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ORIGINAL RESEARCH ARTICLE

ARSENIC INDUCED TOXIC IMPACTS ON THE GROWTH PERFORMANCE AND HAEMATOLOGICAL ASPECTS OF RAHU (*Labeo rohita*)

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ABSTRACT

The effect of environmental pollutants such as heavy metals on aquatic animals especially fish species are lethal. The research work was conducted to determine acute toxicity and sub- lethal (1/3 of LC_{50}) effects of arsenic (As) on growth and haematological parameters of *Labeo rohita*. The fish *L. rohita* was exposed to different concentrations of arsenic for different time periods. Fish were kept under constant water temperature (28^0C), water hardness (300mg/L) and pH (7). The LC_{50} of arsenic for 96hr was calculated i.e. 30.71 mg L^{-1}. Mortality of fish was observed with increase in the concentrations of arsenic for 96 hr exposure. Growth performance was significantly higher ($p< 0.05$) in unstressed fish in comparison with arsenic (sub-lethal toxicity) exposed *L. rohita*, recorded at 14th and 28th day. Growth reduction strongly correlated with arsenic toxicity. The hematological analysis of arsenic exposed *L. rohita* at 14th and 28th day exhibited significant decrease in erythrocyte counts or red blood cells (RBCs), hemoglobin (Hb) and the content of hematocrit (Hct), while noticeable elevation in white blood cells count (WBCs), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were recorded in comparison to control. This study clears that arsenic is highly toxic for fish. The altered blood and growth parameters can be used as biomarkers for arsenic detection in water environment.

INTRODUCTION

Arsenic, a metalloid occurring naturally mostly with ores particularly in gold, discharged in environment by the operations of ore processing mainly (Champeau *et al*., 2017). Arsenic is considered as one of the most dangerous effluent of aquatic environment among metallic pollutants known to be as genotoxic and carcinogenic (Chen *et al*., 2005). Its toxicity caused abnormalities in chromosomes structure through the utilization of drinking water (Wong chien *et al*., 2001).

According to World Health Organization, arsenic is one of the top 10 chemicals of concern for public health (Babich and Beneden, 2019). As industrialization has increased so much, the pollution caused by heavy metals in natural freshwater has become an issue of national importance. The toxicity and accumulation of heavy metals in biota gives considerable attention to aquatic ecosystem (Javed, 2004).

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Fish is sensitive to heavy metal pollution. There are deadly and long lasting impacts over fish fauna through toxicity by heavy metals (Avenant and Marx, 2000). Fish is not only a main protein factor in human diet but also monitor contamination status of aquatic environment (Qadir and Malik, 2011). In pond water if the level of As is very high, it may have strong negative effects on fish, lower their market value and eventually primes to the closure of fish farms (Liao *et al.*, 2003). The fish fauna of native species for example *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* (mori) are going towards extinction and adversely affected by the heavy metal load in water as aquatic organisms are chronically wide-open to acute toxicity concentration of metals (Humtsoe et al., 2007). The large amount of heavy metals from external aquatic environment ad due to the fact that in food chain fish placed at highest place so it can gather metals easily (Mansour and Sidky, 2002).

The severe anemia can be caused by poisonous heavy metals in fresh water fish *L. rohita*, produce changes in hematological indices (Praveena *et al.*, 2013). Acute and sub-lethal arsenic treatments caused significant alteration in hematological profiles of fish (Kavitha et al., 2010).

The health status of fish is assessed by hematological parameters such as RBC, WBC, Ht and Hb (Carvalho and Fernandes, 2006). Blood indices are highly sensitive to physiological changes and health position that's why hematological parameters gives support in diagnosing the structural and functional conditions of animals exposed to pollutants (Saravanan *et al*., 2011). The growth of fish can be adversely affected by heavy metals (Hayat *et al.*, 2007).

Therefore the present work was proposed to analyze the toxic effects of arsenic on growth and hematological parameters of *L. rohita*, which is consumed by humans on large extent.Bangladesh, and Malaysia, documenting the highest prevalence rate is in Cyprus, that is 16% (Ahmad *et al.*, 2019).

METHODOLOGY

Determination of acute toxicity after 96-hour

The research work was conducted in the laboratory of Department of Zoology, Government College Women University (GCWUS) Sialkot. The experiment was carried out in glass aquaria to determined growth performance and haematological parameters of fish *Labeo rohita* under arsenic toxicity. 90 days old fingerlings of fish *L. rohita* were taken by local fish hatchery Bhaigowala, Sialkot. The aquaria were cleaned with chlorinated water before adding fish into it. Eight fish were placed in each aquarium and tested against various concentration of arsenic, separately with three replicates. Constant air flow with air pump fixed with capillary system was maintained throughout the experiment. Pure salt of As was dissolved in water for the preparation of stock solution and required metal concentration of each test dose was prepared using the stock solution.

Probit analysis

During 96 hour trial period, the data on fish mortality was collected. Dead fish were recorded and removed from media and percent mortality was calculated by probit analysis (Hamilton *et al*., 1977). During the experiment water temperature, pH and water hardness were kept constant at 28 °0 C, 7 and 300 mg/L respectively. In each test trial the physico-chemical parameters viz. water temperature, pH, water hardness, carbon dioxide, dissolved oxygen, ammonia, calcium and magnesium were checked on daily basis by following APHA (2005).

Growth performance analysis of sub-lethal arsenic exposed *L. rohita*

The growth parameters viz. wet weight gain (WG), fork length and total length of treated and un-treated *L. rohita* were noted at 14 and 28 day of experiment. The Electronic weighing balance and measuring scale were used for the calculation of weight and length of fish respectively. At the end of experiment, mean increase or decrease in weights gain (WG), length gain (fork and total length), Feed intake (FI), Feed conversion rate (FCR), Feed efficiency rate (FER), Specific growth rate (SGR) and Condition factor (CF) of stressed and unstressed fish were determined by the following formulas:

$WG (g) = W_2 - W_1$

Total length gain (cm) = Final total length – Initial total length

Fork length gain (cm) = Final fork length – Initial fork length

FI (g/fish) = dry feed intake / Number of fish

FCR (g / g) = FI (g) / WG (g)

FER = (g) WG (g) / FI (g)

SGR (% day $^{-1}$) = [ln (W2) ¬- ln (W1)/t × 100]

Survival (%) = [Number of survived fish / initial number of fish] × 100

Where WG is the weight gain of body. W_1 is initial weight (g), W_2 is final weight (g).

Haematological analysis of sub-lethal arsenic exposed fish

The blood samples were obtained from the caudal vein of each fish with sterilized syringe and were preserved in k3EDTA anticoagulant coated vials for the determination of different hematological parameters viz. hemoglobin (Hb), hematocrit (Hct), total leukocyte count (WBCs), total erythrocyte count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). However, hematological parameters viz. hematocrit, mean corpuscular hemoglobin and mean corpscular hemoglobin concentration were derived with the help of formulas given below.

MCH (pg) = Hb (g %) × 10 / RBC (mm ^{3})

Where Hb is hemoglobin and RBC is erythrocyte count.

MCHC (%) = Hb (g %) × 100 / Hct (%)

Where Hct is hematocrit.

MCV (µm) = Hct (%) × 10 / RBC (mm ^{3})

Statistical Analysis

The acute toxicity of arsenic (96-hr LC_{50}) for *L. rohita* was calculated by using Probit analysis method with 95% confidence intervals (Hamilton *et al.*, 1977). Analysis of

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variance (ANOVA) was applied to compare the means of two groups (Steel *et al.*, 1996). Each test was conducted in three replicates.

RESULTS

Acute toxicity studies

During present investigations the acute toxicity (in terms of 96-hr LC_{50}) of arsenic was determined by exposing the fish *L. rohita* to the different concentrations of arsenic. The values of arsenic (in terms of 96-hr LC_{50}) for *L. rohita* was found to be 30.71 mg L^{-1} . Thus arsenic was represented as toxic heavy metal for fish *L. rohita* at the end of trial (Table 1).

Table. 1 Acute toxicity of arsenic (96 hr LC_{50}) exposed *L. rohita* by Probit analysis.

Replicates	96-hr LC_{50} (mg L^{-1})	SE	95.0%Fiducial CI	
			Lower	*Upper*
R1	31.46	0.853	29.79	33.15
R2	31.10	0.846	29.44	32.78
R3	29.42	0.856	27.74	31.11
Overall	30.71	0.8523	29.04	32.40

Growth studies

L. rohita were exposed to one third of calculated LC_{50} of arsenic. Growth parameters results during 28 days experiment, exhibited low values of weight gain, length gain, fork length gain, feed efficiency ratio, standard growth rate and condition factor in treated fish as compared to control fish. However, very little increase witnessed in the values of feed conversion ratio of treated fish (1/3 of LC_{50}). Meanwhile, no mortality was reported during the trial hence, survival rate was 100%.

During study it was observed that metal stress reduces the rate of metabolism in to *L. rohita*, which results in decreased growth parameters viz. weight gain, fork and total length gain.

However, experimental fish displayed higher values of feed conversion ratio (FCR).

Unstressed *L. rohita* showed significantly elevated values for feed intake during each growth trial study period (Figure: 1).

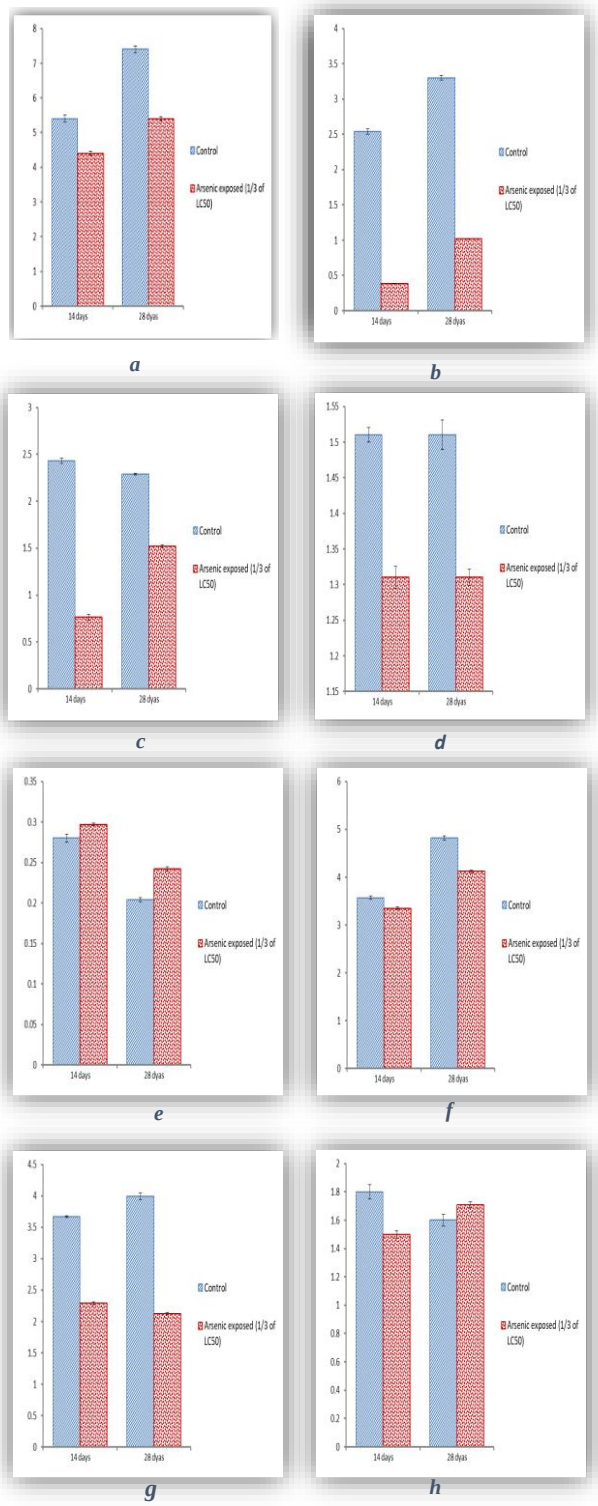

Fig. 1 Comparison (between control and experimental groups) and fluctuations of growth parameters weight (a), total length (b), fork length (c), feed intake (d), feed conversion ratio (e), feed efficiency ratio (f), standard growth rate (g) and condition factor (h) of fish *L. rohita* exposed to 1/3 of LC_{50} arsenic concentration

Haematological studies

In the present study, Generally red blood cells (RBCs), haemoglobin (Hb), contents of hematocrit (Hct) White blood cells (WBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) contents of sub-lethal arsenic exposed fish differed significantly when compared with control fish.

Among all blood parameters, WBCs, MCV, MCH and MCHC were significantly increased in treated fish (arsenic exposed) as compared to the contents of these parameters in control fish (untreated) while the RBCs, Hb and Hct contents in treated fish were reduced significantly when compared with the control fish (Table 2).

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DISCUSSION

Present work depicted that arsenic was represented as toxic heavy metal for fish *L. rohita*.

Toxic effects of arsenic for *Labeo rohita* was significantly variable under the different concentrations of arsenic metal in terms of 96 hr LC_{50} experiments. Kousar and Javed (2014) undertook an experiment, which revealed that arsenic (As) is highly poisonous for aquatic animals as compared to any other metal.

Table: 2 Effects of sub-lethal concentration of arsenic on haematological parameters of *L. rohita* after 14 and 28 days.

Haematological Parameters	Treatments: Sub-lethal (1/3rd of LC_{50}) Arsenic exposed *Labeo rohita*		
	Control	After 14 Days	After 28 Days
RBCsC($\times 10^6$/µL)	3.21±0.01 a	1.38±0.01 b	1.02±0.01 c
Hb(g/dL)	6.11±0.01 a	4.58±0.01 b	4.19±0.01 c
Hct (%)	32.85±0.01a	21.68±0.01b	17.80±0.01c
WBCsC (103/µL)	26.81±0.01c	48.20±0.01b	57.45±0.01a
MCV (fL)	102.34±0.01c	178.84±0.01b	194.12±0.01a
MCH (pg)	19.03±0.01c	33.19±0.01b	41.08±0.01a
MCHC (g/dL)	18.60±0.01b	21.12±0.01ab	23.54±0.01a

Sarnowski (2003) depicted that reduced metabolic rate and growth of fish is due to the disturbance caused by toxic effects of heavy metals (Sarnowski, 2003). Kim and Kang (2004) also investigated that metal stress lowers the rate of growth in fish and there is a secondary association among heavy metal experience and growth. Hansen et al. (2002) disclosed that, reduced growth in fish was observed due to the exposure of sub-lethal metal. The results of Shaw and Handy (2006) research work were in accordance with present study that metal exposure to fish showed significant reduction in body weight gain in comparison with unstressed (control) fish. Hayat et al. (2007) reported that when fish were reared in the acute toxicity medium of (sub-lethal concentrations) metal, ultimately lower values of fork and total length of fish were exhibited.

According to Mohantay *et al*. (2009) heavy metal produces sensitive oxygen (O_2) species which caused oxidative tension, proved to be toxic for fish. It was also evident that during stressful conditions, the growth reduced due to the reduction in feed intake by fresh water fish *L. rohita*. Ptashynski *et al.*, (2002) examined deviation in the values of condition factor (K) of fish due to the metal dietary exposure and concluded that metal exposure had no effect on condition factor and remained unchanged. The inferior ailment in contaminated fish comparative to reference fish often indicated lower values for condition factor (K) derived from Length (total and fork) and weight (W) measurements (Levesque *et al*., 2002; Eastwood and Counture, 2002). Bears *et al*. (2006) concluded that arsenic exposed fish depicted three fold more arsenic deposition in liver than unstressed (control) fish.

The present study reported negative growth outcomes of *L. rohita* (rohu) under the sub-lethal exposure of metal for 28 days trial when compared to control fish. The results also showed that condition factor significantly decreased when compared to controlled fish under sub lethal concentration of arsenic.

Hemoglobin, hematocrit, erythrocytes, WBCs, MCV, mean cell hemoglobin and mean cell heamoglobin concentration are those hematological indices that can be used as indicators of metal pollution in aquatic environment. During present research work, due the toxic effects of arsenic the contents of haematological parameters viz. RBCs, Hb, Hct decreased significantly while on the other hand contents of MCV, MCHC, MCH increased significantly when compared with control medium. Praveena *et al.* (2013) reported that heavy metals caused significant reduction in red blood cells, hemoglobin and packed cell volume. Due to the destructive action of effluents on erythrocytes that may be the reason of less red blood cell count coupled with low hemoglobin content. Heavy metal exposure caused decrease level of hemoglobin and packed cell volume, it clearly suggested that the mechanism of hemodilution is possibly due to damage of gill or impaired osmoregulation.

Mazon *et al*. (2002) also reported significant decrease in hemoglobin, erythrocyte content and hematocrit value in fish exposed to increased copper concentration. Moreover, significant reduction in hematological variables viz. MCHC, RBC and HB in erythrocytes of Zn exposed catfish Hetero clarias was also observed by Kori Siakpere and Ubogu (2008).

Singh *et al.* (2008) also observed that the elevated level of white blood cell counts showed the laceration in body tissue, hard physical stress and as well as leukemia. In many cases, impaired morphology of red blood cells was noted. Heavy metal leads to the high level of white blood cells. Thus it is examined that the hematological parameters are the most sensitive indices in monitoring the toxicity of metal especially at sub lethal concentration exposure.

CONCLUSION

In present investigation arsenic was come out as toxic heavy metal for fish *Labeo rohita*. One third of calculated LC_{50} significantly affected the growth parameters as well as haemotological parameters. Thus there is need to avoid or lessen arsenic utilization to save future generations from the harmful effects of arsenic.

Declaration of interest: The authors declared no conflict of interest.

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