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Impacts of Physico-Chemical Parameters on Fish Grown Under Heavy Metal Stress

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ABSTRACT

The experiment was conducted in glass aquaria to determine the growth potentials of 240-day three fish species *viz*., *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under chronic exposures (1/3 of LC_{50}) of Al, As, Ba, Cr and their mixture (Al + As + Ba + Cr) for 90 days. The growth parameters *viz*. increase in wet weights, fork and total lengths, feed intake, feed conversion efficiency and condition factor of fish were correlated with physico-chemical parameters viz. total ammonia, dissolved oxygen, carbon dioxide, electrical conductivity, sodium, potassium, calcium and magnesium of the test media (water) of different treatments. The ammonia contents of the test media (water), used for growth trials with three fish species, escalated due to aluminum and arsenic toxicity while it remained lowest in the control media. Dissolved oxygen contents of the control media were significantly higher than all other treatments. However, dissolved oxygen contents of water decreased significantly due to arsenic treatment to the fish predicting variable changes in the metabolic rates of fish exposed to either individual metals or metal mixture from that of control. During growth trials, the increase in dissolved oxygen contents of the media used for *Labeo rohita* under chromium stress is indicative of decrease in oxygen consumption by the fish due to onset of acute hypoxia. The average sodium contents of the test media increased significantly due to exposure of *Labeo rohita* to metal mixture. Physico-chemical variables like pH, sodium, calcium and dissolved organic matter are known to cause metallic toxicity.

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Authors' Contributions
MJ planned the research. HA executed the experimental work. AJ performed statistical analysis. SMH helped in writing manuscript and GJ helped in collection of data.

Key words
Heavy metals, stress, fish, growth, physico-chemistry.

INTRODUCTION

Fish is an important source of quality protein and contributes significantly towards global aquaculture production (Naz *et al*., 2012). Fish meat is amongst preferred food items for humans because it is highly nutritive, palatable with high protein innards, low saturated fats and rich in omega-3 fatty acids. Fish is a very good bio-indicator of ecological toxicity and often used to assess quality of water bodies (Dautremepuits *et al*., 2004; Vutukuru *et al*., 2005).

Main cities, tanneries and industries are usually located near water passages and their ultimately gain entry into the rivers or seas. The discharges especially from paints, mining, electroplating, dye and battery making industries contain heavy metals that deteriorate water quality at massive scales (Azmat *et al*., 2012). Similarly, pesticide also enter into the aquatic ecosystems (Mahboob *et al*., 2014), altering the physico-chemical properties of water bodies which may cause toxicity to fish (Shoaib *et al*., 2013). These elements are posing threat to the survival of the fish because these heavy metals can persist in living bodies for a longer period of time and have high potential for bioaccumulation and biomagnification in the food chain (Ozparlak *et al*., 2012). In addition these elements escalate animal's sensitivity towards changes in temperature, dissolved oxygen contents and vice-versa (Gundogdu, 2008).

Fish is an integral part of human food in many parts of the world and has been used as an experimental animal to understand chronic effects of sub-lethal toxicity of several metals. The studies on metallic toxicity make possible to assess the sub-lethal (1/3 LC_{50}) effects on behavior, physiology, biology and growth of numerous animals, to predict possible toxic effects and their consequences on adaptive abilities of these organisms (Pane *et al*., 2004).

Aquatic toxicity is essential for the evaluation of environmentally hazardous pollutants (Ambreen and Javed, 2015). Aluminum (Al) is found in the form of silicates and oxides on Earth (Scancar *et al*., 2004). The solubility of aluminum increased linearly with the decrease in water pH causing. Inorganic aluminum is considered to be most harmful to numerous fish species (Camargo *et al*., 2009). Arsenic is a metalloid element and is abundantly found in the aquatic bodies (Reimer *et al*., 2002). The geogenic and anthropogenic processes

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Table I.- Treatments/sub-lethal concentrations (1/3 LC_{50}) of various metals and their mixture.

Fish species	Treatments/Sub-lethal concentrations (1/3 LC_{50})					
	Aluminum (mgL^{-1})	Arsenic (mgL^{-1})	Barium (mgL^{-1})	Chromium (mgL^{-1})	Mixture (mgL^{-1})	Control (mgL^{-1})
Catla catla	37.96	3.11	51.30	33.86	12.48	0.00
Labeo rohita	46.86	3.76	49.16	48.32	14.39	0.00
Cirrhina mrigala	49.71	3.21	50.23	40.00	14.37	0.00

Growth parameters of the fish in all the aquaria *viz.*, increase/decrease in average wet weights, fork.

increase the availability of arsenic to aquatic organisms, including fish (Hopkins *et al*., 1999). In natural waters, barium is mostly found along with sulphur, oxygen and carbon. Barium compounds are used to prepare paints, rubber, ceramics and glass. However, these barium compounds have the ability to dissolve in aquatic medium that is why they become part of lakes, rivers, soils and food at little quantities. Chromium is found commonly in micro molar concentrations in the aquatic bodies (Zhang *et al*., 1994). However, chromium is considered the most harmful chemical to the aquatic animals, especially the fish (Al-Akel and Shamsi, 1996).

Increased concentrations of heavy metals in to the water bodies have serious consequences on aquatic biota and in order to conserve the native fauna it is essential to check their tolerance limits against metallic ion toxicity. Indian major carps *i.e., Catla catla*, *Labeo rohita* and *Cirrhina mrigala* are the preferred edible fish species which are present in natural freshwater bodies of the country. The present study was planned to study the growth performance of major carps under chronic sub-lethal exposure of metals *viz.* aluminum, arsenic, barium, chromium and their mixture.

MATERIALS AND METHODS

Present study on growth performance of Indian major carps under chronic sub-lethal exposure of selected heavy metals was conducted in glass aquaria (each having dimensions 3ft × 2ft × 2ft length x width x depth). Fish species *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* (240-day old) were used as experiment animals and their growth potential was assessed under chronic exposures (1/3 of LC_{50}) of aluminum (Al), arsenic (As), barium (Ba), chromium (Cr) and their mixture (Al + As + Ba + Cr) for 12 weeks. The control (un-stress) fish were grown in metal free water. The trials of each fish species were accomplished with three replications each.

The fingerlings of all the three fish species were placed in cemented tanks (each having dimensions 12ft x 2.5ft x 3ft length x width x depth) for a period of two weeks for acclimation. After acclimation period, ten fish/species with almost similar weight and size were selected and placed in separate aquaria containing 50 liter tap water and were fed to satiation twice daily at 9:00 a.m. and 05:00 p.m. The feed had following ingredients: Fish meal, 39.01%; Corn gluten (30 % CP), 40.54%; Cotton seed meal, 01.72%; Rice polish, 07.17%; Wheat flour, 05.00%; Oil (sun flower), 03.56%; Vitamin and Mineral Mixture, 03.00%; Digestible protein was 34 % and digestible Energy = 3.00 $Kcalg^{-1}$. Major profile (%) was Lysine, 2.0153; Methionine, 0.8522; Ca^{++}, 2.3740; PO_4, 1.0890; and Na^+, 0.4444

Each fish species in treatment aquaria was exposed to various levels of sub-lethal (1/3 of LC_{50}) concentrations of metals (Table I) determined by Azmat (2011) for a period of 12 weeks. The experiment was conducted under controlled laboratory conditions using static water assay with continuous supply of air. Water temperature (30ºC), pH (7.50) and total hardness (300 mgL^{-1}) were kept constant.

Growth parameters of the fish in all the aquaria viz. increase/decrease in average wet weights, fork and total lengths, condition factor, feed intake and feed conversion efficiency of three fish species were examined on weekly basis throughout the experimental period. The physico-chemical parameters viz. temperature, pH, total hardness, total ammonia, dissolved oxygen, carbon dioxide, electrical conductivity, sodium, potassium, calcium and magnesium of the water media used for 90-day growth trials with each species of fish under sub-lethal exposure of aluminum, arsenic, barium, chromium, metal mixture and control were monitored on daily basis and their weekly means were taken.

The exposure media were repetitively replenished and exchanged partly to sustain the above mentioned sub-lethal concentrations in the test media for all the three fish species for 12 weeks. Metal concentrations of the test media were measured using Perkin Elmer (AAnalyst 400) Atomic Absorption Spectrophotometer.

Statistical analyses of data

The obtained data was subjected to statistical software $SPSS^{10.1}$ and Analysis of Variance (ANOVA) was applied to explain the impacts of physico-chemical parameters of water on the growth performance of three experimental fish species (Steel *et al.*, 1996).

RESULTS

Table II shows effect of water quality parameters such as ammonia, dissolved oxygen, CO_2, electrical conductivity, Na, K, Ca and Mg on fish growth in medium containing heavy metals such as Al, As, Ba, Cr, and metal mixtures.

Aluminum

During present study, total ammonia contents of the test media showed positive correlation with dissolved oxygen, potassium, increase in fish weight and fork length, and feed conversion efficiency of fish while correlation coefficient between total ammonia and electrical conductivity appeared negative but statistically significant. Dissolved oxygen had negatively significant correlations with calcium and feed intake while it was positively significant with magnesium, increase in fish fork length, feed conversion efficiency and condition factor of fish. Carbon dioxide of the test media increased concomitantly with an increase in calcium concentration while it had inversely significant correlations with magnesium, metallic ion concentration and condition factor of fish. Electrical conductivity of water had negative relationship with potassium while that with metallic ion toxicity remained positive but highly significant. Sodium was negatively but significantly correlated with potassium while its correlation coefficient with the extent of metallic toxicity remained positively significant. Potassium of the test media declined significantly with an increase in metallic ions while it showed positively significant correlations with increase in fish weight, fork length and feed conversion efficiency of fish. Calcium exhibited negative but significant correlations with magnesium and metallic ions of the test media while its relationships were significantly direct with feed intake and condition factor of fish. Magnesium contents increased significantly with concomitant increase in metallic ion concentrations of the test media and condition factor of fish while its correlation with feed intake was significantly inverse. Increase in fish weight was significantly and positively correlated with its fork and total length increments, and feed conversion efficiency of fish. Under the influence of aluminum sub-lethal chronic stress, the feed intake by the fish was negatively but significant correlated with feed conversion efficiency and condition factor of fish (Table III).

Arsenic

During 90-day growth trials, arsenic exposure to the fish caused significantly positive relationships of total ammonia with potassium, fish weight, fork and total length increments, and feed conversion efficiency of fish while it was negative but significant with carbon dioxide. Dissolved oxygen contents of the test media decreased significantly with an increase in the concentration of exposed metal to the fish while dissolved oxygen exhibited significantly positive impact on fish weight increments and feed intakes. Carbon dioxide contents of the media had positively significant impacts on electrical conductivity and condition factor of fish while carbon dioxide showed inversely significant relationships with an increase in potassium contents, fish weight, fork and total length increments and feed conversion efficiency of fish. Sodium contents of water showed positively direct relationship with magnesium only. Potassium caused significantly positive impacts on the increase in fish weight, fork and total lengths, and feed conversion efficiency of fish while correlation coefficient between potassium and condition factor of fish remained negatively significant. The correlation coefficient between calcium and magnesium was positively significant. Under sub-lethal arsenic toxicity, an increase in fish weight showed positive correlation with feed conversion efficiency while the same remained negatively significant with condition factor of fish. The condition factor of fish had negative but highly significant correlations with total ammonia, potassium, increase in fork and total lengths and feed conversion efficiency of fish while it was positive with carbon dioxide and electrical conductivity. An increase in arsenic concentration in water caused significant decrease in dissolved oxygen, electrical conductivity and feed intake by the fish (Table IV).

Barium

The sub-lethal concentrations of barium caused significantly positive impacts on dissolved oxygen, fish weight, fork length and total length escalations while it remained inversely significant with carbon dioxide. Both feed conversion efficiency and condition factor of fish decreased while feed intake had positively significant correlation with the total ammonia concentrations of the test media. Dissolved oxygen had negative but significant correlation with carbon dioxide while it was positively significant with fish weight, fork and total length increments, and feed intake. The relationship of carbon dioxide with fish growth parameters *viz.* increase in weight, fork and total lengths, and feed intake remained negative but statistically highly significant. Feed intake caused significant escalations in weight, fork and total

Table II.- Water quality parameters of the test media during 90-day growth trials.

Media used for fish	Metals/Treatments					
	Aluminium	Arsenic	Barium	Chromium	Metal mixture	Control
Total ammonia (mgL^{-1})						
Catla catla	2.73±0.01 **a**	2.40±0.02 **de**	2.37±0.02 **e**	2.52±0.02 **c**	2.60±0.04 **b**	1.35±0.02 **f**
Labeo rohita	2.16±0.01 **d**	2.44±0.02 **b**	2.40±0.01 **bc**	2.38±0.02 **c**	2.55±0.01 **a**	1.37±0.02 **e**
Cirrhina mrigala	2.52±0.01 **a**	2.52±0.01 **a**	2.53±0.02 **a**	2.42±0.03 **b**	2.00±0.13 **c**	1.57±0.02 **d**
Overall Means	**2.47±0.29 a**	**2.45±0.06 ab**	**2.43±0.08 b**	**2.44±0.07 b**	**2.38±0.33 c**	**1.43±0.12 d**
Dissolved oxygen (mgL^{-1})						
Catla catla	5.08±0.01 **cd**	5.03±0.02 **d**	5.09±0.01 **c**	5.03±0.02 **d**	5.15±5.15 **b**	5.75±0.04 **a**
Labeo rohita	4.97±0.01 **c**	4.87±4.87 **d**	4.98±0.03 **c**	5.10±0.01 **b**	4.95±4.95 **c**	5.67±0.07 **a**
Cirrhina mrigala	5.17±0.01 **b**	5.08±5.08 **c**	5.13±0.01 **bc**	4.97±0.07 **d**	5.09±0.03 **c**	5.64±0.03 **a**
Overall Means	**5.07±0.10 b**	**4.99±0.11 d**	**5.07±0.08 b**	**5.03±0.06 c**	**5.06±0.10 bc**	**5.69±0.06 a**
Carbondioxide (mgL^{-1})						
Catla catla	1.84±0.02 **a**	1.84±0.02 **a**	1.76±0.02 **cd**	1.72±0.02 **d**	1.78±0.02 **bc**	1.17±0.02 **e**
Labeo rohita	1.71±0.02 **c**	1.77±0.01 **b**	1.84±0.02 **a**	1.63±0.02 **d**	1.82±0.01 **ab**	1.22±0.01 **e**
Cirrhina mrigala	1.64±0.02 **e**	1.73±0.02 **c**	1.76±0.03 **bc**	1.68±0.03 **d**	1.78±0.03 **ab**	1.23±0.01 **f**
Overall Means	**1.73±0.10 b**	**1.78±0.06 a**	**1.79±0.05 a**	**1.68±0.04 c**	**1.79±0.02 a**	**1.21±0.03 d**
Electrical conductivity (mScm^{-1})						
Catla catla	3.33±0.01 **b**	3.38±0.01 **ab**	3.40±0.03 **ab**	3.14±0.03 **c**	3.40±0.02 **ab**	3.46±0.04 **a**
Labeo rohita	3.38±0.03 **a**	3.26±0.01 **b**	3.42±0.02 **a**	3.43±0.05 **a**	3.43±0.04 **a**	3.44±0.04 **a**
Cirrhina mrigala	3.37±0.02 **ab**	3.29±0.01 **b**	3.36±0.28 **ab**	3.40±0.03 **ab**	3.42±0.03 **a**	3.45±0.02 **a**
Overall Means	**3.36±0.03 bc**	**3.31±0.06 c**	**3.39±0.03 ab**	**3.32±0.16 c**	**3.42±0.01 ab**	**3.45±0.01 a**
Sodium (mgL^{-1})						
Catla catla	310.19±5.54 **a**	319.83±6.39 **a**	319.35±7.03 **a**	319.31±5.19 **a**	308.09±4.95 **a**	319.99±5.51**a**
Labeo rohita	319.90±3.84 **b**	308.38±3.93 **b**	319.70±5.53 **b**	320.02±5.34 **b**	354.42±4.55 **a**	308.47±5.98**b**
Cirrhina mrigala	317.76±5.02 **a**	315.85±11.01 **a**	320.26±5.04 **a**	319.05±3.10 **a**	314.09±6.01 **a**	319.52±6.82**a**
Overall Means	**315.95±5.10 a**	**314.69±5.81 a**	**319.77±0.46 a**	**319.46±0.50 a**	**325.54±7.53 a**	**313.96±6.52a**
Potassium (mgL^{-1})						
Catla catla	10.55±0.01 **abc**	9.92±0.02 **cd**	10.35±0.02 **abcd**	10.33±1.75 **abcd**	9.81±0.02 **d**	10.07±0.03 **bcd**
Labeo rohita	9.80±0.02 **c**	10.21±0.02 **abc**	10.39±0.02 **abc**	10.33±0.04 **abc**	10.39±0.02 **abc**	9.90±0.02 **bc**
Cirrhina mrigala	10.20±0.03 **b**	10.75±0.04 **ab**	10.39±0.02 **ab**	10.45±0.02 **ab**	10.86±0.03 **ab**	10.89±0.01 **a**
Overall Means	**10.18±0.37 a**	**10.29±0.42 a**	**10.38±0.02 a**	**10.37±0.07 a**	**10.35±0.52 a**	**10.29±0.53 a**
Calcium (mgL^{-1})						
Catla catla	26.05±0.02 **a**	24.28±0.04 **ab**	23.71±1.90 **b**	24.07±3.50 **ab**	24.33±1.02 **ab**	24.19±1.44 **ab**
Labeo rohita	25.57±0.06 **a**	25.58±0.05 **a**	24.12±1.90 **a**	24.10±0.43 **a**	23.87±0.05 **a**	24.34±0.56 **a**
Cirrhina mrigala	23.60±0.03 **b**	24.38±2.75 **ab**	24.06±0.03 **ab**	23.96±0.06 **ab**	25.65±0.06 **ab**	24.92±1.43 **ab**
Overall Means	**25.07±1.30 a**	**24.75±0.72 a**	**23.96±0.22 a**	**24.04±0.07 a**	**24.62±0.92 a**	**24.48±0.38 a**
Magnesium (mgL^{-1})						
Catla catla	58.83±0.02 **a**	59.84±3.39 **a**	60.60±3.52 **a**	59.98±4.47 **a**	59.79±3.20 **a**	59.88±0.30 **a**
Labeo rohita	59.52±0.04 **a**	59.15±0.01 **a**	59.94±4.10 **a**	59.97±5.29 **a**	60.14±1.61 **a**	59.84±4.43 **a**
Cirrhina mrigala	59.91±0.02 **a**	59.79±7.07 **a**	60.06±2.02 **a**	60.14±5.47 **a**	59.00±1.31 **a**	59.47±4.64 **a**
Overall Means	**59.42±0.79 a**	**59.60±0.38 a**	**60.20±0.35 a**	**60.03±0.09 a**	**59.64±0.58 a**	**59.73±0.23 a**

Means with same letters in a single row are statistically similar at $p< 0.05$.

Table III. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three fish species under sub-lethal concentration of aluminum.

	Sub-lethal concentration (mgL^{-1})	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Total ammonia	-0.61418													
Dissolved oxygen	0.17499	0.66641												
Carbon dioxide	-0.98191	0.53515	-0.26181											
Electrical conductivity	0.68895	-0.68891	-0.19604	-0.59662										
Sodium	0.65271	-0.63443	-0.15086	-0.64598	0.55217									
Potassium	-0.69797	0.99238	0.57964	0.62359	-0.72493	-0.68197								
Calcium	-0.80892	0.03411	-0.71590	0.84736	-0.35530	-0.3551	0.14578							
Magnesium	0.80570	-0.02801	0.72180	-0.84052	0.34971	0.33909	-0.13898	-0.99880						
Increase in weight	-0.62330	0.82282	0.43570	0.56241	-0.65943	-0.64781	0.82026	0.18157	-0.17081					
Increase in fork length	-0.34773	0.87927	0.74533	0.29205	-0.55564	0.57592	0.84103	-0.20389	0.21638	0.79969				
Increase in total length	0.56036	-0.60722	-0.24284	-0.49189	0.28327	0.37688	-0.60492	-0.24365	0.26337	0.67798	-0.37191			
Feed intake	-0.32454	-0.54640	-0.98320	0.40031	0.08550	0.08154	-0.44840	0.81808	-0.82058	-0.32420	-0.67727	0.15325		
Feed conversion Ratio	-0.09496	0.80463	0.90900	0.01182	-0.39472	-0.39538	0.73399	-0.47647	0.48370	0.75554	0.88935	-0.46990	-0.86417	
Condition factor of fish	0.67572	0.08980	0.73311	-0.73121	0.25663	0.33890	-0.02644	0.93031	0.91615	-0.11687	0.26576	0.02295	-0.82940	0.51827

Critical Value (2 tail) = +/- 0.66422

Table IV. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three fish species under sub-lethal concentration of arsenic.

	Sub-lethal concentration (mgL^{-1})	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Total ammonia	-0.04488													
Dissolved oxygen	-0.90758	0.38127												
Carbon dioxide	-0.28344	-0.81912	-0.08459											
Electrical conductivity	-0.76196	-0.55318	0.46169	0.78743										
Sodium	-0.58404	0.05229	0.43312	0.44339	0.52527									
Potassium	-0.02972	0.96723	0.38272	-0.89640	-0.60846	-0.07591								
Calcium	0.42355	0.03285	-0.46001	0.08424	-0.26407	0.34144	-0.03304							
Magnesium	-0.09141	0.15728	0.00708	0.26595	0.04470	0.78301	0.05032	0.69115						
Increase in weight	-0.40102	0.84422	0.71466	-0.72694	-0.25444	0.06310	0.87938	-0.34438	-0.10897					
Increase in fork length	0.50661	0.76870	-0.17571	-0.91464	-0.90835	-0.38575	0.82490	0.28066	-0.00636	0.50242				
Increase in total length	0.62410	0.71143	-0.32711	-0.84091	-0.95247	-0.40145	0.74047	0.27806	0.06345	0.36371	0.95707			
Feed intake	-0.98956	0.15747	0.94323	0.18177	0.68961	0.59370	0.13844	-0.38857	0.09878	0.50399	-0.40765	-0.54773		
Feed conversion ratio	-0.22746	0.88554	0.58057	-0.82686	-0.42117	-0.05385	0.92556	-0.29487	-0.13986	0.98307	0.62857	0.50782	0.33790	
Condition factor of fish	-0.33893	-0.87491	-0.02687	0.94070	0.84937	0.32779	-0.91312	-0.05450	0.06080	-0.69743	-0.93861	-0.89626	0.22892	-0.80611

Critical Value (2 tail) = +/- 0.66422

Table V. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three fish species under sub-lethal concentration of barium.

	Sub-lethal concentration (mgL^{-1})	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Total ammonia	-0.19010													
Dissolved oxygen	0.70159	0.53339												
Carbon dioxide	-0.75644	-0.21236	-0.86233											
Electrical conductivity	-0.07386	-0.20558	-0.24776	0.35723										
Sodium	-0.02755	0.17759	0.04041	0.32298	0.15272									
Potassium	-0.66066	0.49031	-0.18350	0.33215	-0.16817	0.21923								
Calcium	-0.10110	0.14775	0.12010	0.11332	-0.01480	0.31991	-0.02855							
Magnesium	0.09167	-0.17420	0.09809	-0.35247	0.07028	-0.22048	-0.15381	0.09411						
Increase in weight	0.67304	0.56107	0.96656	-0.74983	-0.03948	0.18458	-0.21315	0.13643	0.04584					
Increase in fork length	0.93047	0.15664	0.89740	-0.87236	-0.10474	-0.01362	-0.45290	-0.10315	0.11398	0.87448				
Increase in total length	0.92759	0.17399	0.88986	-0.84466	-0.08158	-0.01693	-0.50471	-0.10547	0.05327	0.88151	0.99322			
Feed intake	0.55989	0.68723	0.95887	-0.78897	-0.18613	0.04387	-0.05258	-0.03268	0.02626	0.95515	0.81832	0.82163		
Feed conversion ratio	-0.11965	-0.66798	-0.55377	0.57821	0.42332	0.33090	-0.28156	0.50160	0.04041	-0.47159	-0.39488	-0.39388	-0.70932	
Condition factor of fish	0.17099	-0.97944	-0.56273	0.27960	0.22360	-0.13875	-0.52527	-0.15321	0.00747	-0.56846	-0.18527	-0.18527	-0.69900	0.68139

Critical Value (2 tail) = +/- 0.66422

lengths of fish. Increase in fish total length showed positively direct relationship with feed intake also. Feed conversion efficiency of fish had positively significant relationship with condition factor of fish (Table V).

Chromium

Chromium toxicity of the test media showed negatively significant relationships with total ammonia and carbon dioxide while these were positive and highly significant with electrical conductivity, fish weight, fork and total lengths and feed intake by the fish. Total ammonia of the test media had positively significant correlation with carbon dioxide and condition factor of fish while that with electrical conductivity, fish weight, fork and total lengths and feed intake remained inversely significant. Dissolved oxygen contents of the media showed positively significant correlation with fish fork length increments only. Carbon dioxide had negative but significant correlations with electrical conductivity, fish weight, fork and total lengths and feed intake while it remained significantly direct with condition factor of fish. Increase in fish weight had positively significant relationships with the increase in fish fork and total lengths, and feed intake while it was negative but significant with condition factor of fish. Feed intake by the fish, under sub-lethal stress of chromium, caused significantly inverse relationship with fish weight escalations (Table VI).

Metal mixture

The metal mixture toxicity of the test media, used for three fish species, showed positively significant correlations with potassium, increase in weight and feed intakes by the fish. Total ammonia caused negatively significant correlations with potassium, calcium and feed intake while it remained positive and highly significant with fish total length, feed conversion efficiency and condition factor. Dissolved oxygen contents of the test media did not show any significant relationships with either physico-chemical parameters or growth variables studied during these experiments. Carbon dioxide had significantly direct relationship with electrical conductivity. Potassium exhibited significantly positive relationships with increase in fish weight and feed intake while it was negative but highly significant with increase in fish fork and total lengths, and condition factor of fish. Calcium showed significantly positive relationship with feed intake while the same with increase in total lengths and condition factor of fish exhibited negative but highly significant correlation. Increase in fish weight correlated negatively with the increase in fish fork and total lengths while the correlation coefficient between increase in fish weight and feed intake was positively significant. Increase in fish fork length had significantly direct relationship with an increase in total length while it remained negative but highly significant with feed intake by the fish. Increase in total length caused positive correlation with both feed conversion efficiency and condition factor of fish while it remained negative but significant with feed intake. Feed intake by the fish was correlated negatively with feed conversion efficiency and condition factor of fish with statistically significant correlation coefficients. Feed conversion efficiency showed positive correlation with condition factor of fish also (Table VII).

Control fish

In control test media, total ammonia contents showed positively significant relationships with carbon dioxide, potassium, increase in fish weight and feed intake while it remained negatively significant with condition factor of fish. Dissolved oxygen contents of the media had negatively significant correlation with carbon dioxide while its correlation coefficient with fish condition factor was positively significant. Carbon dioxide had negative but significant correlation with feed conversion efficiency and condition factor of fish. Electrical conductivity of water exhibited positively significant correlation with calcium only. Sodium caused significantly positive relationships with fork and total length increments of fish. Potassium contents of the media had positively significant correlation with increase in fish weight, fork length and feed intake while it remained negative but highly significant with condition factor of fish. Increase in fish weight showed significantly direct relationships with the increase in fish fork and total lengths and feed intake by the fish. The correlation coefficient between fish weight increment and condition factor remained negatively significant. Total length increments of fish showed significantly direct relationship with feed conversion efficiency of fish while correlation coefficient between feed intake and condition factor of fish was negative but significant (Table VIII).

DISCUSSION

Aquatic pollution can be checked by assessing the accumulation of heavy metals in fish and its environment (Jabeen and Javed, 2011). The fish are closely linked with water and constitute a significant food item in diet of humans (Naz *et al*., 2012). Growth is a straight forward manifestation of metal’s effect on the fish because it integrates all the impacts within the body (Azmat, 2011). Metals mixture in their mode of action may be additive, antagonistic or synergistic in fish body (Javed and Yaqub, 2010). The ammonia contents of the

Table VI. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three fish species under sub-lethal concentration of chromium.

	Sub-lethal concentration (mgL^{-1})	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Total ammonia	-0.90255													
Dissolved oxygen	0.52650	-0.14240												
Carbon dioxide	-0.89713	0.89584	-0.25462											
Electrical conductivity	0.84965	-0.94009	0.11938	-0.77753										
Sodium	0.08154	0.03653	0.19706	0.16359	-0.06088									
Potassium	-0.00384	0.09864	0.00922	-0.14586	0.05972	-0.62030								
Calcium	0.01495	0.06198	0.17382	0.14095	0.07626	-0.12925	0.08119							
Magnesium	0.00105	0.22376	0.19798	0.12749	0.00617	0.29478	0.50276	0.19843						
Increase in weight	0.92924	-0.92664	0.37519	-0.79762	0.92431	0.05515	-0.11819	0.06259	-0.05641					
Increase in fork length	0.94455	-0.77213	0.66957	-0.85692	0.68496	-0.02452	0.05787	0.15837	-0.03863	0.80678				
Increase in total length	0.96633	-0.84870	0.59049	-0.87044	0.73950	0.05661	-0.09820	-0.03521	-0.14129	0.89604	0.94301			
Feed intake	0.93564	-0.95807	0.26260	-0.85744	0.96440	0.05266	0.02363	-0.00110	0.01030	0.92335	0.80365	0.83491		
Feed conversion ratio	-0.23938	0.30602	0.20950	0.36021	-0.33405	0.04256	-0.39472	0.19138	-0.16833	-0.04347	-0.05710	-0.05710	-0.42243	
Condition factor of fish	-0.62199	0.87004	0.27408	0.71108	-0.82273	-0.03338	0.16342	0.34016	0.23889	-0.71379	-0.53343	-0.53343	-0.79883	0.40170

Critical Value (2 tail) = +/- 0.66422

Table VII. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three fish species under sub-lethal concentration of metal mixture.

	Sub-lethal concentration (mgL^{-1})	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Total ammonia	-0.54487													
Dissolved oxygen	-0.56204	-0.08554												
Carbon dioxide	0.43976	0.18301	-0.34882											
Electrical conductivity	0.41869	-0.14723	-0.54019	0.28669										
Sodium	0.41644	0.28465	-0.33749	0.81430	0.51607									
Potassium	0.88954	-0.84443	-0.29433	0.08119	0.26413	0.03745								
Calcium	0.22563	-0.80254	0.44455	-0.17838	-0.17524	-0.42420	0.55560							
Magnesium	-0.04531	0.23169	0.48785	0.46437	-0.08222	0.59240	-0.17485	-0.02904						
Increase in weight	0.77239	-0.57256	-0.18568	0.19852	0.29030	0.15794	0.80499	0.53592	0.12973					
Increase in fork length	-0.97052	0.57229	0.54152	-0.25308	-0.36877	-0.26036	-0.90803	-0.23732	0.15965	-0.78467				
Increase in total length	-0.65215	0.94621	0.02344	0.24274	-0.15906	0.24914	-0.92318	-0.70865	0.22247	-0.71758	0.71208			
Feed intake	0.66919	-0.96347	-0.01536	-0.17246	0.10775	-0.25247	0.93258	0.75532	-0.22120	0.71568	-0.71757	-0.99057		
Feed conversion ratio	-0.30832	0.79941	-0.00750	0.42371	0.11786	0.49363	-0.61846	-0.46934	0.50658	-0.10587	0.37818	0.74607	-0.75633	
Condition factor of fish	-0.57690	0.90060	0.05506	0.18978	-0.00803	0.34636	-0.85681	-0.69808	0.26680	-0.64525	0.60923	0.93212	-0.94301	0.76666

Critical Value (2 tail) = +/- 0.66422

Table VIII. Correlation coefficients between fish growth and physico-chemical parameters of the water media during growth trials with three control fish species.

	Total ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Electrical conductivity ($mScm^{-1}$)	Sodium (mgL^{-1})	Potassium (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Increase in weight (mg)	Increase in fork length (mm)	Increase in total length (mm)	Feed intake (mg)	Feed conversion ratio (%)
Dissolved oxygen	-0.56876												
Carbon dioxide	0.71409	-0.75909											
Electrical conductivity	-0.03915	0.22708	-0.23505										
Sodium	0.20605	0.21578	-0.25169	0.52910									
Potassium	0.95267	-0.41931	0.52400	0.09529	0.45758								
Calcium	0.19045	0.03287	0.09018	0.67861	0.34399	0.31408							
Magnesium	0.00393	0.33445	0.08832	0.32385	-0.19957	-0.06038	0.14961						
Increase in weight	0.89875	-0.32106	0.39439	0.12484	0.51576	0.97951	0.27177	-0.02521					
Increase in fork length	0.58230	0.05185	-0.08919	0.21530	0.67422	0.75512	0.11463	-0.02152	0.86364				
Increase in total length	0.33632	0.26996	-0.31054	0.17489	0.69972	0.55755	0.06904	-0.01993	0.70112	0.95023			
Feed intake	0.96585	-0.46731	0.57936	0.06056	0.41516	0.99740	0.30626	-0.07004	0.96822	0.71528	0.51098		
Feed conversion ratio	-0.37892	0.61224	-0.78874	0.24951	0.35961	-0.18917	-0.17510	0.15292	0.00709	0.49216	0.67790	-0.24297	
Condition factor of fish	-0.92666	0.73847	-0.85519	0.04459	-0.13657	-0.83952	-0.24112	0.11425	-0.72602	-0.30264	-0.03756	-0.86907	0.65615

Critical Value (2 tail) = +/- 0.66422

test media (water), used for growth trials with three fish species, escalated due to aluminum and arsenic toxicity while it remained lowest in the control media. Dissolved oxygen contents of the control media were significantly higher than all other treatments. However, dissolved oxygen contents of water decreased significantly due to arsenic treatment to the fish predicting variable changes in the metabolic rates of fish exposed to either individual metals or metal mixture from that of control. Hayat (2009) during his studies on metals' chronic toxicity reported reduction in dissolved oxygen contents while increase in ammonia excretion by fish. This shows significant effects of heavy metals toxicity on fish respiration (James *et al*., 2009). These respiratory enzymes are also inhibited in the fish when exposed to heavy metals (Balavenkatasubbiah *et al.,* 1984). The increase in dissolved oxygen contents of the media used for *Labeo rohita* under chromium stress is indicative of decrease in oxygen consumption by the fish due to onset of acute hypoxia. McGeer *et al*. (2000) reported significant effect of copper exposure on the daily feed consumption and appetite in *Oncorhynchus mykiss*. The inhibitory effect of metals on the growth of fish has also been reported due to their ability to take food under variable chronic stress of metals (Puvaneswari and Karuppasamy, 2007). Furthermore, the drop in fish metabolism as a protective adaptation to pledge low intake of metal, during chronic exposure, cannot be ruled out. Fish can reduce oxygen consumption during chronic exposures due to the factors that could inhibit respiration. In fish, gills are the important osmoregulatory and respiratory organs and cellular damage provoked by various metals may damage the respiratory surface area. Therefore, alteration in respiratory activity has been employed as an indicator of stress in organisms living in polluted waters (Dharmalata and Joshi, 2002). Heavy metals like nickel, cadmium, chromium and lead can decrease the affinity of hemoglobin for oxygen binding ability also. Therefore, fish erythrocytes would become more permeable to metals and became fragile (Witeska and Kosciuk, 2003) causing significant decrease in oxygen consumption by the fish (Sarkar, 1999). Sherwood *et al*. (2000) reported adverse effects of zinc, cadmium and copper on the growth rate, feed consumption and feed conversion efficiency of yellow perch (*Perca flavescens*). Higher concentrations of either individual metals or metal mixture caused increase in carbondioxide contents of the test media significantly showing concentration and metal species specific stress induced in three fish species to liberate significantly more carbon dioxide in water through respiration and thus, increasing the carbon dioxide concentrations of the test media (Abdullah, 2007). The average sodium contents of the test media increased significantly due to exposure of *Labeo rohita* to metal mixture. Physico-chemical variables like pH, sodium, calcium and dissolved organic matter are known to cause metallic toxicity to the aquatic organisms, including fish (Niyogi and Wood, 2004). The observed uptake of metals by the fish during sub-lethal exposures engross branchial toxicity with metals that interferes with active sodium uptake (Bury *et al*., 2003; Pyle and Wood, 2008). Yaqub and Javed (2012) reported influence of various physico-chemical parameters on bioaccumulation of metals by fish. Therefore, exposure of metals upset the homeostasis in fish. However, fish physiology is adjusted to recompense the specific effects by altering the mode of chemical reactions to re-establish the equilibrium in fish body (Beyers *et al*., 1999).

REFERENCES

Abdullah, S., 2007. *Studies on acute toxicity of metals to the fish viz.* Catla catla, Labeo rohita *and* Cirrhina mrigala. Ph.D. thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan, 1-191.

Al-Akel, A.S. and Shamsi, M.J.K., 1996. Hexavalent chromium: toxicity and impact on carbohydrate metabolism and haematological parameters of carp (*Cyprinus carpio* L.) from Saudi Arabia. *Aquat. Sci*., **58**: 24 – 30.

Ambreen, F. and Javed, M., 2015. Assessment of acute toxicity of pesticides mixtures for *Cyprinus carpio* and *Ctenopharyngodon idella*. *Pakistan J. Zool*., **47**: 133-139.

Azmat, H., Javed, M. and Jabeen, G., 2012. Acute toxicity of aluminum to fish (*Catla catla, Labeo rohita* and *Cirrhina mrigala*). *Pak. Vet. J*., **32**: 85 – 87.

Azmat, H., 2011. *Studies on 96-hr LC_{50} and lethal responses of major carps in relation to their growth and bioaccumulation of waterborne metals*. Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan, 1 – 275.

Balavenkatasubbiah, M., Rani, A. U., Geethanjali, K., Pyrushottam, K. R. and Ramamurthy, R., 1984. Effect of cupric chloride on oxidative metabolism in the freshwater fish, *Tilapia mossambica*. *Ecotoxicol. environ. Saf*., **8**: 289 – 291.

Beyers, D. W., Rice, J.A., Clements, W. H. and Henry, C. J., 1999. Estimating physiological cost of chemical exposure: Integrating energetics and stress to quantify toxic effects in fish. *Can. J. Fish. aquat. Sci*., **56**: 814 – 822.

Bury, N.R., Walker, P. A. and Glover, C. N., 2003. Nutritive metal uptake in teleost fish. *J. exp. Biol*., **206**: 11 – 23.

Camargo, M. M. P., Fernandes, M. N. and Martinez, C. B. R., 2009. How aluminum exposure promotes osmoregulatory disturbances in the neotropical freshwater fish *Prochilus lineatus*. *Aquat. Toxicol*., **94**: 40 – 46.

Dautremepuits, C., Betoulle, S., Paris-Palacios, S. and Vernet, G., 2004. Immunology related perturbations induced by copper and chiston in carp (*Cyprinus carpio*). *Arch. environ. Contam. Toxicol*., **47**:370-378.

Dharmalata and Joshi, N., 2002. Toxicity and respiratory response of Heteropneustes fossilis exposed to zinc chloride and fly ash leachate. *Himalayan J. environ. Zool*., **16**: 87 – 90.

Gundogdu, A., 2008. Acute toxicity of zinc and copper for rainbow trout (*Oreochromis niloticus*). *J. Fish. Sci*., **2**: 711–721.

Hayat, S., 2009. *Studies on the growth performance and meat quality of metal stressed fish reared under semi-intensive pond culture system*. Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan, pp. 1-195.

Hopkins, W.A., Rowe, C. L. and Congodon, J. D., 1999. Elevated trace element concentrations and standard metabolic rates in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. *Environ. Toxicol. Chem*., **18**: 1258 – 1263.

Jabeen, G. and Javed, M., 2011. Evaluation of arsenic toxicity to biota in River Ravi (Pakistan) aquatic ecosystem. *Int. J. Agric. Biol*., **13**: 929 – 934.

James, R., Sampath, K., Nagarajan, R., Vellaisamy, P. and Manikandan, M. M., 2009. Effect of dietary Spirulina on reduction of copper toxicity and improvement of growth, blood parameters and phosphatases activities in carp, *Cirrhina mrigala*. *Ind. J. exp. Biol*., **47**: 754–759.

Javed, M. and Yaqub, S., 2010. *Studies on toxic effects of metal mixtures on fish*. First Annual Research Report. Project # PSF/Res./P-Au/Envr/(85).

Mahboob, S., Ghazala, Al-Ghanim, K.A., Sutana, S., Al-Misned, F. and Ahmed, Z., 2014. Fish cholinesterases as biomarkers of sublethal effects of organophosphorus and carbamates in tissues of *Labeo rohita*. *Pakistan J. Zool*., **46**: 121-127.

McGeer, J. C., Szebedinszky, C., McDonald, D. G. and Wood, C. M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 1: Iono-regulatory disturbance and metabolic costs. *Aquatic Toxicol*., **50**: 231 – 243.

Naz, S., Javed, M., Tahir, A. and Azmat, H., 2012. Impact of physic-chemical variables of test media on growth performance of metal stressed major carps. *Pakistan J. Zool*., **44**: 1291 – 1296.

Niyogi, S. and Wood, C. M., 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. *Environ. Sci. Technol*., **38**: 6177 – 6192.

Ozparlak, H., Arslan, G. and Arslan, E., 2012. Determination of some metal levels in muscle tissue of nine fish species from Beysehir lake, Turkey. *Turk. J. Fish. aquat. Sci*., **12**: 761 – 770.

Pane, E. F., Haque, A., Goss, G. G. and Wood, C. M., 2004. The physiological consequences of exposure to chronic, sublethal waterborne nickel in rainbow trout (*Oncorhynchus mykiss*): exercise vs resting physiology. *J. exp. Biol*., **207**: 1249 – 1261.

Puvaneswari, S. and Karuppasamy, R., 2007. Accumulation of cadmium and its effects on the survival and growth of larvae of *Heteropneustes fossilis* (Bloch, 1794). *J. Fish. aquat. Sci*., **2**: 27 – 37.

Pyle, G. and Wood, C. M., 2008. Radiotracer studies on waterborne copper uptake, distribution and toxicity in rainbow trout and yellow perch: A comparative analysis. *Hum. Ecol. Risk Assess*., **14**: 243 – 265.

Reimer, K. J., Koch, I., Ollson, C.A., Cullen, W. R., Granchinho, S., Andrews, P., Jamieson, H. and Walker, S. R., 2002. *Arsenic Report # 72*. Environmental Sscience Group, Royal Military College, Kingston, Ontario, Kingston.

Sarkar, S. K., 1999. Effect of two heavy metals (copper sulphate and cadmium sulphate) on the oxygen consumption of the fish, *Cyprinus carpio*. *U. P. J. Zool*., **1**: 13 – 16.

Scancar, J., Stibilj, V. and Milacic, R., 2004. Determination of aluminum in Slovenian foodstuff and its leachbility from aluminum cookware. *Fd. Chem*., **85**: 151 – 157.

Sherwood, G. D., Rasmussen, J. B., Rowan, D. J., Brodeur, J. and Hontella, A., 2000. Bioenergetics costs of heavy metal exposure in yellow perch (*Perca flavescens*): in situ estimates with a radiotracer (^{137}Cs) technique. *Can. J. Fish. aquat. Sci*., **57**: 441 – 450.

Shoaib, N., Pirzada, J. A. S. and Khalid, H., 2013. Toxicity of synthetic pyrethroid pesticides, fenpropathrin and fenvalerate, on killifish Aphanius dispar juveniles. *Pakistan J. Zool*., **45**: 1160 – 1164.

Steel, R. G. D., Torrie, J. H. and Dinkkey, D. A., 1996. *Principles and procedures of statistics*. 2nd Ed., McGraw Hill Book Co. Singapore.

Vutukuru, S. S., 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *Int. J. environ. Res. Publ. Hlth*., **2**: 456 – 462.

Witeska, M. and Kosciuk, B., 2003: Changes in common carp blood after short-term zinc exposure. *Environ. Sci. Pollut. Res*., **3**: 15 – 24.

Yaqub, S. and Javed, M., 2012. Acute toxicity of waterborne and dietary cadmium and cobalt for fish. *Int. J. Agric. Biol*., **14**: 276 – 280.

Zhang, J., Huang, W. W., Liu, M.G. and Cui, J. Z., 1994. Eco-social impact and chemical regimes of large Chinese rivers: a short discussion. *Water Res*., **28**: 609 – 617.