamda Azmat
Department of Zoology and Fisheries, University of Agriculture, Faisalabad-38040, Pakistan

Abstract.- Growth responses of *Catla catla, Labeo rohita and Cirrhina mrigala* were determined, separately, under chronic exposure of mixture of metals (Fe+Zn+Pb+Ni+Mn) at sub-lethal concentrations (1/3rd of LC_{50}) for 12 weeks (01-06-2010 to 01-09-2010). Fish were kept under constant water temperature, pH and total hardness of 30ºC, 7 and 200mg/L, respectively. The physico-chemical parameters were monitored on daily basis throughout test period. The relationships among growth parameters of fish and water quality characteristics of metal mixture (MM) stressed media were computed by using correlation method. Physico-chemistry of the test media (water) used for different treatments exerted significant impacts on fish growth, feed intake, condition factor and feed conversion efficiency (FCE) of fish also. Significantly better feed intake enhanced the ammonia (NH_3) production and excretion by the fish resulting into non-significantly positive relationship of fish weight increments with contents of the test media. Significantly higher feed intake resulted in excessive excretion of NH_3 by the fish to cause significant impact on its growth. Sodium (Na) and potassium (K) showed significantly positive correlation with ammonia concentrations of the test media, indicating excessive release of Na and K by the fish under stress of MM that resulted in significantly more excretion of NH_3 by the fish.

Key Words: Fish growth, physico-chemical parameters, metal mixture stress, major carps.

INTRODUCTION

Major carps and other cyprinids contribute significantly towards global aquaculture production. Freshwater fish have been an important source of quality proteins for human beings. The fish are intimately associated with water and constitute an important food item in human diet. Fish are largely used to evaluate the quality of aquatic bodies and some of their physiological change can be considered as biological indicator of environmental pollution (Javed, 2012). Throughout the world, natural aquatic bodies have extensively been contaminated with heavy metals that are originated from domestic, industrial and other man made activities (Azmat *et al.*, 2012; Yaqub, 2012). Increasing population and establishment of industries in the urban areas of the Punjab (Pakistan) have resulted in the discharge of heavy metals and their compounds through industrial effluents and domestic sewage into the rivers. In industrial waste-water they are usually found in mixtures which are specific to a particular pollution source. Fe, Zn, Pb, Ni and Mn are representative heavy metals that are referred to as selected water quality indicators (Hayat, 2009). Majority of the studies of harmful effects of metals on fish deal with single metal species (Abdel-Baki *et al.*, 2011; Godwin *et al.*, 2011; Azmat *et al.*, 2012; Yaqub and Javed, 2012; Javed, 2012) while the aquatic organisms are usually exposed to mixtures of various metals.

Water quality characteristic of aquatic environment arise from a multitude of physical, chemical and biological interactions. Water quality parameters such as pH, Na, Ca and organic matter affect the toxicity of metals in the aquatic environments (Niyogi and Wood, 2004). Metal bio-availability is measured by biotic (feeding behavior and exchange surface) and abotic (temperature, pH) factors (Chowdhury and Blust, 2002). Quality of water may be changed due to different types of chemicals like biological and physical pollutants originating from different industrial and agricultural sources (Andhale and Zambare, 2012). Dissolved oxygen concentrations, temperature, salinity and presences of other metals may also modify metals toxicity to the fish (Witeska and Jezierska, 2003). Any disturbances could result in reduced fish metabolic rate and hence reduced growth. As a consequence, metal mixture-exposed fish showing

* Corresponding author's e-mail: sam_ali_3@yahoo.com
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higher mortality rates may result in the reduction of fish population. This necessitated the planning of a research project to determine relationships of growth parameters of fish with physico-chemistry of the test media. This data can be useful for future planning regarding sustainability of natural habitats ad conservations of indigenous fish species.

MATERIALS AND METHODS

The tests were conducted on 90-days old juveniles of major carps *viz*., *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* obtained from the Fish Seed Hatchery, Faisalabad. The test fish species were acclimated to laboratory condition for 48-hour before starting the test. The fish in good condition and state of health, after acclimated period, were selected for experiment that was conducted in glass aquaria of 70-L water capacity. One third of metal mixture (Fe+Zn+Pb+Ni+Mn) LC_{50} concentrations viz. 14.09±0.55, 20.65±0.61 and 14.45±1.09, mg L^{-1} (Javed and Yaqub, 2010) were used as sub-lethal levels for *Catla catla*, *Labeo rohita and Cirrhina mrigala*, respectively. Stock solutions of metals (iron, lead, manganese, nickel and zinc) from reagent grade $FeCl_2.4H_2O$, $ZnCl_2$, $PbCl_2$, $NiCl_2.6H_2O$ and $MnCl_2.H_2O$ (Sigma Aldrich) which were prepared in de-ionized water to the desired metal concentrations. The metal mixture (Fe+Zn+Pb+Ni+Mn) was prepared by mixing appropriate quantities of stock solutions on metallic ion equivalence basis. Test media were supplied with air pump fixed with capillary system. The growth trials of each species of fish, under sub-lethal concentrations, were conducted for a period of 12 weeks.

Determination of fish growth

During growth studies, one group of each fish species was kept un-stressed as a control, while the other groups were exposed, separately, to the sub-lethal metal mixture concentrations ($1/3^{rd}$ of LC_{50}) in the glass aquaria. During each grown trials fish were fed, to satiation, twice a day with the following feed: Corn Gluten (30% Crude Protein), 39.27; Wheat flour, 5%; Fish meal, 40%; Rice polish, 7.51%; Vitamin and mineral mixture (Methionine, 0.82%; Lysine, 1.93%; Ca^{++}, 2.26%; Na^{+}, 0.32%; PO_4^{--}, 1.20%), 5%; Oil (Sun flower), 3.22%; Digestible protein (DP) was 35% and Digestible energy (DE) was 2.90 $Kcalg^{-1}$.

During growth trials of 12 weeks, all the three fish species were investigated weekly for their increase in average weights (g), fork and total lengths (mm), feed conversion efficiency, condition factor and feed intakes (g). No fish species showed any mortality during the entire period of growth trials under sub-lethal levels of metal mixture.

Determination of physico-chemical variables of the test media

Water temperature, electrical conductivity, pH and dissolved oxygen of the test media were determined twice a day by using digital meters, viz. HANNA HI-8053, HI-8733, HI-8520 and HI-9146, respectively. However, total hardness, total ammonia, chlorides, sodium and potassium concentrations in each test medium were determined by the methods of A.P.H.A. (1998). Water pH (7), total hardness ($200mgL^{-1}$) and temperature (30°C) of water were kept constant during each trial for fish. The pH of the test media was maintained by adding NaOH and HCl to increase and decrease pH, respectively. In order to maintain the total hardness of water, salts of $MgSO_4$ and $CaSO_4$ were added to increase the hardness while EDTA was used to decrease the water hardness. Water temperature was maintained as $30^{o}C$ by using automatic heaters.

Statistical analyses of data

The parameters of growth and water chemistry of the tests were subjected to statistical analyses by following Steel *et al*. (1996) through Micro-Computer. Correlation analyses were also computed to find-out relationships among various parameters defined for this study.

RESULTS

Catla catla

The weight increments of *Catla catla* showed positively significant correlation with feed conversion efficiency and condition factor. The correlation between feed conversion efficiency and feed intake was negative but highly significant. Condition factor of fish was inversely ($p<0.05$)

influenced by the potassium contents of the media. The relationship between carbon dioxide and dissolved oxygen appeared positive and significant. However, the relationship between magnesium and calcium was significantly inverse (Table I).

Labeo rohita

The feed conversion efficiency of *Labeo rohita* was negatively but significantly correlated with feed intake. Total ammonia contents of the media increased with concomitant increase in feed intake. Dissolved oxygen concentrations affected the condition factor of fish positively and significantly. The correlation coefficient for the relationship between calcium and total ammonia was significantly inverse. The relationship between carbon dioxide and dissolved oxygen appeared positively significant while that between magnesium and calcium was inverse but statistically significant (Table I).

Cirrhina mrigala

Chronic exposure of metals mixtures to *Cirrhina mrigala* caused inverse correlation between feed conversion efficiency and feed intake of this fish. The correlations of carbon dioxide with total ammonia and dissolved oxygen of water were positively significant. However, the relationship between magnesium and calcium contents of the test media (water) was inversely significant (Table I).

Control fish

The weight increments of control fish were positively correlated with feed conversion efficiency, dissolved oxygen and calcium. Feed intake of fish showed significantly inverse relationships with total ammonia and dissolved oxygen contents of the test media while feed conversion efficiency exhibited significantly direct relationships with total ammonia and dissolved oxygen contents of the media. The relationship between potassium and total ammonia was significantly inverse. The feed conversion efficiency of fish exhibited significantly positive correlations with dissolved oxygen and carbon dioxide while correlation coefficient between dissolved oxygen and electrical conductivity was negatively significant. Carbon dioxide in water had significantly direct relationships with electrical conductivity and magnesium contents while it was significantly inverse with calcium. Sodium had significantly positive correlation with potassium while that between calcium and magnesium appeared negatively significant (Table I).

DISCUSSION

The environmental contamination can be assessed by analyzing the pattern of metal accumulation in various body tissues/organs of fish (Jabeen, 2012). The growth of fish is commonly used as a receptive and unswerving end point in chronic studies to predict toxic effects of different biochemical and physiological processes, which are more revealing to assess the effects on specific processes associated with feeding, assimilation, excretion and metabolism in fish (Bhavan and Geraldine, 2000). The water quality characteristics of metallic ion exposure media, used during growth trials, exerted significant impacts on growth, feed intake, condition factor and feed conversion efficiency of fish.

Therefore, pollutant intoxication should provoke biochemical changes before initiation of fish growth reduction. Subathra and Karuppasamy (2007) reported reduced feeding due to increase in the concentration of Cu exposure to the fish, *Mystus vittatus*. The exposure of metals mixtures to the fish caused significant correlation of fish growth with ammonia contents of the medium. Significantly higher feed intake resulted in excessive excretion of ammonia by the fish to cause significant impact on fish growth. Among the physico-chemical variables, ammonia exerted significantly negative impacts on fish growth. Hayat (2009) while working on chronic toxicity of metals, Fe+Zn+Pb+Ni+Mn, and their mixture to the fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala)* investigated oxygen reduction in the stress media that enhanced the ammonia excretion by the fish. The higher level of ammonia in comminuted water affected the feed intake, growth and physiology of the fish, *Clarias gariepinus* (Schram *et al*., 2010).

Table I.- **Relationship of fish growth with physico-chemical parameters of the test medium (water).**

	Fish Weight	Feed intake	FCE	K Factor	NH_3	DO	CO_2	EC	Na	K	Ca
Catla catla											
Feed intake	-0.26658										
FCE	**0.54678**	**-0.93827**									
K Factor	**0.51096**	0.20495	0.05590								
NH_3	0.13551	0.17819	-0.10725	0.11691							
DO	0.11239	-0.09051	0.08988	-0.26600	0.18770						
CO_2	0.27249	0.20047	-0.07093	0.05833	0.32955	**0.80398**					
EC	0.06899	-0.14660	0.18082	-.0.00081	-0.07193	0.15968	0.13614				
Na	0.15293	-0.28203	0.27531	-0.09771	-0.03394	-0.08904	-0.23288	-0.35493			
K	-0.09262	-0.17348	0.07484	**-0.45373**	0.35520	0.19816	0.03485	0.18397	0.08794		
Ca	-0.16689	0.07902	-0.14052	-0.39486	-0.20979	0.06210	-0.08121	0.00301	0.08536	-0.02836	
Mg	0.26001	-0.01090	0.12333	0.35166	0/03754	0.11260	0.42589	0.12436	-0.18365	-0.08150	**-0.79516**
Labeo rohita											
Feed intake	0.05324										
FCE	0.10675	**-0.94652**									
K Factor	0.10168	-0.02154	0.00954								
NH_3	0.22268	**0.53105**	-0.43739	-0.12977							
DO	-.0.16441	-0.34656	0.26771	**0.46347**	-.0.32462						
CO_2	0.09000	-0.41668	0.39783	0.44355	-0.31828	**0.78086**					
EC	0.07520	-0.29722	0.30983	0.21997	0.18444	0.17696	0.38232				
Na	0.15469	0.07320	0.06439	0.20549	0.32675	0.12474	-.03716	-.0.17708			
K	0.00302	-0.35686	028551	-0.12715	-0.01684	-0.07191	-0.18661	0.10688	0.32479		
Ca	0.13062	-0.30153	0.24795	-0.34665	**-.0.52001**	0.10114	0.17265	-0.15870	-0.12984	0.07463	
Mg	0.00698	0.11120	-0.13686	0.36468	.39828	0.04431	-0.06221	0.31255	0.21721	0.19817	**-0.83539**
Cirrhina mrigala											
Feed intake	0.11638										
FCE	0.42900	**-0.78128**									
K Factor	0.08546	-0.27137	0.27342								
NH_3	0.00138	0.2614	-0.08385	0.26565							
DO	0.32805	0.29967	-0.10765	-0.5722	0.61749						
CO_2	0.21053	0.25999	-0.10941	0.12890	**0.62466**	**0.92137**					
EC	-0.00282	-0.8262	0.11797	-0.27794	0.07193	-0.02357	-0.00309				
Na	0.10036	0.0901	0.06468	-0.209592	-0.13615	-0.35342	-0.36514	-0.10354			
K	0.08531	-0.15597	0.11100	-0.15954	0.18602	0.44736	0.29498	0.08548	-0.37315		
Ca	-0.28688	0.09356	0.29183	0.00558	0.05876	-0.29088	-0.24774	0.23391	-0.19859	-0.253528	
Mg	0.29058	-0.14388	0.29858	0.0165	-0.10459	0.29591	0.27167	-0.15055	0.07225	0.39424	**-0.93892**

Critical value (2tail 0.05) ± 0.45425

Continued

	Fish Weight	Feed intake	FCE	K Factor	NH_3	DO	CO_2	EC	Na	K	Ca
Control fish											
Feed intake	**-0.721**										
FCE	**0.85763**	**-0.88560**									
K Factor	0.17110.	0.18811	-0.24988								
NH_3	0.11617	-0.28891	**0.43972**	**-0.43160**							
DO	**0.54907**	**-0.42599**	**0.41021**	-0.29342	**0.40045**						
CO_2	**-0.46773**	-0.21265	0.00537	-0.18404	**0.47407**	-0.23302					
EC	-0.28736	-0.16011	0.17789	-0.03966	0.34615	**-0.60786**	**0.72722**				
Na	0.22279	-0.23432	0.12571	0.09312	-0.31793	-0.01813	-0.13960	-0.09035			
K	-0.02265	-0.16970	0.04664	0.05631	-0.11968	-0.13249	0.21315	0.14014	**0.54086**		
Ca	**0.50514**	0.09834	0.16824	0.14300	-0.50132	-0.19592	**-0.78587**	-0.27074	0.22003	-0.05665	
Mg	**-0.39444**	-0.08851	-0.12023	-0.01521	0.16794	-0.11271	**0.58190**	0.38824	-0.08233	0.03133	**-0.58503**

Critical value (2tail 0.05) ± 0.39521

FCE Feed conversion efficiency; K Factor Condition factor; NH_3 Total ammonia (mg L^{-1}); DO Dissolved oxygen (mg L^{-1}); CO_2 Carbon dioxide (mg L^{-1}); EC Electrical Conductivity (mS cm^{-1}); Na Sodium (mg L^{-1}); K Potassium (mg L^{-1}); Ca Calcium (mg L^{-1}); Mg Magnesium (mg L^{-1})

The relationship between carbon dioxide and dissolved oxygen appeared positively significant while that between magnesium and calcium was inverse but statistically significant for all fish species. Salam *et al*. (2002) correlated the zinc, iron, copper, manganese, sodium, potassium and magnesium toxicities with the length and weight of *Cirrhinus mrigala*. Results showed that sodium had significantly positive correlation with the increase in weight while zinc showed negative impacts. However, iron, magnesium, manganese and copper had isometric relationships with the increased body length of fish.

Dissolved oxygen concentrations were positively correlated with the carbon dioxide contents of the treated media, while negative correlation was found between dissolved oxygen concentrations and carbon dioxide in the control media. Increase in feed intake by the fish resulted escalation in ammonia contents of the test media used for both *Catla catla* and *Labeo rohita*. However, dissolved oxygen contents of the media decreased significantly due to enhanced excretion of ammonia by the fish body. De Boeck *et al*. (1995) reported reduced oxygen contents of water-borne copper exposure media used for *Cyprinus carpio*. Ammonia excretion in fish also declined at lower oxygen concentrations. Therefore, measurements of critical oxygen concentrations and ammonia nitrogen excretion appeared useful indicators of metal stress to the fish. Sodium and potassium contents of the test media showed significantly positive correlation with ammonia concentrations of the test media, indicating excessive release of sodium and potassium by the fish under the stress of metals in a mixture form that resulted in significantly more excretion of ammonia. Abdullah (2007) reported significant imbalance of sodium and potassium levels in fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) under the exposure of various metals.

CONCLUSIONS

Dissolved oxygen contents of the media decreased significantly due to enhanced excretion of ammonia by the fish. Fish weight increments showed non-significantly positive relationships with the ammonia contents of the test media showing the impacts of various mixtures to cause significant changes in feed intake (due to metals mixture stress) that ultimately reflected in terms of significant changes in the feed conversion efficiency of fish. Therefore, measurements of critical oxygen concentrations and ammonia nitrogen excretion appeared useful indicators of metal mixture stress to the fish.

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