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Effect of Water Chemistry on Growth Performance of Some Freshwater Fish Exposed to Some Heavy Metals Mixture

Saima Naz, Muhammad Javed
Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan
Corresponding author email: mansaima23@gmail.com
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Abstract This studied was conducted to evaluate the correlation between water physico-chemical parameters and the growth performance of two fish species viz. *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. Fish were exposed to sub-lethal (1/3rd of LC_{50}) concentrations of metal mixture under controlled laboratory condition for 90 days. All physical and chemical parameters were determined twice a day through out experimental period. Water pH (7.25), total hardness (225 mg/L as $CaCO_3$) and temperature (30°C) of water were kept constant during each trail for fish. Correlation and regression analyses were computed to find-out relationships among various parameters defined for this study. During growth periods of 90 day, both fish species were monitored weekly for their increase in average wet weights (g), fork and total lengths (mm), specific growth rate, condition factor, feed intakes and feed conversion efficiency. No fish species showed any mortality during the entire period of growth trails under chronic stress of metal mixture. The relationships among all phyisco-chemical and growth parameters were investigated after 90-day growth trail. The obtained results showed that physico-chemistry of metal mixture stressed media exerted significant effect on both fish growth. Significantly better feed intake increased the ammonia production and excretion by both fish resulting fish weight increments positively non-significant correlated with contents of stressed media and also cause significant effect on growth of fish. Total ammonia content of test media exhibited significantly direct relationship with dissolve oxygen. However, calcium showed significantly inverse correlation with magnesium by the both fish species. It is also concluded that not only toxicity of metals but also the deficiency of consumed oxygen cause frequent deaths of fish in water due to biological decomposition of heavy metals.
Keywords Metal mixture stress, Fish growth, Physico-chemical parameters

Introduction

Several parts of Pakistan are in front of water contamination quandary which is mounting gradually around industrial and urban areas. Sewage, pesticides, fertilizers and heavy metals are key environmental toxins contaminated the freshwater system. A serious threat is simulated to the biota by heavy metals when they get to aquatic ecosystem, due to their constant and relentless existence (Naz et al., 2012; Begam, 2004). Over the recent years, in numerous developing countries, drastically growing costal pollution has multiplied environmental problems. High metal concentrations in the aquatic environment, especially in the coastal sediments are resultant of the discharge of industrial wastes (Ni et al., 2005). To evaluate the healthiness of aquatic organisms, fish are commonly used (Farkas et al., 2002). In contrast to water or sediment, fish accumulate metals more and take up these in their body organs too (Khaled, 2004; Olaifa et al., 2004). Zinc is a self-important toxicant to fish (Everall et al., 1989). Disruption of gill tissue, hypoxia, ion regulation and disturbance of acid base balance occurs due to zinc (Hogstrand et al., 1994). In the cellular structures, nickel somehow plays its role for morphological conversions and chromosomal anomalies (Coen et al., 2001). Lead is recognized for amending the hematological system by holding back the activities of several enzymes which take part in heme biosynthesis (Schobert and Jahn, 2002). Iron is the most vital element for hemoglobin and myoglobin development in fish as well as it also plays a fundamental role for the growth of aquatic organisms. Unfortunately, increased industrial effluents polluted the natural ecosystem and it enhanced at momentous contamination stage (Hussain et al., 2011). The sub-lethal exposure of manganese not only affected the survival of the fish but may also reduce growth (Nowak and Duda, 1996).

Quality of water available for fish farming must be described for any proper-prepared plan for aquaculture as water is a basic requirement for fish farming (Summerfelt, 2000). Physical, biological, and chemical contents of water define the quality of water. Even in the absence of pollution, the water quality of lakes and rivers alters with the geographic areas and seasons (Lawson, 2011). Water quality guidelines give crucial scientific information about parameters concerning water quality and ecologically appropriate toxicological threshold values for the protection of specific water uses (Abowei, 2010). Temperature, pH, rainfall, dissolved oxygen, salinity and carbon dioxide are important physico-chemical parameters affecting aquatic environment which are supposed to be the limiting factors for the continued existence of aquatic life (flora and fauna). Stumpy water flow, industrial discharges and municipal effluents deteriorate the quality of water (Chitmanat and Traichaiyaporn, 2010). The objective of this work was to assess important water related physical and chemical factors and their effects on fish growth.

1 Results

1.1 Fish growth under chronic stress of metal mixture

The exposure of fish to sub-lethal concentrations of metal mixture caused significant impacts on the average wet weight increments of two fish species. Weight increments of treated fish species varied significantly. However, *Hypophthalmichthys molitrix* attainted significantly higher weight, fork and total lengths than the *Ctenophyarygodon idella*. Feed intakes and specific growth rate fish species varied significantly due to exposure of metal mixture. Regarding overall performance of treated fish species, *Ctenopharyngodon idella* exhibited significantly better condition factor of 1.59±0.04. *Ctenopharyngodon idella* showed significantly higher average feed intake than that of *Hypophthalmichthys molitrix* with significant differences among them. The feed conversion efficiency of two treated fish species varied significantly (Table 1). The metal mixture stressed fish exhibited lower weights than control. Fork and total lengths, specific growth rate, feed intake, condition factor and feed conversion efficiency also showed the same trend as that observed for average weight increments.

1.2 Physico-chemical studies

The data on physico-chemical parameters of the test media estimated during the trail revealed that the mean values of total hardness and electrical conductivity were higher in treated media than that of control media. However, the treated fish media had lower concentrations of dissolved oxygen, total ammonia, carbon dioxide, sodium and magnesium

Table 1 Growth performance of two fish species exposed to sub-lethal concentrations of metal mixture.

Species	Growth parameters						
	Increase in weight (g)	Increase in fork lengths (mm)	Increase in total length (mm)	Average specific growth rates	Feed intake (g)	Condition factor	FCE (%)
C. idella	11.50±0.02 b	11.88±0.02 b	11.96±0.05 b	13.14±0.03 b	18.50±0.50 a	1.59±0.04 a	62.14±0.05 b
H. molitrix	11.88±0.02 a	11.90±0.02 a	11.98±0.02 a	15.16±0.14 a	18.46±0.03 b	1.56±0.03 b	64.36±0.08 a
Treatments							
Metal mixture stressed fish	11.69±0.26 b	11.89±0.02 b	11.97±0.01 b	14.15±1.14 b	18.48±0.02 b	1.59±0.01 b	63.25±1.56 b
Control	27.02±0.02 a	19.20±0.04 a	20.01±0.01 a	29.43±0.03 a	22.10±0.02 a	2.08±0.04 a	122.26±0.01 a

Note: Condition factor (K)=W x $10^5 \div L^3$ where W=wet weight (g); L=Wet total length (mm); FCE%=Gain in weight (g)/feed intake (g) x100' Means with similar column are statistically non-significant at $p<0.05$

than media used for control fish. The concentrations of calcium and potassium in both mediums used for treated and control fish remained almost same during these growth trails (Table 2).

1.2.1 *Ctenopharyngodon idella*

The feed conversion efficiency of *Ctenopharyngodon idella* showed positively significant correlation with fish weight increments while feed conversion efficiency had negative but significant correlation with feed intake. Total ammonia contents of the test media exhibited significantly direct relationships with dissolved oxygen and carbondioxide. Magnesium showed positively significant relationship with electrical conductivity while that with calcium was negatively significant (Table 3).

1.2.2 *Hypophthalmichthys molitrix*

The feed conversion efficiency and condition factor of *Hypophthalmichthys molitrix* were correlated significantly with feed intake of fish while that with condition factor was statistically positive. Total ammonia contents of the test media were positively and significantly correlated with dissolved oxygen and electrical conductivity of the media. The relationship of dissolved oxygen with carbondioxide was positively significant also. Carbondioxide showed positively significant correlation with sodium. The correlation between sodium and potassium was positively significant also. However, calcium showed significantly inverse correlation with magnesium contents of the test media (Table 3).

1.2.3 Control fish

The wet weight increments of fish were positively correlated with feed intake and feed conversion efficiency (FCE). Feed intake of fish exhibited significantly inverse relationships with total ammonia and dissolved oxygen contents of the test media while feed conversion efficiency showed significantly direct relationships with total ammonia and dissolved oxygen contents of the control media. The relationship between potassium and total ammonia was significantly inverse. The feed conversion efficiency (FCE) of fish exhibited significantly positive correlations with dissolved oxygen and carbondioxide while correlation coefficient between dissolved oxygen and electrical conductivity was negatively significant. Carbondioxide 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 3).

2 Discussion

The environmental contamination can be evaluated by investigating the pattern of different metals accumulation in numerous body organs and tissues of fish (Azmat and Javed, 2011). The fish growth is generally used as a amenable and a reliable end point in chronic studies to envisage toxic influences of various biochemical and physiological processes, which are more divulging to review the effects on specific processes viz. feeding, excretion, assimilation and metabolism in fish (Bhavan and Geraldine, 2000). The control fish depicted significantly maximum growth in terms of average increase in weight as compared to the treated (Javed, 2012). Moreover, the growth of rainbow trout was adversely affected due to exposure of Cu+Zn+Cr+Ni+Fe mixtures (Kazlauskiene and Stasiunaite, 1999). Vosyliene and Jankaite (2006) investigated the acute and long term toxicity of a heavy metal model mixture (HMMM) of Cu, Zn, Pb, Ni, Cr and Mn on rainbow trout by using a set of biological parameters. Significant changes were found in the weight of liver and hepatosomatic index of fish while no significant changes were observed in the weight gains of fish. During the present investigation, both treated and control fish performed differently as far as their feed intakes were concerned. In the treated fish, significant relationship was found between its weight escalations and feed intake while in case of control fish, the relationship computed between their weights increments and feed intake was negative but statistically non-significant. Significant impacts on the growth and feed conversion efficiency were reported in eels when they were exposed to dietary cobalt (Heinsbroek et al., 2007). Vosyliene et al. (2003) reported significant influence on the feed intake which was exerted due to toxic impacts of metals on the fish (DeBoeck et al., 1997). Swift and simplified information allied to the advantages gained by the fish is conveyed by condition factor which seizes immense connotation, for the reason that diminution in condition factor causes hypoxia that entails negative effect on the feed intake of fish, moreover lessens

Table 2 Mean values of physico-chemistry variables determined during growth trails for treated and control fish species

	Temperature (°C)	pH	Total hardness (mg/L)	Total Ammonia (mg/L)	Dissolved oxygen (mg/L)	Carbon dioxide (mg/L)	Electrical conductivity (mS/cm)	Sodium (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)
Ctenopharyngodon idella	30.58±0.70	7.24±0.09	225.18±0.74	1.77±0.31	5.11±0.32	1.69±0.27	327.20±33.00	285.92±5.26	7.08±0.38	36.71±2.98	66.16±4.64
Hypophthalmichthys molitrix	29.79±0.74	7.21±0.08	225.63±0.25	1.88±0.30	5.33±0.29	1.65±0.24	329.90±15.36	285.33±9.01	7.11±0.30	36.46±1.91	65.60±2.25
Control	30.90±0.46	7.23±0.05	224.56±0.43	2.31±0.28	5.47±0.30	1.91±0.32	301.12±33.57	304.00±9.58	7.20±0.50	36.89±1.56	75.81±3.00

Table 3 Relationships among growth parameters and physico-chemistry of the test media (water)

	Fish Weight	Feed intake	FCE	K Factor	NH_3	DO	CO_2	EC	Na	K	Ca
Ctenopharyngodon idella											
Feed intake	-0.32049										
FCE	0.59079	-0.93282									
K Factor	0.15632	-0.22695	0.27898								
NH_3	-0.42583	0.09770	-0.23405	0.07333							
DO	-0.25228	0.14805	-0.27390	0.24504	0.51249						
CO_2	-0.00507	-0.07893	-0.03852	0.22596	0.76013	0.79954					
EC	-0.00507	0.12071	-0.12409	0.28506	-0.17009	0.09685	-0.01414				
Na	-0.25172	0.30924	-0.34967	-0.23365	0.02293	-0.13326	-0.15881	-0.28025			
K	-0.08174	-0.19700	0.12222	0.34270	0.13259	0.50227	0.33513	0.01077	-0.08536		
Ca	0.05098	-0.03852	0.12047	-0.35680	0.02198	-0.12769	0.11899	-0.32590	0.27974	0.00435	
Mg	0.00868	-0.10149	-0.00094	0.35944	0.25478	0.34924	0.25746	0.53009	-0.20854	0.05458	-0.75494
Hypophthalmichthys molitrix											
Feed intake	-0.09199										

FCE	0.31244	-0.94723									
K Factor	0.26892	0.46066	-0.35265								
NH_3	-0.13702	0.14312	-0.18065	-0.22472							
DO	-0.17004	0.25809	-0.29409	-0.31880	0.78304						
CO_2	-0.27722	0.00947	-0.03785	-0.28716	0.72263	0.85136					
EC	0.03940	-0.05408	0.11659	0.04496	-0.32516	-0.17427	-0.16380				
Na	0.19079	0.10430	0.00609	0.19227	0.27756	0.26713	0.51527	-0.07719			
K	-0.16685	0.11178	-0.03373	-0.10628	0.32427	0.22541	0.36449	0.17406	0.56579		
Ca	0.02665	0.01007	-0.03334	0.29746	-0.42915	-0.47416	-0.30942	0.20381	0.16126	-0.12402	
Mg	-0.20345	-0.00780	0.02005	-0.28186	0.24623	0.39283	0.34801	0.07441	-0.11719	0.30966	-0.86223
Critical value (2 tail 0.05) ± 0.45425											
Control											
Feed intake	-0.7228										
FCE	0.85764	-0.88561									
K Factor	-0.17111.	0.18812	-0.24989								
NH_3	0.11618	-0.28893	0.43973	-0.43161							
DO	0.54901	-0.42598	0.41022	-0.29343	0.40044						
CO_2	-0.46774	-0.21264	0.00539	-0.18401	0.47408	-0.23301					
EC	-0.28737	-0.16012	0.17788	-0.03967	0.34619	-0.60787	0.72721				
Na	0.22278	-0.23434	0.12574	0.09313	-0.31797	-0.01812	-0.13963	-0.09031			
K	-0.02264	-0.16971	0.04664	0.05632	-0.11969	-0.13246	0.21314	0.14013	0.54087		
Ca	0.50513	0.09836	0.16826	0.14301	-0.50133	-0.19591	-0.78589	-0.27073	0.22001	-0.05661	
Mg	-0.39445	-0.08853	-0.12021	-0.01522	0.16795	-0.11272	0.58191	0.38825	-0.08232	0.03132	-0.58502
Critical value (2 tail 0.05) ± 0.39521											

Note: FEC=Feed conversion efficiency; K Factor=Condition factor; NH3=Total ammonia (mg/L1); DO=Dissolved oxygen (mg/L); CO_2=Carbon Dioxide; E.C.=Electrical conductivity (mS/cm); Na=Sodium (mg/L); K=Potassium (mg/L); Ca=Calcium (mg/L); Mg=Magnesium (mg/L) condition factor and growth also (Randall and Yang, 2004). Ali et al. (2003) observed significant variations in specific growth rate and condition factor and weight gains of *Oreochromis niloticus* grown under different sub-lethal concentration of water-borne Cu. Dethloff et al. (2001) found no significant difference in condition factor values of rainbow trout collected from different sites polluted with Cr, Cd and Se.

In the present investigation, physico-chemical parameters viz. temperature, pH, total hardness, calcium, magnesium, sodium, potassium, total ammonia, carbon-dioxide and electrical conductivity were significantly variable during the study period in the control and treated test media. Increase in ammonia excretion due to significant decline in dissolved oxygen contents of the water media was reported by Naz et al. (2012) while investigating chronic toxicity of metals mixture to freshwater fish species (*Catla catla*, *Labeo rohita*, *Cirrhina mrigala*). The exposure of metals mixtures to the fish species caused significant effect growth with ammonia contents of the test medium. Significantly higher feed intake by the fish resulted in excessive excretion of ammonia which effect on fish growth. Among the physico-chemical variables, ammonia contents of treated media exerted negative impacts on fish growth. Hayat (2009) while studying on chronic toxicity of heavy metals, Mn+ Fe+Zn+Pb+Ni+ and their mixture to the three fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) reported oxygen reduction in the treated media that increased the ammonia excretion by the all three fish. The higher level of ammonia in polluted water affected the growth, feed intake and physiology of the fish, *Clarias gariepinus* (Schram et al., 2010).

The relationship between dissolved oxygen and carbon dioxide had positively significant while that between magnesium and calcium was inverse but statistically significant for all fish species. This is in line with the findings of (Shafiq et al., 2012) who reported that fish weight escalations were positively and significantly correlated with total ammonia but negatively and significantly correlated with magnesium contents. Sudden changes in pH result in fish mortality as an increase in temperature would enhance value of pH more than recommended range (Summerfelt, 2000). Dissolved oxygen of test media were significantly positive correlated with the carbon dioxide contents, while negative correlation was found between dissolved oxygen concentrations and carbon dioxide in the control media. Shereena and Logaswamy (2008) studied the impact of heavy metals (copper sulphate, cadmium carbonate, zinc sulphate and lead nitrate) on the oxygen consumption of Tilapia mossambica. They reported decrease in oxygen consumption by the fish under metal stressed conditions. At higher metal concentration, the carbon-dioxide of test media has increased.

3 Conclusion

Higher growth in terms of increase in wet weight, fork and total lengths, specific growth rate, feed intake, condition factor and feed conversion efficiency was significantly attained by control fish as compared to treated fish. Feed conversion efficiency and condition factor of fish were interrelated and the effects of chronic exposure of metal mixture on the two parameters were significantly pronounced. Dissolved oxygen in waters depends upon water temperature, partial pressure of oxygen in atmosphere and salt contents in waters. Fish wet weight increments exhibited positive non-significantly relationships with the ammonia contents of the treated media showing the effect of various metal mixtures to cause changes in feed intake that eventually reflected in terms of significant changes in the feed conversion efficiency of fish.

4 Material and method

The tests were conducted on 90-days old juveniles of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* obtained from the Fish Seed Hatchery, Faisalabad. The test fish species were acclimated to laboratory condition for 2 days before starting the test. Healthy and good fish, after acclimated period, were selected and conducted in 70-L glass aquaria. One third of metal mixture (Zn+Pb+Ni+Mn+Fe) LC_{50} concentrations viz. 28.78 and 30.91 mg/L (Javed and Yaqub, 2010) were used as sub-lethal levels for *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, respectively. Stock solutions of iron, lead, manganese, nickel and zinc were prepared ,separately, by dissolving required quantities of their chlorides viz. $FeCl_2 \cdot 4H_2O$, $PbCl_2$, $MnCl_2 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$ and $ZnCl_2$ (Sigma Aldrich), respectively in deionizer water.

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. Each test concentrations for every fish species was tested with three replications.

4.1 Studies on fish growth

During growth studies, one group of each fish species was kept un-stressed as a control, while the other groups (10 fish per each aquarium) with following average weights, fork and total lengths were exposed, separately, to the sub-lethal metal mixture concentrations (1/3rd of LC_{50}) in the glass aquaria.

Fish species	Average weight (g)	Average fork length (mm)	Average total length (mm)
C. idella	3.12±1.75	59.42±10.85	62.71±11.96
H. molitrix	4.68±2.07	77.16±11.80	82.19±13.24

During each grown trials, fish were fed with commercial feed having Crude protein: 30%, Digestible protein: 35% and Digestible energy: 2 900 kcal/kg, throughout the trial. The feed was offered twice a day to visible satiation. During growth trials of 12 weeks, the two fish species were investigated weekly for their increase in average weights (g), fork and total lengths (mm). Fish species did not show any mortality during the whole duration of growth trials under sub-lethal concentrations of metal mixture.

4.2 Physico-chemistry of test media (water)

Total hardness, total ammonia, carbon dioxide, sodium and potassium concentrations in each test medium were determined by the methods of APHA (1998). However, pH, water temperature, dissolved oxygen and electrical conductivity of the test media were determined twice a day by using digital meters, viz. HI-8733, HANNA HI-8053, HI-9146 and HI-8520, respectively. Water pH (7.25±0.02), total hardness (225±2.32) mg/L and temperature (30±0.84)°C of water were kept constant during each trial for fish. The pH of the test media was maintained by adding sodium hydroxide (NaOH) and hydrochloric acid (HCl) to increase and decrease pH, respectively. In order to maintain the total hardness of water, salts of $MgSO_4$ and $CaSO_4$ were used to increase the hardness, while EDTA was added to decrease the hardness of water.

4.3 Statistical analyses of data

The growth and physico-chemical parameters of the test media 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 water quality parameters included in this study.

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