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Growth Performance of Metals Mixture Stressed Fish in Earthen Ponds

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ABSTRACT

The present research was undertaken to elucidate the effects of sub-lethal concentrations of metals (Zn+Pb+Mn) mixture on the growth performance of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla*. Fish were given sub-lethal stress under controlled laboratory conditions, separately, for a period of 90 days and then they were transferred to the semi-intensive ponds. The growth of five metals mixture stressed fish species was monitored for a period of 14 fortnights in earthen ponds and compared with the control group. Data were collected on fortnightly basis to check the growth performance of each fish species in terms of average wet weight, fork and total length increments, specific growth rates and feed conversion ratios under the composite culture system. Highly significant differences at $p<0.01$ were observed for average wet weight (g), fork and total length (mm) increments in metals mixture stressed fish as compared to control group and also among five fish species. The control fish showed significantly better specific growth rate than that of metals mixture stressed fish. Due to varying growth potential, species specific variations were found among all fish species for specific growth rates. Among all the mixture stressed fish species reared in earthen ponds, maximum increase in average wet weight and also specific growth rate were shown by *Cirrhina mrigala*, whereas, *Ctenopharyngodon idella* exhibited maximum average fork and total length increments, respectively. However, statistically higher average wet weight increment and specific growth rate were found as 611.75±1.52 g and 291.31±2.26 for *Labeo rohita* reared in control pond. Among all the five control fish species, the maximum average fork and total length increments were shown by *Cirrhina mrigala*. Highly significant differences were recorded for feed conversion ratios of stressed and control fish ponds. The feed conversion ratio of metals mixture stressed fish pond was lower (1:7.94) in comparison to the control fish pond, for which it was computed as 1:8.44. Feed conversion ratios of ponds varied significantly among various fortnights of the study period.

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SA executed experimental work analyzed the data and wrote the article. MJ supervised the work.

Key words
Metals mixture stress, Semi-intensive ponds, zinc, lead, manganese, *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita*, *Catla catla*

INTRODUCTION

Among various pollutants of aquatic ecosystems, heavy metals are specifically harmful in their mode of action due to their propensity of bio-magnification in food chain. Sewage disposal, wastes from industries and soil leaching are believed to be the major causes for upsetting the natural aquatic ecosystems. A tremendous use of fertilizers based on metal compounds in agricultural uprising has also resulted in progressive increase in the metallic ion toxicity in fresh waters due to run-off events into the aquatic bodies (Prasath and Arivoli, 2008). Therefore, contamination of freshwater ecosystems with a wide range of pollutants has now become a subject of debate in the world (Rauf *et al.*, 2009). On the ecological stability of the natural aquatic bodies, metal contamination may cause destructive effects and hence interrupting the density and diversity of aquatic fauna (Hayat and Javed, 2008). Fish could be used as discriminative bioindicators of trace metals, as they not only amass metals in their bodies but also contribute towards water contamination by creating changes in their various essential functions (Dobrowolskir and Skowronska, 2006). Even at less concentrations, heavy metals can adversely affect the life of fish, resulting into several forms of interruptions in its health status and well-beings (Vosyliene and Jankaite, 2006). Water-borne heavy metals are very much noxious to the early stages of development of fish (Kazlauskiene and Vosyliene, 2008). In addition, sub-lethal concentrations of different metals in water can lessen the fish growth due to obstruction of digestive tract development or loss of appetite due to stressful conditions exerted by metals (Stasiunaite, 2005).

Fish growth rate is very susceptible to environmental factors and is highly variable. Thus, growth rate measurements can be used to get knowledge on the performance of fish. Fish growth is related to alterations in morphometric traits, biochemical and chemical body configuration (Rowe, 2003). Any type of interruption in utilization of food or energy production as influenced by toxicants, such as heavy metals, would be indicative of fish growth rates (Hansen *et al.*, 2002). To

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observe the bioavailability of water-bound metals, carps could act as suitable detecting organisms in freshwater habitats. *Cirrhina mrigala*, *Catla catla*, *Labeo rohita*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are the most cultured species of polyculture because of appropriate weather conditions in Pakistan for their rearing on one hand and consumer preference on the other. However, the production of carps in inland waters has been declined due to reduction in their growth capability susceptible to heavy metals contamination.

Zinc, lead, manganese, iron and nickel are pervasive, naturally existing metals that are related to the industrial and metal mining activities (Abdullah *et al*., 2007). The effects of heavy metals depend on the mobility of each metal through environmental compartments and the pathways by which metals reach aquatic ecosystem and humans. Therefore, real concentrations of zinc, lead and manganese in the natural waters of Pakistan vary with area, site and location. The concentrations of these metals in river and tributary waters were significantly higher than the safe limits described by USA and Pakistan Environmental Protection Agency (2008), for sustainable conservation of aquatic habitats. In most of the ecotoxicological researches, single metal effects on fish have been assessed while studies on fish biological responses towards the heavy metals mixture are scanty (Kazlauskiene and Burba, 1997). However, these studies have revealed that, the impact of metal mixtures can vary in their toxicity to fish from the single metal effects. The impact of two or more metals mixtures may be synergistic, additive or antagonistic (Jezierska and Witeska, 2001). In our natural riverine systems, heavy metals are found in the form of mixtures, not as single metal. Hence, it is vital to examine the growth performance of fish, as a result of heavy metals mixture stress, which are on the verge of extinction in the rivers of Pakistan especially Punjab. Therefore, this study was conducted to evaluate the growth performance of metals (Zn+Pb+Mn) mixture stressed fish under semi-intensive pond culture system.

MATERIALS AND METHODS

Metals mixture stress under laboratory conditions

The research project was conducted at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. The fingerlings (90-day age) of five fish species viz. *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* were purchased from local outlet, brought to the laboratory and acclimatized for ten days in cemented tanks. Healthy fingerlings of similar weight and size of each fish species were selected, stocked in glass aquaria and divided into two groups. In each group, the total fish density was kept as 65, while the stocking density of all the five fish species viz. *H. molitrix*, *C. idella*, *C. mrigala*, *L. rohita* and *C. catla* was 7, 10, 13, 26 and 9, respectively. Chemically pure chloride compounds of zinc ($ZnCl_2$), lead ($PbCl_2$) and manganese ($MnCl_2.4H_2O$) were dissolved separately in deionized water for stock solutions, while for mixture preparation, stock solutions of these metals were mixed on ion equivalence basis (1:1:1). One group of fish was exposed to sub-lethal ($1/3^{rd}$ of LC_{50}) concentrations of Zn+Pb+Mn mixture regarded as "stressed group", while the other group was kept in metal free water regarded as "control group". Each species of fish *viz. H. molitrix*, *C. idella*, *C. mrigala*, *L. rohita* and *C. catla* were exposed to sub-lethal concentrations of 17.85, 18.84, 18.21, 21.03 and 18.59 mgL^{-1}, respectively as determined by Javed and Yaqoob, 2011 in aquaria, separately, for 90 days. The aquaria washed and solutions were changed on weekly basis. The whole experiment was conducted at constant temperature (28ºC), pH (7.50) and total hardness (225 mgL^{-1}) of water. Constant aeration was provided to all the test media with an air pump fixed with a capillary system. Fish were fed with pelleted feed (32% digestible protein and 3.00 Kcal g^{-1} of digestible energy) twice a day until satiation.

Growth studies in semi-intensive pond culture system

The Zn+Pb+Mn mixture stressed and control fish were stocked in earthen ponds (0.012 ha), separately, at the rate of 65 fish per pond with the stocking density of 10, 15, 20, 40 and 15 percent for *H. molitrix*, *C. idella*, *C. mrigala*, *L. rohita* and *C. catla*, respectively. Both stressed and control fish ponds were fertilized with broiler droppings at the rate of 0.17 g nitrogen per 100 g fish weight daily, in order to promote the pond biota for fish consumption. The fish were also provided supplementary feed, at the rate of three percent of their wet body weight initially and then decreased gradually with decline in water temperature. However, no feed was offered to the fish at water temperature below 25 ºC. The fish were grown in earthen ponds for 14 fortnights. From each pond, random sampling (n=5) of each fish species was done by using drag net, on fortnightly basis, and their wet weights (g), fork and total lengths (mm) were measured and recorded. After recording the data, fish were released back into their respective ponds.

The following growth parameters of each fish species were monitored on fortnightly basis: increase in wet weights; fork lengths and total lengths, specific growth rates and feed conversion ratios of ponds.

Specific growth rates (SGR) and feed conversion ratios (FCR) were computed by using formulae:

SGR = Increase in wet weight×100/time duration

and

FCR = 1 g feed given: Fish weight increment in g

where,

Feed given = Total fish biomass × feeding rate / 100

Statistical analyses of data

The statistical differences among different parameters of growth were analyzed through Factorial Experiments (RCBD) by following Steel *et al*. (1996). Tukey's/Student Newman-Keul tests were used for the comparison of mean values. The computer packages *i.e.* MSTATC and MICROSTAT were used for these analyses.

RESULTS

The experiment was conducted to evaluate the growth performance of Zn+Pb+Mn mixture stressed fish species *viz. Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* in semi-intensive ponds, under metal free environment.

Wet weight (g)

Table I shows analysis of variance on average increase in wet weights of both stressed and control five fish species under semi-intensive pond culture system. Statistically highly significant ($p<0.01$) differences were observed between the stressed and control fish as well as among five fish species for their average wet weight increments. The control fish showed significantly better (561.08±12.28 g) growth in terms of average increase in wet weight than that of metals mixture stressed (367.04±7.90 g) fish. However, among the five stressed fish species, *Cirrhina mrigala* attained significantly higher (424.98±1.18 g) whereas, *Ctenopharyngodon idella* exhibited lower (342.12±1.51 g) average wet weight increments. The control fish species showed average wet weight increments of 486.00±1.18 (*H. molitrix*), 543.42±2.61 (*C. idella*), 558.47±2.32 (*C. mrigala*), 611.75±1.52 (*L. rohita*) and 605.76±1.80 g (*C. catla*), respectively.

Fork length (mm)

The average fork length increments of stressed and control fish exhibited statistically highly significant variations at $p<0.01$ (Table I). The Zn+Pb+Mn mixture stressed fish exhibited lower average fork length increment as 282.66±4.41 mm than the control fish, for which the same was recorded as 414.72±9.99 mm. All the five fish species showed statistically highly significant variations for average increase in their fork lengths. The fish growth regarding average fork length increments in metals mixture stressed pond followed the order: *C. idella* > *L. rohita* > *C. mrigala* > *C. catla* > *H. molitrix*. However, the maximum and minimum average fork length increments were recorded as 473.95±2.82 and 357.29±1.34 mm for *C. mrigala* and *H. molitrix*, respectively, reared in control pond.

Total length (mm)

Statistically highly significant ($p<0.01$) differences were observed between stressed and control fish and also among five fish species for their average total length increments (Table I). The control fish showed higher (433.83±9.61 mm) average total length gain as compared to the metals mixture stressed (302.10±4.71 mm) fish, in semi-intensive ponds. Among the metals mixture stressed five fish species, minimum and maximum average total length increments were shown by *H. molitrix* and *C. idella*, respectively. In the control fish pond, *Cirrhina mrigala* gained higher average total length increment of 489.63±2.28 mm, while *H. molitrix* exhibited significantly lower average increase in its total length, which was recorded as 377.01±2.30 mm.

Specific growth rates (SGR)

Analysis of variance on specific growth rate revealed that there existed statistically highly significant differences at $p<0.01$ between stressed and control fish, as well as among all the five fish species. In semi-intensive ponds, the control fish exhibited higher (267.18±5.91) specific growth rate as compared to Zn+Pb+Mn mixture stressed fish (174.78±3.84). Among all the five metals mixture stressed fish species in pond, *Cirrhina mrigala* exhibited significantly better specific growth rate as compared to the other fish species. However, in control pond, the specific growth rates of fish followed the order: *L. rohita* ≥ *C. catla* > *C. mrigala* > *C. idella* > *H. molitrix* (Table I).

Feed conversion ratios (FCR)

Table II shows analysis of variance on feed conversion ratio of ponds that revealed highly significant ($p<0.01$) differences between metals mixture stressed and control fish. The feed conversion ratio of control fish pond was significantly higher (1:8.44) as compared to the metals mixture stressed fish pond, for which it was computed as 1:7.94. Also there existed statistically highly significant differences at $p<0.01$ among various fortnights of the study period. The feed conversion ratio of metals mixture stressed fish pond was significantly higher during 1^{st} fortnight as 1:23.36 at a water temperature of 37.22°C, while it was lower during 10^{th}

Table I. Average increase in wet weights, fork lengths, total lengths and specific growth rates of five fish species.

	Hypophthalmichthys molitrix	*Ctenopharyngodon idella*	*Cirrhina mrigala*	*Labeo rohita*	*Catla catla*	*Overall means±SD
Average increase in wet weights (g±SD)						
Stressed fish	353.76±0.91 **b**	342.12 ±1.51 **c**	424.98±1.18 **a**	357.83±1.46 **b**	356.49±1.43 **b**	**367.04±7.90 b**
Control fish	486.00±1.18 **e**	543.42±2.61 **d**	558.47±2.32 **c**	611.75±1.52 **a**	605.76±1.80 **b**	**561.08±12.28 a**
Average increase in fork lengths (mm±SD)						
Stressed fish	258.43±1.03 **e**	303.19±1.72 **a**	286.01±1.52 **c**	295.27±2.29 **b**	270.39±1.75 **d**	**282.66±4.41 b**
Control fish	357.29±1.34 **e**	406.18±2.92 **d**	473.95±2.82 **a**	414.68±2.30 **c**	421.50±1.82 **b**	**414.72±9.99 a**
Average increase in total lengths (mm±SD)						
Stressed fish	279.29±0.63 **e**	326.18±1.14 **a**	306.06±1.67 **c**	313.62±1.76 **b**	285.35±2.24 **d**	**302.10±4.71 b**
Control fish	377.01±2.30 **d**	429.89±2.91 **c**	489.63±2.28 **a**	432.16±1.17 **c**	440.44±2.90 **b**	**433.83±9.61 a**
Specific growth rates (±SD)						
Stressed fish	168.46±0.60 **b**	162.91±1.13 **c**	202.37±1.97 **a**	170.40±2.90 **b**	169.76±2.91 **b**	**174.78±3.84 b**
Control fish	231.43±2.63 **d**	258.77±3.03 **c**	265.94±2.32 **b**	291.31±2.26 **a**	288.46±1.74 **a**	**267.18±5.91 a**

Means with similar letters within a single row and *column are statistically non-significant at $p<0.05$.

fortnight as 1:3.31 at 28.00ºC water temperature, respectively. The maximum and minimum feed conversion ratios shown by the fish reared under control pond were 1:24.97 and 1:3.35 during 1st and 6th fortnights of the study period, respectively. At the end of growth trial, the total increase in fish biomass of Zn+Pb+Mn mixture stressed fish pond was computed as 23936.98 g while in control fish pond it was computed as 37573.41 g.

DISCUSSION

The essential and non-essential metals present in water are acquired by the fish via different routes. However, the tendency of metals to bio-accumulate in the fish vary significantly from species to species due to their inherent potential to tolerate against various metals (Kousar and Javed, 2014). The elevated concentrations of zinc, could induce acid-base disturbances, hypoxia, growth retardation and eventually death of fish (Hogstrand *et al*., 1994). Lead is present in water bodies in a broad range of chemical and physical forms that could adversely influence the fish behavior (Jackson *et al*., 2005). Digestive enzymes liberation and the net weight gain severely affected as a result of lead exposure to the fish (Jain *et al*., 1996). An increased manganese concentration may have chronic impacts on the growth performance of fish (Partridge and Lymbery, 2009). However, the metals occur in the form of various mixtures in aquatic environments leading to multiple responses in fish caused by the synergistic or antagonistic effects of single metals in the mixture (Ambreen *et al*., 2015). The sub-lethal metals mixture exposure can cause various behavioural, developmental and physiological alterations in fish leading to their reduced growth potential (Syvokiene *et al*., 2003). It was concluded that, under the semi-intensive pond culture system, the control fish exhibited significantly higher growth regarding their average wet weight, fork and total length increments as compared to Zn+Pb+Mn mixture stressed fish. Vosyliene *et al*. (2003) investigated the impact of a model mixture of heavy metals (Cu+Zn+Ni+Cd+Pb+Mn) on *Oncorhynchus mykiss*. They concluded that, the growth of fish was significantly altered due to long-term exposure of sub-lethal concentrations of metals mixture. Ramesha *et al*. (2003) also reported that Hg+Cd mixture caused a chronic stress to the fish that eventually altered the growth rate of *Cyprinus carpio*. A significant reduction in the growth of *Cirrhina mrigala* was assessed by Javed (2015a), when the fish was subjected to chronic mixed (waterborne+dietary) exposure of metals, as compared to the control fish. The polluted fish most oftenly showed minimum values for their weight and length estimations. These lowered values of weight and length increments were due to the heavy metal toxicants that exist in naturally contaminated environs (Eastwood and Couture, 2002). An experiment on *Cirrhina mrigala*, stressed with sub-lethal metals (Mn+Zn+Pb+Ni+Fe) mixture was conducted by Hussain *et al*. (2010) in order to monitor the growth performance of fish in earthen ponds. They observed that *Cirrhina mrigala* kept under controlled environment exhibited higher average wet weight, fork and total length increments as compared to metals mixture stressed fish, in semi-intensive ponds. Their results are parallel to the present findings as significant reductions in average wet weights, fork and total lengths of Zn+Pb+Mn mixture stressed fish were

Table II. Growth performance of fish.

Fortnight #	Water temperature (˚C)	Total fish biomass (g)	Increase in biomass (g)	Feeding rate (%)	Feed given (g)	FCR
Stressed						
Initial	37.22±2.48	1612.95	-	3	48.39	-
1	37.18±2.52	5393.50	3780.55	3	161.81	1:23.36 **a**
2	36.20±2.35	8528.96	3135.46	3	255.87	1:12.25 **b**
3	35.51±2.27	11104.80	2575.84	3	333.14	1:7.73 **c**
4	34.87±2.09	13113.13	2008.33	2	262.26	1:7.66 **c**
5	33.06±2.03	14490.91	1377.78	2	289.82	1:4.75 **e**
6	33.48±1.95	15893.91	1403.00	2	317.88	1:4.41 **f**
7	32.92±1.82	17280.90	1386.99	2	345.62	1:4.01 **g**
8	32.67±1.79	17908.14	627.24	1	179.08	1:3.50 **h**
9	31.54±1.74	18822.51	914.37	1	188.23	1:4.86 **e**
10	29.00±1.68	19467.57	645.06	1	194.68	1:3.31 **h**
11	26.60±1.34	21133.47	1665.90	1	211.33	1:7.88 **c**
12	24.62±1.04	22507.09	1373.62	1	225.07	1:6.10 **d**
13	21.59±1.09	23850.48	1343.39	-	-	-
14	20.11±1.22	25549.93	1699.45	-	-	-
Total			23936.98		3013.18	**1:7.94**
Control						
Initial	38.18±2.61	1743.53	-	3	52.31	-
1	37.56±2.58	6951.51	5207.98	3	208.55	1:24.97 **a**
2	36.83±2.25	11240.58	4289.07	3	337.22	1:12.72 **b**
3	35.78±2.39	15395.38	4154.80	3	461.86	1:9.00 **d**
4	34.19±2.14	19222.56	3827.18	2	384.45	1:9.95 **c**
5	33.44±2.07	22388.13	3165.57	2	447.76	1:7.07 **e**
6	32.71±1.98	23996.42	1608.29	2	479.93	1:3.35 **i**
7	32.93±1.93	26048.18	2051.76	2	520.96	1:3.94 **h**
8	31.99±1.85	27496.12	1447.94	1	274.96	1:5.27 **g**
9	32.34±1.72	29135.68	1639.56	1	291.36	1:5.63 **f**
10	30.85±1.68	30884.45	1748.77	1	308.84	1:5.66 **f**
11	27.65±1.27	33145.19	2260.74	1	331.45	1:6.82 **e**
12	25.63±1.35	35078.90	1933.71	1	350.79	1:5.51 **fg**
13	24.44±1.18	37182.28	2103.38	-	-	-
14	19.87±1.06	39316.94	2134.66	-	-	-
Total			37573.41		4450.44	**1:8.44**

Means with similar letters in a single column are statistically non-significant at $p<0.05$.
+ FCR = Feed Conversion Ratio

observed as compared to the control fish, in semi-intensive ponds. The results of present experiment are in line to the findings of Naz *et al.* (2013), who evaluated the species specific growth responses of *Labeo rohita*, *Catla catla*, *Cirrhina mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*, under sub-lethal (1/3rd of LC_{50}) exposure concentrations of Zn+Pb+Mn mixture. They observed that among five mixture stressed fish species, *C. mrigala* attained significantly maximum wet weight increment while *L. rohita* gained higher wet weight increment in control environment.

During present investigation, the specific growth rate shown by the control fish was higher as compared to the metals mixture stressed fish, under semi-intensive pond culture system. These results are in accordance to Vosyliene (2002) who reported that when *Oncorhynchus mykiss* was exposed to heavy metals model mixture (Mn+Cd+Pb+Cr+Ni+Zn+Cu), a lesser specific growth rate of the fish was recorded. Collins *et al.* (2001) also reported a decline in specific growth rate of rainbow trout when subjected to sub-lethal copper concentrations. Tsai and Liao (2006) also demonstrated that specific growth rate of *Oreochromis mossambicus* (tilapia) was inversely proportional to the concentrations of arsenic. The copper exposed *Clarias gariepinus* (African walking catfish) showed a decline in specific growth rate as compared to the control fish (Hoyle *et al*., 2007). Regarding specific growth rates, there also existed species specific differences among all the five species in both control as well as stressed fish ponds. The present results showed that in Zn+Pb+Mn mixture stressed fish pond, *C. mrigala* exhibited better specific growth rate as compared to the other fish species while in control fish pond, *L. rohita* and *C. catla* attained higher specific growth rates among five fish species with statistically non-significant differences between them. These results are in confirmation to the findings of Javed (2015), when he reared Zn+Pb+Ni mixture stressed *Cirrhina mrigala*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla* under composite pond culture conditions.

The feed conversion ratio of metals mixture stressed fish pond was lesser than the control fish pond, during present experiment. It was observed that as a result of metals mixture stress, the feed intake of fish declines. Hence, the feed conversion ratio of the metals mixture stressed fish also becomes reduced. The results of Hansen *et al*. (2004) are parallel to the present findings, as reduced feed conversion ratio of rainbow trout was observed under the stress of metals mixture. Ameer e*t al*. (2013) estimated low feed conversion ratios of *Catla catla* and *Labeo rohita* under sub-lethal dual exposure of heavy metals. Hussain *et al*. (2011) reported that, in comparison to control (metal free) environment, a decline in feed conversion ratios were observed for major carps, when they were subjected to sub-lethal concentrations of iron.

CONCLUSIONS

The Zn+Pb+Mn mixture stressed fish reared in earthen ponds exhibited statistically lower average wet weight, fork and total length increments as well as specific growth rates in comparison to the control fish. There also existed species specific variations, due to varying growth potential, among all the five fish species grown in both metals mixture stressed and control fish ponds. The fish, *Ctenopharyngodon idella* was found more sensitive toward metals mixture intoxication and hence revealed lesser growth in semi-intensive pond culture system. However, *Cirrhina mrigala* was found more tolerant towards Zn+Pb+Mn mixture toxicity as it showed higher growth in terms of average wet weight increment and specific growth rate in earthen pond. In control fish pond, maximum and minimum average increase in wet weights and specific growth rates were exhibited by *Labeo rohita* and *Hypophthalmichthys molitrix*, respectively. The feed conversion ratio and increase in fish biomass of Zn+Pb+Mn mixture stressed pond was found lower as compared to control fish pond.

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