Original Research

BULLETIN OF ENVIRONMENTAL STUDIES

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Growth performance of *Catla catla* fish in earthen pond under metal stressed conditions

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Abstract

The present research investigates the growth performance of *Catla catla* fish under chronic exposure of metal-mixture (i.e., Ni, Pb and Mn) in a semi-intensive pond culture system. Ninety days old fingerlings were submitted to sub-lethal concentration of metal mixture for 90 days and shifted to earthen ponds. Treated and control ponds were fertilized with poultry droppings on the basis of its nitrogen contents at the rate of 0.17g nitrogen for 100 g of fish weight daily. Control fish exhibited higher growth rates in earthen ponds than the stressed fish. Metal mixture caused a significant reduction in average wet weight, fork and total length of treated fish. Condition factor values of treated fish were greater than the control fish. Untreated fish showed higher feed (digestible protein) given than that of contaminated ones, whereas the higher feed conversion ratios were observed for control fish. We conclude that the metal-mixture causes toxic effects and decreases overall growth of fish. Urgent measures are required to prevent such contamination and regular monitoring of freshwater bodies to enhance the quality and quantity of fish biomass.

Article Information

Edited by:
Mariam Sabeeh, UET, Taxila, Pakistan
Muhammad Arslan, UFZ, Germany

Reviewed by:
Dadan Wardhana Hasanuddin,
XXXXXXXX

Article History:
Received; September 23, 2016
Received in revised form; May 28, 2016

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Keywords: *Catla catla*; metal stress; mixture toxicity; physicochemical variables

Introduction

Aquatic pollution is a serious worldwide problem especially in developing countries (Kazi et al., 2016; Shahid et al., 2015a). Freshwater bodies are being polluted by several point and non-point sources including chemicals and metals that cause stress to aquatic organisms (Begum, 2004; Khare and Singh, 2002; Shahid et al., 2015b). It has been suggested that the rapid increase in population, urbanization, industrial revolution and lack of environmental awareness are contaminating freshwater bodies with heavy metals such as nickel, zinc, lead and manganese etc. A huge amount of wastewater from industries and urban areas is discharged into freshwater bodies without any prior treatment. These effluents contain poisonous and toxic chemicals including heavy metals that are harmful because they can integrate into the food web and cause detrimental effects to aquatic life and humans (Chaharlang et al., 2012; Iqbal et al., 2017; Iqbal et al., 2016). Elevated amounts of heavy metals may increase the risk of diseases such as cancer, cardiovascular disorders, mental abnormalities and kidney failure (Ali et al., 2013; Copat et al., 2013).

Heavy metals constitute a core group of aquatic pollutants and accumulate in the aquatic ecosystems (Vutukuru, 2005). Among aquatic organisms, fish is one of the most important sensitive taxa. Some trace metals including nickel, cobalt, manganese, lead, and zinc are essential for the normal physiological functions of fish. However, excessive amounts of these metals become toxic to aquatic organisms (Javed, 2003). Higher concentrations of heavy metals may affect the metabolic rate that results in reduced growth. Furthermore, it can also reduce the feed uptake (Hart et al., 2002). Heavy metals enter in the environment by different natural and man-made activities and target the aquatic life in different ways. For instance, Pb concentration in biological tissues corresponds to the environmental pollution levels and varies significantly with geographical area and demographic factors. Lead accumulation causes reproductive disturbance, neurotoxicity and hemotoxicity in fish (Rodamilans et al., 1996). Exposure of lead to aquatic animals can cause behavioral abnormalities and defective cognitive functions (Palaniappan et al., 2008). It can accumulate in fish tissues like kidney, gills, liver, bones and scales during chronic exposure (Dallas and Day, 1993). Similarly, Ni causes respiratory toxicity and significantly increases the ventilation rate and oxygen consumption that may lead to swelling of the gills (Hughes et al., 1979; Pane et al., 2004). However, the mechanism of Ni toxicity to aquatic organisms is much clear (Blewett and Leonard, 2017). Likewise, a higher Mn concentration induces suffocation, paralysis and cauterization of gill lamellae fresh water fish (Schweiger, 1957). Additionally, it may significantly reduce the total erythrocyte count, number of erythrocytes/1000 blood cells and relative liver weight (Agrawal and Srivastava, 1980). In natural ecosystem, metals are present in a mixture form which is more toxic than a single metal alone.

Pakistan is one of the developing countries facing water pollution and food scarcity (Fahim, 2017; Iqbal et al., 2017; Kirby et al., 2017). With growing population of Pakistan, it is necessary to provide the sufficient and healthy food. Fish is one of the important sources of quality protein, commonly used and aqua cultured in Pakistan (Naz et al., 2008).The most important freshwater culturable fish species of Pakistan are the major Indian carps i.e. Rohu (*Labeo rohita*), Thaila (*Catla catla*) and Mori (*Cirrhinus mrigala*). Some exotic species such as Gulfarm (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitix*). However *Catla catla* is very important due to its taste, nutritional quality, size and rapid growth (Cauchie et al., 2000). Keeping in view the toxic effects of heavy metals, the present research was conducted to evaluate the growth performance of *Catla catla* exposed to metals' mixture under semi-intensive polyculture pond system.

Materials and Methods

Test Organisms

The research work was conducted at the Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. The 90 days old *Catla catla* fingerlings were brought to the laboratory and acclimatized for 10 days in cemented tanks. Stock of fish species was divided into two groups *viz;* treated and control and kept separate in glass aquaria.

Fish exposure to metals' mixture

Chemically pure chloride compounds of lead, nickel and manganese were dissolved in deionized water and stock solutions were prepared. These stock solutions were mixed on ionic basis to prepare metal mixture. Fish individuals were exposed to sub-lethal (1/3rd of LC_{50}) concentrations of the mixture. However, the control group of fish was kept un-stressed in clean water. The fish was exposed to 22.87 mg L^{-1} metal-mixture for 90 days at constant water temperature (28˚C), pH (7.50) and total hardness (225 mgL^{-1}) (Naz and Javed, 2013). Constant aeration was provided to all the test media (water) with an air pump fixed with a capillary system. The fish was fed, to-satiation, with the feed (32% digestible protein and 3.00 $Kcalg^{-1}$ of energy) twice a day. During sub-lethal metal mixture exposure period, the physico-chemical variables of water including temperature, pH, total hardness, dissolved oxygen, electrical conductivity, total alkalinity, total ammonia, sodium, potassium, nitrates, phosphates, chlorides, plankton biomass and light penetration were analyzed twice a day following the standard methods for examination of water prescribed by American Public Health Association (2005).

Fish Growth

The exposed and control fish were stocked in earthen ponds (0.012 ha) separately, with 65 fish in each pond and stocking density of *Catla catla* was 10% for growth studies. Both treated and control fish ponds were fertilized with poultry droppings at the rate of 0.17g nitrogen per 100g fish weight daily in order to promote the pond biota for fish consumption. The fish were also provided supplementary feed equal to 3% of their wet body weight initially and then decreased gradually with decline in water temperature. However, no feed was offered at water temperature below 25˚C. The fish were grown in earthen ponds for 210 days. From each pond, random sampling (n=7) of each fish species was done by using drag net, on fortnightly basis, and their growth parameters measured. After recording the data, fish were released back into their respective ponds. The following growth parameters of fish were monitored on fortnightly basis:

1. Increase in fish wet weights (mm)
2. Increase in fork lengths (mm)
3. Increase in total lengths (mm)
4. Specific growth rates (mm)
5. Condition factor
6. Feed conversion ratios of ponds

Specific Growth Rate was measured using the following formulae:

$$SGR = \frac{\text{Increase in Weight}\,)\times 100}{14}$$

The condition factor was calculated using the following equation:

$$K = \frac{\text{Average weight} \times 10^5}{\text{Average fork length}^3}$$

Physicochemical parameters

All of the physicochemical parameters were monitored fortnightly. Water temperature, dissolved oxygen, pH and electrical conductivity by the digital meters were determined by digital meter HANNA HI-9146. Whereas sodium and potassium were determined using Flame Photometer (PFPI) (Richards, 1954). Light penetration was determined with the help of Secchi's disc while total ammonia was measured using the spectrophotometer. For estimation of total alkalinity, methyl orange indicator method (APHA, 2005) was used, whereas total hardness was measured by titration of water sample with EDTA using Erichrome Black T (EBT), as indicator. Phosphates and nitrates were also determined with the help of

Bausch and Lomb spectronic-21. Additionally, the planktonic biomass was determined indirectly, from the total solids and total dissolved solids (Bajguz and Hayat, 2009).

Statistical Analysis

Statistical analysis such as mean, standard deviation, standard error of the data obtained on different parameters of fish growth and physico-chemistry was carried out using R statistical language (version 3.0.3; R Core Team, 2014). Additionally, for the comparison of both groups, Welch's t-test was performed.

Results

Increase in wet weight (mm)

To find out the effect of heavy metal toxicity, increase in wet weight of fish was analyzed. Initial and final wet weight of control group was 21.71± 1.11 and 628.78±1.65g respectively. In contrast, initial wet weight of treated fish was 22.14±2.98 but the final wet weight (354.51±2.76g) was significantly lower than the controls (Welch two sample t-tests, p-value < 0.05; Figure 1). Among all fortnights, the maximum increase in average wet weight of treated and control fish were recorded as 86.57 and 106.58g respectively during the 1st fortnight. Whereas the minimum increase in average weight i.e., 2.52 and 8.24 g were observed during 9th fortnight respectively.

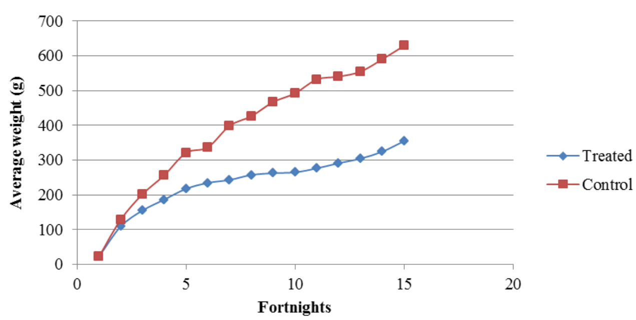

Figure 1: Average increase in wet weight (g) of treated and controls of *Catla catla.*

Increase in Average Fork Length (mm)

In case of fork length, a significant effect of metal toxicity was observed. Average initial fork length of control fish was recorded as 97.79±2.09mm. At the end of experiment, the average final fork length was 520.19±1.30mm. Maximum increase in fork length was 82.37mm during the 1st fortnight, whereas the minimum was 10.81mm and recorded during the 11th fortnight of the experiment. In contrast, the treated organisms showed average final fork length (361.12±0.89mm) that was significantly lower than the controls (Welch two sample t-tests, p-value < 0.05; Figure 2). The maximum increase in average fork length in treated *Catla catla* was recorded as 65.01mm during the 1st fortnight, whereas the minimum increase was 3.22mm observed in the 10th fortnight of the trial.

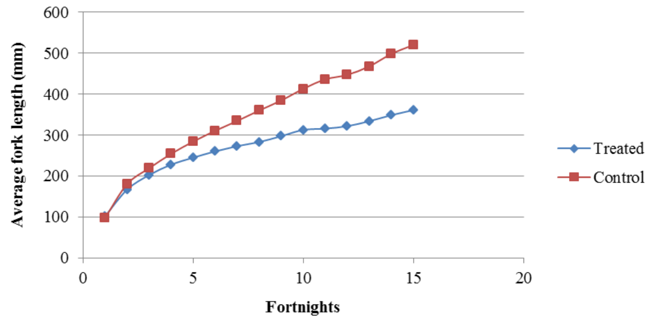

Figure 2: Average increases in fork lengths (mm) of treated and control of *Catla catla*

Increase in Average Total Length (mm)

Initial total length of control fish was recorded as 113.04±2.91mm and the final total length was 554.11 ± 1.98mm. Maximum and minimum increase in total length was 95.10 and 9.25 mm in the1st and 11th fortnight respectively. In comparison, the initial total length of treated *Catla catla* was recorded as 117.54±2.87mm that is similar to that of control, however the final total length (396.12±2.08mm) was significantly lower (Welch two sample t-tests, p-value < 0.05; Figure 3). The maximum increase in total length in treated fish was observed to be 76.91mm during the 1st fortnight of the trial. However, the minimum increment was 7.45mm in the 11th fortnight.

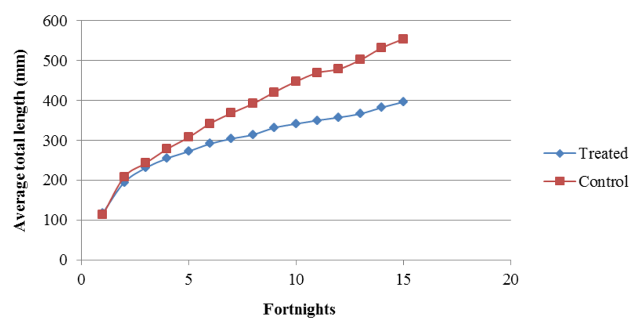

Figure 3: Average increases in total lengths (mm) of treated and control of *Catla catla.*

Specific Growth Rate (SGR)

The specific growth rate of control and treated individuals were calculated on fortnightly basis. It was observed that exposed fish exhibited significantly lower specific growth rates as compared to control fish (Welch two sample t-tests, p-value < 0.05; Figure 4). For control fish, the maximum and minimum value of specific growth rate was 761.23 and 58.86 in 1st and 11th fortnight respectively. In contrast, the maximum SGR of treated *Catla catla* was 618.36 during 1st fortnight whereas the minimum was recorded as 18.00 during 9th fortnight.

Condition factor

Both treated and control *Catla catla* showed variations in their condition factor values. Condition factor values for treated fish were found to be greater than that of control; however the difference was not significant (Welch two sample t-tests, p-value > 0.05; Figure 5). Maximum and minimum condition factor values for control fish were recorded as 2.32 and 0.45 in initial and 14th fortnight respectively. Whereas the treated *Catla catla* showed maximum and minimum condition factor values of 2.34 and 0.75 during 2nd and 14th fortnight respectively.

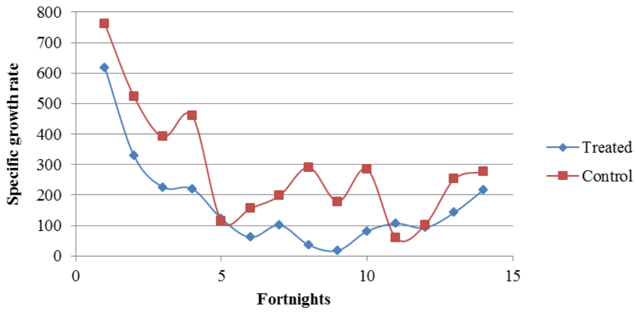

Figure 4: Variations in specific growth rates (SGR) of treated and control of *Catla catla.*

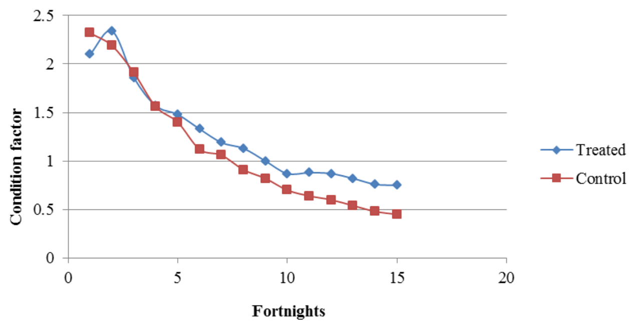

Figure 5: Variations in condition factor of treated and control of *Catla catla.*

Feed Conversion Ratio (FCR)

The maximum feed conversion ratios for both treated and control pond were observed during 1st fortnight as 24.30 and 25.30, respectively. The minimum feed conversion ratios for the same ponds were 2.00 and 4.00 during 9th and 7th fortnight respectively. However there was no significant difference among both groups (Welch two sample t-tests, p-value > 0.05; Figure 6).

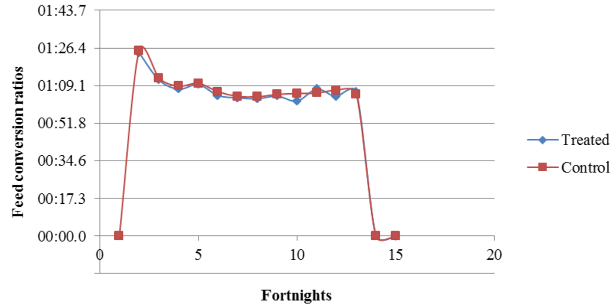

Figure 6: Variation in feed conversion ratio of treated and control fish species

Discussion

This study aimed at determining the growth performance of *Catla catla* fish in earthen pond under metal stressed conditions. The metal exposed fish showed significantly lower growth in terms of wet weight, total length, fork length as compared to control. It is well established that that the heavy metals can affect the survival ability, reproduction, growth rate and physiological functions in fish (Amundsen et al., 1997; Dallas and Day, 1993; Hughes et al., 1979; Palaniappan et al., 2008; Pane et al., 2004). Similar findings have been reported by Hussain et al. (2010). They studied the growth performance of *Cirrhina mrigala* under sub-lethal exposure of metal mixture of heavy metals viz. Fe, Zn, Pb, Ni and Mn. Results showed significantly reduced growth rate under sub-lethal chronic exposure of mixture. Moiseenko and Kudryavtseva (2001) investigated the effects of metal mixture *viz*; nickel, copper, aluminum, strontium, cobalt, manganese, zinc, lead and mercury on the growth performance of fish. The increased accumulation of metals in the fish body organs caused reduced growth rates in fish. Similarly, Javed (2006) studied the growth responses of fish *Labeo rohita* and *Cirrhina mrigala* during chronic exposure of nickel and manganese. During stress period, both species exhibited decrease in weight, total and fork length. Likewise, Verslycke et al. (2003) and Jezierska et al. (2000) also reported reduced growth rate and less survival of fish.

In present study, fork and total length was significantly affected by metals. Exposed organisms showed significantly less increase in total and fork length as compared to controls. Coetzee et al. (2002) reported relationship between metal concentration and increase in total length of fish. The heavy metals were generally lower in fish with large size. Similarly, Kausar and Salim (2006) determined the effect of metal toxicity and water parameters weight, fork and total lengths *Labeo rohita*.

During present investigation, feed conversion ratio varied significantly between treated and control fish and the highest observed in control fish as compared to the metal stressed fish. Although the difference between both groups was not significant. Ye et al. (2006) investigated the toxic effects of dietary metals mixture (calcium and phosphorous) to juvenile fish. They found that fish diet without phosphorous supplement showed decreased growth, loss of appetite and poor feed conversion efficiency. The effect of copper and cadmium on the metabolic activity of growing rainbow trout was significant. They observed that exposure of fish to sub-lethal concentrations of metal mixture resulted in the reduction of feed conversion efficiency of the metal stressed fish. Control fish showed better condition factor as compared to the treated fish species. The same results were reported by Heydari et al. (2011). They studied the accumulation of Cadmium and Lead in the liver and muscles of stellate sturgeon (*Acipenser stellatus*). Significant negative correlation was observed between lead concentrations and condition factor of fish. Similar results were also found out by Farkas et al. (2002) while studying the relationship between growth rate and heavy metal concentration in body organs of *Abramis brama*.

Conclusions

From the obtained results it is concluded that the sub-lethal concentrations of metal-mixture significantly affected the growth performance of *Catla catla* fish in earthen pond. Exposed organisms showed significantly lower values of weight, fork and total length than that of control fish. Higher feed conversion ratios were observed for control fish species than that of treated fish. The study confirms the toxic nature of metal-mixture and the results reflect that the prevention of water contamination with heavy metals is necessary to enhance the quality and quantity of fish biomass.

Compliance with ethical standards

Conflict of Interest

The authors declare that they have no conflict of interests.

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