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Growth performance of major carps during exposure of zinc and bioaccumulation in fish body organs

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Abstract

Growth is an index of metals influence on a fish. This investigation aimed to determine growth responses of major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during exposure to sublethal concentration to zinc. The variations among fish age, species, treatment (zinc) of growth parameters viz. weight increment, fork lengths, and condition factor were statistically significant at $P<0.01$. The control fish age group 90,120,150 and 180 day showed better feed conversion ratio 0.234, 0.168, 0.132 and 0.29 as compared to that of zinc exposed fish which showed range 0.01 to 0.23. Three fish species showed significantly higher amount of zinc in liver than other organs. *Labeo rohita* showed significantly higher trend for zinc accumulation in body organs than as compared to *Cirrhina mrigala* and *Catla catla*. Among body organs; skin, muscle, scales displayed significantly lesser trend for zinc accumulation. It is concluded that metal intoxication significantly reduced the growth of all three fish species and may also show good indicator of metals present in surrounding environment.

Keywords: Bioaccumulation, Chronic sublethal exposure, Heavy metals, Major carps, Zinc

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Introduction

Recent era is well aware about pollution and its effect especially the river pollution with heavy metal is affecting aquatic life badly. Many awareness programs have been started to observe and abatement of river pollution with heavy metal. Discharging of all sorts of highly obnoxious matter into freshwater is an affront to civilized values and damaging sustainability to the environment. In aquatic ecosystem, Fish can be used as an indicator of freshwater pollution through heavy metals as they inhabit diverse tropic levels. Fish absorbs dissolved

heavy metals from the ambient water that can accumulate in several tissues and organs and even be bio-magnified in the food web. Environmental and health issues resulting from metals present in aquatic ecosystem are very well known (Shah et al., 2020). Decrease in fish species can be linked with industrial and domestic pollutants, ecological degradation and change in habitat in rivers (Atique and An, 2019).

In the absorption procedure, there are four likely ways for the substance (such as metals) to come in a fish; as food ingested, simple circulation of the metals in ionic form over gill pores, drinking water and through skin absorption (Frank et al., 1998; Khan et al., 2018). Most abundant vertebrate are fish which affect human through food intake (Lee et al., 2019). The accumulation of heavy metals is dose and time dependent (Annabi et al., 2013). The trace metals are vital for common physiological procedures; unusually high concentration can be lethal to aquatic organisms (Javed, 2003). The most common pollutants include manganese, Iron, nickel and zinc which are found in Punjab Province Rivers (Javed and Mahmood, 2001). Feed intake and fish productivity decreases in the presence of heavy metals (Sfakianakis et al., 2015). Alvarado et al. (2021) determined the heavy metal concentrations in the organs/tissues of carp and Catfish. Results showed that metal concentrations (Zn, Cu, Cd and Pb) were highest in liver whereas metal concentrations (Hg and As) were highest both in liver and muscle tissue.

Lorenten and Maage (1999) studied the bioaccumulation of trace elements zinc, manganese, iron and copper from first feeding of fingerling of Atlantic salmon (*Salmo salar*). Selenium become highly toxic when present in high amount in aquatic environment (Li et al., 2020). Zinc, Molybdenum and copper was proved to be the most toxic heavy metals causing increased hazard rate to mortality up to 96 hr and shorter life expectancy (Gouva et al., 2020). Zinc and iron were deposited to body with growth at all stages. High influx of industrial and urban effluents into the Riverine system of Pakistan necessitates the importance to investigate the growth responses of major carps, which are adversely affected due to heavy loads of metals and bioaccumulation of zinc during chronic exposures to suggest measures for the rehabilitation of the aquatic habitats.

Material and Methods

The research was conducted at Fisheries Research Farms, University of Agriculture, Faisalabad. The experimental organisms were fingerlings major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* starting from the age of 90, 120, 150 and 180 days. Four age groups of three fish species were reared separately for 30-day interval under sub-lethal concentration of zinc with three replications. The fish were kept under laboratory conditions in 500 liter cemented tank for two weeks prior to the experiment. After this acclimatization period, the fish were transferred in 50 liter glass aquaria for growth trials. Five fish of each species were kept in each aquarium containing sublethal concentration of zinc. Chemically pure chloride of zinc ($ZnCl_2$) was used to prepare stock solutions for desired dilutions. In control aquarium no metal was added. The condition factors (weight-length relationship) of the fish were examined before starting the experiment. The fish in good state of health, after acclimatization period, were exposed to the following sub-lethal levels of zinc as mentioned (Table 1) for a 30-day growth trial.

Table-1: Concentration of sublethal Zinc (Mg/Litre) after 30 day growth trial

Species of fish	Sub-lethal zinc Concentrations (mg/litre)
Catla catla	10.41
Labeo rohita	13.33
Cirrhina mrigala	16.67

(Javed and Abdullah, 2003)

Fish growth studies
Prior to the experiment, initially the growth parameters viz. wet body weight, fork and total length of fingerlings were measured and recorded. After sublethal metal (zinc) exposures of 30-days for each age group, the feed intake, increase or decrease in average weight, length and feed conversion ratios were measured. During each trial, fish was feed to satiation daily (6 days a week) at 9:00 and 17:00 hours with the feed of digestible energy 2.90 Kcal g-1 (35 percent digestible protein).

Condition factors of fish were determined with the following formula:

$$K = \frac{W \times 10^5}{L^3}$$

Here,
W = wet weight in gram
L = wet fork length in millimeter
Number 10^5 is condition factor near the unity (Carlander, 1970)

Determination of Zinc in fish
A pre-weighed fish body organ samples were transferred to a beaker of 100ml and concentrated HNO_3 of 20ml was added to it. The beaker was enclosed with a watch glass and low heat was applied on a hot plate until the solid material dissolved. Perchloric acid (72%) was added @ 10 ml per sample after cooling and heated gently again initially and then vigorously until a clear, colourless solution resulted. When the volume was reduced to about 3ml, cooled and transferred through filter paper to a 50ml volumetric flask and made up the volume. The filtrate was stored in Teflon bottles for the determination of zinc concentration through Atomic Absorption Spectrophotometer (Perkin Elmer-5100) by following the method as defined in SMEWW (1989).

Statistical analysis
The data obtained from this experiment, were analyzed statistically using MSTAT software (Nissen, 1989). Analysis of variance was accomplished to find out statistical differences between variables of study.

Results

Growth performance of three fish species in chronic exposure of the Zinc
The experiment was conducted to observe the growth performance of three fish species includes *Catla catla, Labeo rohita* and *Cirrhina mrigala* reared in chronic exposure of zinc at sub-lethal concentrations for four age groups.

i. 90-day fish
Table 2 represents growth performance of 90-day fish after treatment in comparison of control group. During this trial, *Catla catla*, *Labeo rohita* and

Cirrhina mrigala gained the weights of 0.59, 1.03 and 1.05 g respectively. The average total feed intake by *Catla catla* was maximum as this fish consumed 66.95 g feed. Regarding the fork length, the positive increment was found in all three fish species. There was increase in total length in all three fish species with the highest average increment of 0.80 mm in both *Catla catla* and *Cirrhina mrigala*.

Table-2: Growth performance of 90-day fish during chronic exposure of zinc and control for 30 days

	Catla catla		*Labeo rohita*		*Cirrhina mrigala*	
	Treated	Control	Treated	Control	Treated	Control
Survival rate	100%	100%	100%	100%	100%	100%
Weight (+ or -)(g)	0.59	1.12	1.03	1.78	1.05	1.38
Feed intake	66.95	91.14	51.25	91.14	47.93	91.14
Feed conversion ratio	0.01	0.234	0.02	0.234	0.02	0.234
Fork length (mm)	0.20	0.20	0.40	0.00	0.60	0.00
Total length (mm)	0.80	1.40	0.60	0.60	0.80	1.00

ii. 120-day fish
During this trial both *Labeo rohita* and *Cirrhina mrigala* gained the average weight of 0.68 g. The total feed intake by *Catla catla* was maximum by consuming 47.99 g feed. The fork length increments were similar in both *Catla catla* and *Cirrhina mrigala* (0.60 mm) while in *Labeo rohita* it was 0.40 mm. However, total length increment was positive in all three fish species with highest increase in total length of 1.80 mm for *Catla catla* (Table 3).

Table-3: Growth performance of 120-day fish during chronic exposure of zinc and control for 30 days

	Catla catla		Labeo rohita		Cirrhina mrigala	
	Treated	Control	Treated	Control	Treated	Control
Survival rate	100%	100%	100%	100%	100%	100%
Weight (+or-)(g)	0.54	0.42	0.68	0.51	0.68	1.82
Feed intake	47.99	81.67	42.71	81.67	44.23	81.67
Feed conversion ratio	0.01	0.168	0.01	0.168	0.01	0.168
Fork length(mm)	0.60	0.20	0.40	0.60	0.60	2.40
Total length(mm)	1.80	0.40	0.40	0.60	1.20	0.60

Table-4: Growth performance of 150-day fish during chronic exposure of zinc and control for 30 days

	Catla catla		Labeo rohita		Cirrhina mrigala	
	Treated	Control	Treated	Control	Treated	Control
Survival rate	100%	100%	100%	100%	100%	100%
Weight (+or-)(g)	-2.72	0.20	-1.72	0.35	-1.52	0.74
Feed intake	59.84	48.72	53.56	48.72	56.18	48.72
Feed conversion ratio	-0.04	0.132	-0.03	0.132	-0.03	0.132
Fork length(mm)	0.00	0.20	-0.20	0.20	0.00	0.20
Total length(mm)	0.00	0.20	0.00	0.80	0.00	0.80

iii.   150-day fish

A negative growth performance was observed for the three fish species and maximum weight loss was observed in *Catla catla* i.e. - 2.72 g. The total feed intake was maximum for *Labeo rohita* by consuming 59.84g.

Fork length of *Catla catla* and *Cirrhina mrigala* did not change while the same for *Labeo rohita* decrease as - 0.20 mm. regarding the total length, there were observed no change in all the three fish species (Table 4).

iv.   180-day fish

During this growth trial a decline in weight were observed for all the three fish species. The maximum decrease was observed by *Catla catla* i.e. -1.94 g. Maximum feed consumption was observed by *Cirrhina mrigala* consuming 98.72 g. Fork length of *Cirrhina mrigala* did not change while the same for *Catla catla* and *Labeo rohita* were -0.60 and -0.20 mm respectively. *Labeo rohita* and *Cirrhina mrigala* did not show change in term of total length gain (Table 5).

Table-5: Growth performance of 180-day fish during chronic exposure of zinc and control for 30 days

	Catla catla		Labeo rohita		Cirrhina mrigala	
	Treated	Control	Treated	Control	Treated	Control
Survival rate	100%	100%	100%	100%	100%	100%
Weight (+or-)(g)	-1.94	1.42	-1.59	2.18	-1.07	2.08
Feed intake	98.44	99.11	95.17	99.11	98.72	99.11
Feed conversion ratio	-0.02	0.286	-0.02	0.286	-0.01	0.286
Fork length(mm)	-0.60	1.20	-0.20	0.40	0.00	0.00
Total length(mm)	-0.40	0.80	0.00	0.20	0.00	0.20

Analysis of variance was carried out the difference for growth parameters include weight, fork length, and total length of fish species under chronic exposure to zinc. The results of ANOVA (Table 6) on weight(g) and fork length(mm) showed significant response towards fish species, age and treatment at p<0.01. However for total length (mm) responses were significant towards fish species and age at p<0.01, whereas non-significant for treatment. Most important is that the interaction (species*treatment*age) is strongly significant at the level of 0.01, which shows that with change in any parameter there were significant differences in growth parameters.

Table-6: Analysis of variance for weight, total length and fork length effected by three fish species of four age groups in control and treated group during chronic exposure to zinc.

Source of variation	*df*	Mean square		
		Weight Length	Fork Leng	Total
Species	2	178.33**	413.49**	572.66**
Treatment	1	138.38**	731.50**	106.67
Age	3	2033.31**	3828.02**	2520.77**
Species × Treatment	2	115.39**	234.38*	77.90
Species × Age	6	30.72**	160.75**	302.83**
Group × Age	3	7.30	82.02*	160.92
Species × Treatment × Age	6	155.71**	519.47**	753.93**
Error	216	7.79	25.73	103.90

**Significant at the level of 0.01, *Significant at the level 0.05

Condition factor for three fish species during these trials varied significantly at the level of 0.01. The interaction (species x age) was significant at the level of 0.01 also. Condition factor for 180-day zinc exposed fish was 2.326 while the same lowest was 2.009 for 90-day fish (Table 7).

Table-7: Analysis of variance on condition factors (K) of three fish species under chronic exposure of zinc.

S.O.V.	Mean square	S.E.	F. Value	Probability
Fish species	0.849	0.0266	30.0614	p<0.01
Fish age	0.575	0.0307	20.3469	p<0.01
Fish species ×Fish age	0.339	0.0531	12.0153	p<0.01
Treatment	0.045	0.0217	1.6016	N.S
Error	0.028			

Accumulation of metal in body organs of fish
Table 8 displays zinc accumulation patterns in body organs of three fish species during growth trials for four age groups.

i. **90-day fish**
During 30-day trial liver was the organ which showed significantly highest accumulation of zinc as 835.00, 1100.00, 756.25 ug g^{-1} in *Catla catla Labeo rohita* and *Cirrhina mrigala* correspondingly. As far as the mean accumulation of zinc in all three fish species was concerned *Labeo rohita* displayed the maximum average accumulation (477.87± 384.12 ug g^{-1}). The differences among all the fish species were statistically significant (Table 8).

Table-8: Accumulation of zinc (ug per gm) in three fish species during chronic exposure

Fish organs	Fish species		
	Catla catla	*Labeo rohita*	*Cirrhina mrigala*
90-day fish			
Gills	193.02	318.97	176.15
Kidney	481.90	803.57	686.36
Liver	835.00	1100.00	756.25
Skin	150.80	166.27	242.24
Muscle	65.08	174.54	219.81
Scales	125.73	303.85	356.36
Mean ±S.D.	308.59±296.02 c	477.87±384.12 a	406.19±252.21 b
120-day fish			
Gills	190.24	304.72	462.88
Kidney	243.10	483.55	669.01
Liver	1313.10	515.50	1105.66
Skin	182.64	111.15	271.97
Muscle	115.07	113.70	128.96
Scales	116.37	286.88	314.37
Mean ±S.D	360.09±469.40 b	302.58±173.58 c	492.14±352.28 a

Means values with similar letters indicates statistical similarity at the level of 0.05.

ii. **120-day fish**
Both liver and kidney appeared as organs for maximum zinc accumulation while the same were minimum for muscle in three fish species. *Cirrhina mrigala* of this age group showed maximum tendency for zinc accumulation (Table 8).

iii. **150-day fish**
Liver of 150 day *Labeo rohita* showed the highest accumulation of zinc (1834.53 ug g^{-1}). The muscle of all three fish species shows the lowest levels of zinc accumulation. However, the mean accumulation patterns in three fish species varied significantly with the highest concentration (594.01±722.32 ug g^{-1}) of *Labeo rohita* (Table 9).

iv. **180-day fish**
Catla catla and *Cirrhina mrigala* were the species that showed maximum accumulation of zinc in liver while kidney of *Labeo rohita* appeared as organ that concentrate maximum zinc as 793.62 ug g^{-1}. However, fish muscle showed minimum accumulation of zinc as 130.50, 96.19, 46.08 ug g^{-1} in *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* correspondingly (Table 9).

Table-9: Zinc Accumulation (ug per gm) in three fish species under chronic exposure

Fish organs	Fish species		
	Catla catla	*Labeo rohita*	*Cirrhina mrigala*
150-day fish			
Gills	165.78	164.30	162.80
Kidney	625.64	1126.13	716.50
Liver	1011.90	1834.53	1329.00
Skin	190.33	167.44	422.50
Muscle	98.11	126.42	138.54
Scales	297.80	145.22	425.44
Mean ±S.D.	398.26±353.76 c	594.01±722.32 a	532.46±443.56 b
180-day fish			
Gills	159.86	215.28	168.86
Kidney	397.45	793.62	512.82
Liver	1286.65	610.83	1023.10
Skin	148.23	113.73	199.58
Muscle	46.08	96.19	130.50
Scales	122.56	185.93	153.86
Mean ±SD	360.14±469.02 b	335.93±292.90 c	364.79±352.24 a

Means values with similar letters indicates statistical similarity at the level of 0.05.

Discussion

During the course of the experiments three fish species in the treated group showed a reduction in their growth at all age groups in comparison to the control. Fish growth was found reduced significantly in Nile tilapia when exposed to sublethal concentration of Zinc (Abdel-Tawwab et al., 2016). Heavy metal intoxication was found to results in toxic impacts and ecological damages (Moon et al., 2019). Feed intake and fish productivity decreases in the presence of heavy metals (Sfakianakis et al.,

2015). The adrenergic responses consequences in the raise of circulating adrenaline which organizes the body for exercise by increasing ventilation rate, cardiac output and blood flow to muscle, and mobilizing substrates aimed at aerobic metabolism, and second common reaction to stress is the reduced growth. Perhaps this is not surprising that metals can cause reduction in growth (Elling et al., 1999). *Cirrhina mrigala* and *Labeo rohita* showed better growth performance when compared with fish of all four age groups i.e. *Catla catla* showed higher sensitivity towards zinc contamination as far as growth performance is concerned. khanom et al. (2020) studied muscular tissue bioaccumulation of heavy metals including Pb, Cd, Cr, Zn, Cu and Mn in two fish species (*Gudusia chapra* and *Eutropiichthys vacha*) and found that Zn was at a higher quantity, while Cd at a lower amount in both species in Padma River.

During chronic exposure to zinc for all trials, bioaccumulation of zinc occurred maximum in liver for all fish age group except 180-day *Labeo rohita* and lowest concentration of zinc observed in muscle and as described by Zhao et al that higher accumulation of metals in liver related to its function of metabolism that required to maintain life (Zhao et al., 2012), which is in confirmatory to following results. Abdel-Tawwab et al. (2016) also found that Zinc was found maximum in liver and kidney which was lowest in muscle. Alvarado et al. (2021) determined the heavy metal concentrations in the organs/tissues of Carp and Catfish cultured in Lake Chapala and found that liver had a higher concentrating capacity for Zn, Cu, Cd and Pb were highest in liver and muscle tissue. According to Bervoets et al. (2001) concentrations of zinc, lead, copper, and cadmium was measured in food, water, sediment, and *Gasterosteus aculeatus* tissues. Metal accumulation level in tissues was associated to the levels in water, sediment and food whereas gill/liver showed highest metal accumulation as compared to muscle.

Conclusion

Regarding metal toxicity, Zinc affect growth parameters (weight increment, fork lengths and condition factors) significantly ($P<0.01$). The control fish showed better feed conversion ratio as compared to that of zinc exposed fish. Liver showed significantly higher tendency to Zinc accumulation and significantly least trend in muscle than other organs of three fish species. *Labeo rohita* showed significantly higher trend to accumulation of zinc in body organs followed *Cirrhina mrigala* and *Catla catla*. It is concluded that metal intoxication significantly reduced the growth of all three fish species and may also show good indicator of metals present in surrounding environment.

Acknowledgement

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Contribution of Authors

Malik IR: Performed research work, data collection, data analysis and manuscript write up
Nayyab S, Cheema JS, Imran K & Nisar K: Helped in editing and write up of manuscript
Mirza MR, Muzammil S & Hayat S: Helped in editing of manuscript and literature review
Javed M: Conceived the idea and designed research methodology