Iranian Journal of Fisheries Sciences 21(2) 605-618 2022
DOI: 10.22092/ijfs.2022.126708

Research Article

Metals mixture effects on growth performance and their bioaccumulation in fish

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Received: January 2019 Accepted: July 2020

Abstract

A study was conducted to determine the growth performance and bioaccumulation and tolerance of metal mixture toxins in five fish species including *Labeo rohita, Catla catla*, *Cirrhina mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* separately, under chronic exposure of metal mixture (Fe + Zn + Pb) at sub-lethal concentrations (1/3 of LC_{50}) for 12 weeks period. Moreover, response of different body organs of fish towards bioaccumulation of metals under metals mixture stress was also determined. It was found that the relationships among growth parameters of metals mixture stressed as well as unstressed (control) fish were significantly different. Results showed that, the sub-lethal exposure of metals mixture induced substantial impacts on the wet weight gain (g) and length (mm) of fish species, with following trend; *C. catla*> *C. idella*> *C. mrigala*> *L. rohita* >*H. molitrix*. However, growth rate of fish under control treatment (no stress) was significantly higher compared to the fish exposed to metals mixture. It was also observed that the condition factor was positively correlated with fish growth and expressed the extent of fish well-beings. The bioaccumulation pattern in the body organs was observed with the following trend: liver> kidney> gills> skin> fins > muscles> bones. This study concluded that metal mixtures seriously affect the growth and development of aquatic organisms.

Keywords: Fish, Heavy metals, Chronic toxicity, Sub-lethal metal concentration, Growth response, Fe + Zn + Pb mixture

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Introduction

From last several decades, rapid population growth, economic development, urbanization, agriculture development and industrial revolution has disturbed the natural balance of biogeochemical cycles and ultimately have prompted the exploitation of toxic metals in the surroundings of human being (Ali and Khan, 2018a; Rajeshkumar *et al.*, 2018; Schuler and Relyea, 2018). Freshwater bodies mainly lakes, rivers and streams are exposed to water pollution due to several anthropogenic endeavors and have created concerns about the water quality (Yi *et al.*, 2017; Al-Imarah *et al.*, 2018). In developing countries, most of the water bodies have become polluted due to the discharge of untreated industrial and urban wastes (Jabeen and Javed, 2011; Islam *et al.*, 2017). In Pakistan, as a developing country, the water quality of inland water bodies especially in Punjab province is downgrading continuously as untreated discharge of industrial wastes, domestic and agriculture runoff is directly or indirectly being disposed-off in open freshwater bodies (Javed and Mahmood, 2001; Javed, 2012).

Pollution of freshwater ecosystems and subsequently aquatic fauna mainly fish with heavy metals is a stern ecological issue. Fish has a high trophic level in aquatic food chain so can relatively bioaccumulate high concentrations of heavy metals in body (Siraj *et al.*, 2014; Ali and Khan, 2018c, b) that ultimately result in trophic transfer of these metals in the human food chains. A well-known example of trophic transfer of mercury to human beings by the consumption of mercury contaminated fish caused the Minamata disease, a major environmental disaster of the 1950s in Japan (Ali and Khan, 2017). Fish is an important part of human food chain, so there is a dire need to regularly monitor the freshwater fish for heavy metal contamination (Islam *et al.*, 2017; Ali and Khan, 2018c; Pal and Maiti, 2018). Metal pollutants can enter into the fish body either directly through the skin and gills from the ambient water and sediments (abiotic environment) or indirectly from its food through the gut and ultimately accumulate in various tissues (David *et al.*, 2012; Ali and Khan, 2018a, c).

Iron is an essential micronutrient as it is an integral component of proteins involved in oxygen transfer during cellular respiration. Fish need a balance uptake of iron to avoid the effects due to deficiency and potential toxicity. Lead is found in a wide range of both chemical and physical forms in aquatic environment that affect the fish determinately if its concentration is higher than endorsed limits. Inorganic form in oxidized states is the most commonly occurring form of lead in the aquatic environment (Jackson *et al.*, 2005). Zinc could be readily accumulated in fish organs from contaminated water. Zinc is also an important micronutrient as it requires in the body for normal function of an immune system. With aging the response of immune becomes impaired

[DOR: 20.1001.1.15622916.2022.21.2.13.1]

which may be partly associated with changes related to Zn deficiency (Ibs and Rink, 2003).

The toxicity effects of metals combination can diverge from single metal on living organisms. Precise mixture composition, concentration and time interval of fish exposure determines the metal mixture toxicity (Vosylienė *et al.*, 2003; Javed, 2012). Considering the detrimental properties of heavy metals and their combination in the water ecosystems, the present study was conducted to determine the effect of metals mixture (Fe+Zn+Pb) on some fish species including *Hypophthalmichthys molitrix, Ctenopharyngodon idella, Labeo rohita, Catla catla,* and *Cirrhina mrigala* in terms of growth performance and bioaccumulation in fish body under laboratory conditions to predict remedied measures for sustainable conservation of these fish species in Pakistan.

Materials and methods

The study was conducted in Zoology Laboratory, Department of Zoology and Fisheries, The University of Agriculture, Faisalabad, Pakistan. Fingerlings of fish species (*C. catla*, *L. rohita*, *C. mrigala*, *C. idella*, and *H. molitrix*) were obtained from Fish Seed Hatchery, Faisalabad and kept under laboratory condition for a period of 12 weeks to acclimatize prior to start the experiment. The initial total length (mm), fork length (mm) and average wet weight (g) of each fish species were measured prior to the experiments (Table 1). Stock solutions of metals required for desired metals mixture (Fe+Zn+Pb) were prepared by chemically dissolving the chlorides of each pure metal in deionized water, separately. Experiments were run by dividing each fish species into two groups; one unstressed group as a control and other stressed group was exposed to already determined sub-lethal metals mixture concentrations in a ratio of (1:1:1) according to Yaqub and Javed (2012) (Table 2).

Table 1: Initial wet weight, total length, and fork length (Mean±S.D) of fish species.

Fish Species	Weight (g)	Total length (mm)	Fork length (mm)
Catla catla	3.29±1.84	65.89±12.08	57.09±11.85
Labeo rohita	5.64±2.02	84.16±11.23	75.07±11.83
Cirrhina mrigala	4.51±2.01	81.57±11.37	72.92±11.81
Ctenopharyngodon idella	4.09±1.64	80.08±12.02	70.34±12.26
Hypophthalmychthys molitrix	3.80±11.98	74.83±11.74	65.03±11.52

Table 2: Sub-lethal concentrations (mg L^{-1}) of metals mixture (Fe+Zn+Pb) exposed to the five fish species.

Fish Species	Concentrations (mg L^{-1})
Catla catla	28.65±2.51
Labeo rohita	31.88±2.41
Cirrhina mrigala	24.84±2.34
Ctenopharyngodon idella	30.70±1.24
Hypophthalmychthys molitrix	23.01±2.20

During growth experiments on studied fish species, the exposure media were restocked partially in glass aquaria in order to regulate the required sub-lethal concentration of metals mixture. Stressed groups having ten specimens of each species were exposed to sub-lethal concentrations of metals mixture, separately in the aquaria containing 60-litre water for 12 weeks. The growth experiments (12-week) of each fish species were controlled with three times repetitions. Fish was fed to satiation twice a day with feed having composition of 290 kcal g^{-1} digestible energy and 35% digestible protein. The growth parameters including feed intake, total and fork lengths, wet weight, condition factors and feed conversion effectiveness were observed on weekly basis. Mortality rate was zero for each of fish species in the whole duration of growth experiments under the chronic stress of metals mixture.

Fish organs i.e. liver, kidney, bones, gills, skin, muscle and fins were separated and digested out in perchloric acid ($HClO_4$) and nitric acid mixture (HNO_3) in 1:3 V/V ratio by following APHA (1989) at the end of 12-week growth experiment to explore the accumulation patterns of heavy metals mixture in different fish organs.

Concentrations of iron, zinc and lead in different fish body tissues were determined by Atomic Absorption Spectrophotometer (Analyst-400, Perkin Elmer). The physico-chemical parameters of water quality such as dissolved oxygen, water temperature, total ammonia, total hardness, potassium and sodium were checked by following the methods of APHA (1998) on daily basis (Table 3).

The condition factor (K) and feed conversion efficiency (FCE) were calculated according to the following equations.

Equation *I*: $K = (W \times 10^5) \div L^3$

Where K = Condition factor; W = Wet fish weight (g); L = Wet fork length (mm)

Equation *II*: $FCE = \left({W_i}/{F_i} \right) \times 100$

Where FCE = Feed conversion efficiency; W_i = Increase in weight (g); F_i = Feed intake (g)

Statistical analysis

The method of Steel *et al.* (1996) was implemented by using Micro-computer to statistically analyze the data collected on water quality and fish growth parameters. For the evaluation of mean values for different parameters and significance of their mean values, the one-way analysis of variance (ANOVA) and Newman-Keul tests were applied.

Table 3: Physico-chemical variables (Mean ± S.D) of water determined during growth trails with different fish species under chronic exposure of metals mixture (Fe+Zn+Pb).

Fish Species	Total Ammonia (mg L^{-1})	Electrical Conduc-tivity (mS cm^{-1})	Calcium (mg L^{-1})	Sodium (mg L^{1})	Dissolved oxygen (mg L^{-1})	Potassium (mg L^{-1})	Total hardness (mg L^{-1})	Temperature (° C)	pH	Magnesium (mg L^{-1})
Catla catla	1.59± 0.35	3.38± 0.32	35.08± 1.99	297.75± 6.28	5.58± 0.32	8.46± 0.46	225.41± 0.41	29.52± 0.16	7.37± 0.23	68.36± 2.36
Labeo rohita	1.34± 1.61	3.45± 0.33	37.50± 34.80	295.00± 8.08	5.87± 5.56	7.34± 7.55	225.32± 0.34	29.54± 0.69	7.45± 0.42	64.13± 7.96
Cirrhina mrigala	1.51± 0.14	3.48± 0.28	38.20± 1.99	294.58± 3.65	5.48± 0.22	7.32± 0.07	225.21± 0.47	29.61± 0.19	7.15± 0.18	63.24± 3.01
Ctenopharyngodon idella	1.80± 0.28	3.32± 0.28	35.83± 2.10	295.58± 5.05	5.49± 0.23	7.64± 0.25	225.27± 0.47	30.01± 0.58	7.49± 0.49	67.42± 2.28
Hypophthalmychthys molitrix	1.74± 0.19	3.31± 0.28	35.58± 2.92	298.50± 4.87	5.66± 0.22	7.60± 0.22	225.12± 0.45	30.00± 0.40	7.20± 0.13	66.86± 4.54

Results

Fish growth under chronic stress of metals mixture

Significant effects of metal mixture were observed on the average wet weight increments of fish species viz. *H. molitrix*, *C. idella*, *L. rohita*, *C. catla*, and *C. mrigala*, when exposed to sub-lethal concentrations of the mixture of iron, zinc and lead heavy metals. The average weight increments of stressed fish species revealed significant variation as 11.88±0.02 g, 11.50±0.02 g, 11.81±0.05 g, 11.45±0.04 g, 12.03±0.03 g for *H. molitrix*, *C. idella*, *L. rohita*, *C. catla*, and *C. mrigala*, respectively. Among studied fish species, *C. mrigala* exhibited significantly least sensitivity to metals mixture as depicted by increments of their average weight. Metals mixture stressed fish species exhibited significant variations as far as their feed intakes were concerned. *C. idella* showed significantly higher average feed intake (18.50±0.50 g) among the treated fish species, followed by that of *H. molitrix* (18.46±0.03 g), *C. mrigala* (17.90±0.08 g), *L. rohita* (17.08±0.08 g) and *C. catla* (16.80±0.02 g) with significant differences among them. *L. rohita* attained significantly higher average condition factor (2.01±0.02) and significantly lower condition factor was obtained in *C. mrigala* (1.57±0.07). The overall maximum feed conversion efficiency of 69.15±0.01% was recorded for *L. rohita* while it was significantly minimum as 62.14±0.05% in *C. idella*. Moreover, significant variations among the performances exhibited by five treated fish species were also observed. On comparison of two treatments for all species, the metals mixture (Fe+Zn+Pb) in stressed fish attained lower weight (11.73±0.25 g) than the control fish (24.40±2.00 g). Other growth parameters including feed

[DOR: 20.1001.1.15622916.2022.21.2.13.1]

intake, feed conversion efficiency and condition factor also presented the same trend as observed for average wet weight of fish species (Table 4).

Table 4: Growth performance of five fish species exposed to sub-lethal concentrations of metals mixture (Mean ± S.D).

Fish Species	Growth parameters			
	Increase in weight (g)	**Feed intake (g)**	**Condition factor (*K*)**	**FCE (%)**
Catla catla	11.45±0.04[e†]	16.80±0.02[e]	1.80 ±0.04[b]	68.15±0.15[b]
Labeo rohita	11.81±0.05[c]	17.08±0.08[d]	2.01±0.02[a]	69.15±0.01[a]
Cirrhina mrigala	12.03±0.03[a]	17.90±0.08[c]	1.57±0.07[d]	67.21±0.04[c]
Ctenopharyngodon idella	11.50±0.02[d]	18.50±0.50[a]	1.59±0.03[c]	62.14±0.05[e]
Hypophthalmichthys molitrix	11.88±0.02[b]	18.46±0.03[b]	1.59±0.02[c]	64.36±0.08[d]
Treatments				
Metal mixture stressed fish	11.73±0.25[b]	17.75±0.78[b]	1.71±0.91[b]	66.21±2.88[b]
Control	24.40±2.00[a]	22.08±1.38[a]	2.14±0.26[a]	109.75±10.21[a]

[†] Means (± S. D) with different letters in a single column for each factor (Species and Treatment) represents statistically significant difference according to the Student Newman-Keuls tests (p<0.05).

Uptake and accumulation of metals in fish

The average value of Fe, Zn, and Pb concentrations detected in different fish tissues/organs (skin, gills, bones, fins, muscles, kidney and liver) before the exposure of chronic stress of metals mixture were measured (Table 5). After 12-week growth experiments, a significant increase in the concentrations of metals mixture was detected in the studied organs. The Fe+Zn+Pb metals mixture exhibited a strong impact on the bioaccumulation of these metals in different organs/tissues of fish species (Fig. 1a). Liver showed the highest affinity towards accumulation of iron, zinc and lead. Fish bones exhibited significantly the least bioaccumulation for zinc and lead while iron was observed least in muscle tissue.

Overall, a significant accumulation of iron, zinc and lead in fish organs was observed with the following trend: liver> kidney> gills> bones (Fig. 1b). Among fish species, significantly higher accumulations of metals were observed in *C. idella* than the other four species (Fig. 2).

Table 5: Metals concentrations (µg g^{-1}) (Mean ± S.D) in fish organs before chronic exposure.

Metals	Fish species	Organs						
		Kidney	**Liver**	**Skin**	**Muscle**	**Fins**	**Gills**	**Bones**
Fe	*C. catla*	12.05±0.45^{e†}	16.73±0.14^{c}	20.72±0.30^{c}	8.48±0.32^{d}	9.24±0.52^{d}	45.71±0.36^{b}	10.30±0.23^{e}
	L. rohita	47.12±0.04^{a}	81.00±3.96^{b}	15.10±0.12^{d}	15.98±0.50^{c}	15.67±0.34^{c}	52.67±0.70^{a}	14.73±0.07^{d}
	C. mrigala	20.54±0.25^{d}	8.86±0.01^{d}	20.48±3.69^{c}	32.10±0.26^{a}	19.11±1.50^{b}	15.16±0.28^{c}	17.07±0.81^{cd}
	C. idella	41.11±1.20^{b}	83.33±0.09^{b}	27.11±0.27^{b}	17.12±1.30^{b}	19.01±0.32^{b}	47.22±2.17^{b}	19.45±0.12^{bc}
	H. molitrix	37.22±1.67^{c}	86.58±0.35^{a}	30.82±0.40^{a}	15.50±2.44^{c}	22.79±0.24^{a}	54.63±3.33^{a}	28.32±0.41^{a}
	Overall Means	**31.61±14.72^{C‡}**	**55.30±38.95^{A}**	**22.84±6.16^{D}**	**17.84±9.08^{E}**	**17.17±5.10^{E}**	**43.08±16.04^{B}**	**17.97±6.70^{E}**
Zn	*C. catla*	12.18±0.03^{d}	14.60±0.07^{b}	2.51±0.17^{d}	3.28±0.03^{d}	4.12±0.11^{e}	6.79±0.16^{d}	8.22±1.67^{d}
	L. rohita	28.58±1.51^{a}	21.25±3.54^{a}	9.04±0.79^{b}	10.68±1.25^{b}	7.45±1.25^{c}	11.37±0.81^{b}	12.56±1.80^{c}
	C. mrigala	13.17±3.00^{bcd}	15.14±1.57^{b}	9.26±0.63^{b}	12.24±0.43^{a}	8.19±0.66^{bc}	8.92±1.37^{c}	13.41±0.54^{bc}
	C. idella	10.75±3.54^{e}	8.04±1.31^{d}	6.77±1.21^{c}	6.18±0.61^{c}	9.48±0.89^{ab}	14.00±1.12^{a}	16.37±0.30^{a}
	H. molitrix	12.29±2.01^{cd}	9.56±1.80^{cd}	9.89±0.84^{ab}	6.21±0.94^{c}	6.41±0.92^{d}	5.25±1.58^{e}	5.29±0.16^{e}
	Overall Means	**15.39±7.42^{A}**	**13.72±5.22^{B}**	**7.49±3.03^{E}**	**7.72±3.66^{E}**	**7.13±2.02^{E}**	**9.26±3.51^{D}**	**11.17±4.40^{C}**
Pb	*C. catla*	33.58±4.72^{b}	68.15±3.16^{a}	35.96±2.45^{a}	11.02±0.18^{ab}	67.95±4.15^{a}	48.15±2.06^{a}	13.92±1.89^{a}
	L. rohita	49.29±4.03^{a}	39.90±3.84^{b}	4.94±0.52^{e}	4.44±0.27^{d}	22.10±2.07^{de}	31.28±1.36^{b}	9.64±0.31^{cd}
	C. mrigala	13.43±2.31^{e}	17.09±0.59^{de}	25.31±0.27^{b}	6.82±0.68^{cd}	44.00±3.07^{b}	26.67±3.09^{c}	11.02±0.28^{b}
	C. idella	22.08±0.70^{cd}	16.23±1.27^{e}	14.08±1.06^{cd}	9.31±0.40^{bc}	19.40±0.43^{e}	14.28±2.20^{e}	6.98±0.54^{d}
	H. molitrix	19.44±3.17^{d}	21.29±3.43^{c}	11.39±0.34^{d}	9.82±0.77^{bc}	30.70±4.82^{c}	20.39±1.08^{d}	7.38±0.11^{d}
	Overall Means	**27.57±14.18^{C}**	**32.53±22.10^{B}**	**18.34±12.30^{D}**	**8.28±2.64^{E}**	**36.83±19.86^{A}**	**28.15±12.89^{C}**	**9.79±2.84^{E}**

†Means (±S.D) with different letters (a, b, c) in a single column for each metal (Fe, Zn, and Pb) represents statistically significant difference of metal accumulation for the same organ among different fish species according to the Student Newman-Keuls tests (p<0.05).

‡Overall means (±S.D) with different letters (A, B, C) in a single row for each metal (Fe, Zn, and Pb) represents statistically significant difference of overall metal accumulation among different organs according to the Student Newman-Keuls tests (p<0.05).

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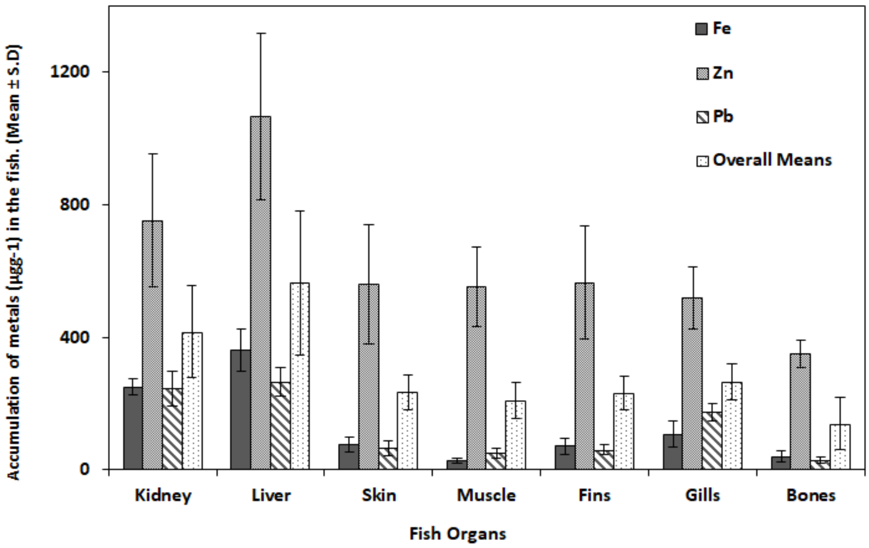

Figure 1a: Accumulation patterns of metals (µg g^{-1}) in fish organs during chronic exposure of metals mixture (Fe+Zn+Pb).

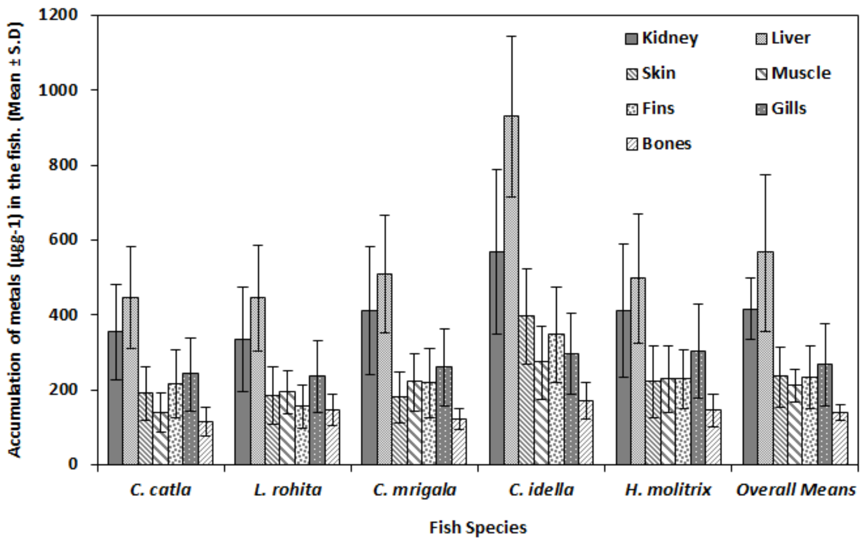

Figure 1b: Accumulation patterns of metals (µg g^{-1}) in fish organs among different fish species.

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Discussion

During the present study, bioaccumulation of heavy metals (Fe, Zn, and Pb) was determined among different fish species and fish organs, when provided in a mixture (Fe + Zn + Pb) form. The toxic effects of heavy metals mixture were also determined on a variety of physiological and biochemical processes in terms of fish growth. To determine the hazardous effects of toxicants on bioenergetics related processes in a living organism such as feeding, excretion, metabolism, and assimilation, the chronic exposure of heavy metals is quite helpful (Bhavan and Geraldine, 2000). In aquatic ecosystems, fish being at a top of trophic level of aquatic food chain are mainly assumed to be affected by heavy metals mixture even at low concentration, when exposed for a long time period (Syvokiene *et al.*, 2003).

The obtained results showed that heavy metals mixture had a significant effect on growth parameters of all studied fish species as compared to the control treatment. The mixture of heavy metals significantly reduced the wet weight gain, fork length and total length. Response of growth parameters in fish has been proved to be sensitive and authentic indicators under waterborne chronic exposure of metals mixtures (Javed, 2012). These findings are in line with Rajeshkumar *et al*. (2017), who found that sub-lethal dose of metals mixture had adverse effect on fish growth and metabolic activities. Metal mixture concentrations at sub-lethal level caused substantial influences on wet weight gain of fish species. Likewise, it has been shown that *C. mrigala* showed substantially lower growth rate as compared to unstressed control group, in terms of wet weight, fork and total lengths when exposed to metals mixture (Zn+Fe+Pb+Ni+Mn) at sub-lethal concentration (Hussain *et al.*, 2010). Vosyliene *et al.* (2003) reported that fish exhibited significant response towards lethal effects of metals mixture (Zn+Cu+Ni+Cr+Cd+Mn+Pb) in terms of behavior, development, growth rate and respiration at larval stage. Metals mixture of Zn+Pb+Ni has also been reported to have similar adverse effects on fish growth, condition factor values and metal accumulation in fish organs (Javed, 2015). Detrimental effects of studied metals have also been reported in other studies. For instance, a significant effect of Zn was observed on growth of *Danio rerio* (Salvaggio *et al*., 2016). Similarly, Pb+Mn mixture has been reported to adversely affect the growth of *C. catla, L. rohita, C. mrigala, C. idella and H. molitrix* under acute exposure (Naz *et al.*, 2020). The results of this study are also coherent with Ayegbusi *et al*. (2018), who found a decreased growth rate of *Clarias gariepinus* when exposed to chronic exposure of Pb. Like Pb and Zn, Fe also has been reported to affect different biological parameters in fish species under chronic exposure (Gemaque *et al*., 2019).

It has been shown that the condition factor is a reliable parameter to assess the growth and physiological well-being of fish species growing on a particular diet and environment (Caldarone *et al.*, 2012). The results demonstrated that among all the fish species, *L. rohita* showed the highest condition factor while, *C. mrigala* showed the lowest value under the heavy metals mixture stress. Nevertheless, the unstressed (control) fish species groups showed eloquently higher values for condition factor in comparison to stressed fish group. It has been suggested that chronic exposure of toxicants modify the behavior and physiology of fish, which reduce the growth of fish ultimately

(Niyogi *et al.*, 2006). The obtained results are coherent with Jezierska and Witeska (2001), who observed significant changes in feed intake as well as growth rate in fish induced by metals mixture stress.

The present study showed that gills, kidney and liver are the three prime sites for the bioaccumulation of metals while their loads were significantly lower in the fish muscle. Meanwhile, liver was observed to have more ability to accumulate metals (Fe, Zn and Pb) followed by kidney and gills tissues of fish during organ-wise accumulation analysis of residual metals. Abdel-Tawwab *et al*. (2016) showed similar findings and concluded that Zn accumulation was lowest in muscles as compared to the liver, gills and kidney, while, fish muscles showed lowest affinity towards metal accumulation in comparison with liver, kidney and gills in all studied fish species. Similarly, Rajeshkumar *et al*. (2017) demonstrated severe adverse effects of heavy metals mixture in fish liver. Azmat *et al.* (2012) obtained similar results and concluded that bioaccumulation of heavy metal was highest in kidney and liver. Variable patterns of bioaccumulation of metals (lead, zinc, iron, manganese and nickel) observed in different organs of fish exhibited different physiological roles in each fish organ (Crafford and Avenant-Oldewage, 2010). The study demonstrated that heavy metals mixture at chronic levels are toxic to fish and those accumulate in different organs of fish in different ratios. Furthermore, reduced growth and increase in size was also evidenced from this study.

Amongst all the treated fish species including *C. catla, L. rohita, C. mrigala, C. idella* and *H. molitrix,* significantly higher mean weight was exhibited by *C. mrigala* while that of lowest was attained by *C. catla.* However, the growth rate was significantly lower in each fish species exposed to metal mixture stress than that of unstressed fish species. It was also concluded that the significantly variable condition factor values that correlated precisely with fish growth expressed the extent of fish well-being. During organ-wise analysis of metal accumulation, liver was found as the prime site of metals (iron, zinc and lead) accumulation with more ability to let the metals penetrate followed by kidney and gills.

Acknowledgments

The authors are thankful to staff members of toxicology laboratory of University of Agriculture Faisalabad laboratory for their help and co-operation to conduct this study.

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[DOR: 20.1001.1.15622916.2022.21.2.13.1]