

## EFFECT OF COMBINED EXPOSURE OF WATER-BORNE AND DIETARY NICKEL ON THE GROWTH PERFORMANCE OF CIRRHINA MRIGALA

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### ABSTRACT

An experiment under CRD was arranged to study the growth performance of *Cirrhina mrigala* as affected by a combined exposure of water-borne and dietary nickel at constant water temperature, pH and total hardness of 30°C, 7.0 and 200 mg L<sup>-1</sup>, respectively. Three age groups viz. 45-, 65- and 90-day were used to determine their response in terms of wet weight, fork and total length gains. Chemically extra pure chloride compound of nickel was used to prepare stock solutions of desired dilution. The wet weight gain by the fish, *Cirrhina mrigala* showed statistically significant variations between treated and control mediums. Fish with nickel treatment gained significantly lower weight, fork and total length than that of the control fish. Feed intake by the fish reared in untreated medium (control) was significantly maximum as compare to treated medium that showed significantly better feed conversion ratio in control than treated fish. The condition factor varied significantly during 12 weeks of the study period. The 90-day fish showed significantly better growth as compared to 65- and 45-day fish. Correlation coefficients among physico-chemical variables and increase in weight (grown under water-borne and dietary nickel) showed positively significant correlation with total ammonia but negatively significant correlation with magnesium contents.

**Key words:** water-borne, dietary, nickel, chronic exposure, growth, fish.

### INTRODUCTION

The aquatic environment, where fish and other aquatic organisms live, is subjected to different types of pollutants which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living aquatic organisms. Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda 2001; Witeska, 2003). It has also been linked as one major factor of disease outbreaks, low productivity, decreased growth and decrease in reproductive ability and mortality in aquaculture (Hansen *et al.*, 2001)

Fish are often at the top of aquatic food chain and may concentrate metals in their bodies from the water (Mansour and Sidky, 2002). Carps significantly contribute towards total global aquaculture production. Fish may absorb dissolved heavy metals that may accumulate in various tissues and organs and even be biomagnified in the food-chain/web. In absorption process the possible routes for metals to enter in a fish are: the food ingested; simple diffusion of metallic ions through gill pores; through drinking water; and by skin adsorption (Abdullah., *et al* 2011 ).

Stress in fish may be induced by various abiotic environmental factors (changes in water temperature, pH, O<sub>2</sub> concentration and pollution). Changes in environmental quality can therefore, be a major

determinant of year-class strength and eventually the long-term dynamics of many fish populations. The linear relationship were found between increasing pH/ alkalinity and decreasing accumulation of metals at variable exposure periods. (Adhikari *et al.*, 2006). Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Oshode *et al.*, 2008). The farmed fish contain more protein and lipids contents than that of natural catch (Mahboob *et al.* 2004, Hussain *et al.* 2011). Aquatic ecosystems are very vulnerable to water pollution. Concern over the environmental, aquatic and human health impacts associated with heavy metals distributions and concentrations in aquatic environments have a long history (Udosen, 2006).

Nickel is common component of natural fresh waters due to the erosion and weathering. Both deficiency and excess of nickel has been shown to reduced survival and growth in fish. Among carps *Catla catla* is more sensitive to nickel followed by *Labeo rohita* and *Cirrhina mrigala* ( Javid *et al.*, 2007). Nickel may be both the toxicant and a micronutrient, but its essentiality to aquatic animals is not established. Interactions between bronchial and gastrointestinal routes of metal uptake are important for understanding metal regulation and essentiality in aquatic animals (Chowdhury *et al.*, 2002). Nickel is a natural element in the earth's makeup. This must be a factor in assessing its ability to harm the environment. Although trace metals like Ni are essential for normal physiological process, aquatic ecotoxicity

testing has shown that  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  and  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  fall into the "harmful" classification where their abnormally high concentrations can become toxic and disturb the homeostasis of an animal (Farkas *et al.*, 2001).

Keeping in view the toxic effects of nickel on aquatic animals that caused serious illness and disorders, the present research work was conducted to investigate the chronic effect of dietary and water borne nickel on the growth performance of fish, *Cirrhina mrigala*.

## MATERIALS AND METHODS

Prior to the experiment, the fish of three age groups viz. 45-, 65- and 90-day age groups were kept in cemented tanks under laboratory conditions for 14 days. CRD design of statistic were used to study the growth parameters viz. wet body weight; fork and total lengths were measured and recorded before and at the end of the experiment. After acclimation period, fish was transferred to 70-liter glass aquarium for growth trials. Twenty fish were kept in each aquarium containing sub-lethal concentrations of nickel. Physico-chemical parameters viz. water temperature (30°C), pH (7.0) and total hardness (200 mg L<sup>-1</sup>) were kept constant throughout the study period. Continuous air was supplied to all the test and control mediums with an air pump through capillary system. Chemically extra pure chloride compound of nickel was used to prepare stock solutions of desired dilution. The sub-lethal water-borne and dietary nickel exposure concentrations for 45-, 65- and 90-day fish were 21.93 mgL<sup>-1</sup>, 79.11 µgg<sup>-1</sup>, 26.41 mgL<sup>-1</sup>, 83.15 µgg<sup>-1</sup>, 29.43 mgL<sup>-1</sup>, 88.80 µgg<sup>-1</sup>, respectively (Javed, 2008). In control aquarium no metal was added. Throughout the experimental period, fish were fed to satiation daily at 09:00 and 17:00 hours with the feed of Digestible Energy 24 Kcal/g (30% Digestible Protein). During sub-lethal exposure of water-borne and dietary nickel, the feed intake, increase or decrease in average wet weights, fork and total lengths and feed conversion ratios were monitored on weekly basis. After obtaining data, fish were released back into their respective aquaria. Three replicates were used for this experiment. The studied growth parameters were increase (+) or decrease (-) in wet weight (g), fork and total lengths (mm) of both treated and control fish. Feed intake, feed conversion ratio and condition factor of fish.

Water temperature and dissolved oxygen were measured and recorded by electronic meter HANNA HI-9146 while pH and electrical conductivity was measured by the digital meters WTW inolab. However, total ammonia, hardness, calcium, magnesium, CO<sub>2</sub> were measured by following the method of A. P. H. A. (1998).

**Data Analysis:** Statistical techniques like mean values, standard deviations, ANOVA and correlations were used to analyzed the collected data (Steel *et al.*, 1996).

## RESULTS AND DISCUSSION

This experiment was conducted to examine the growth performance of *Cirrhina mrigala* as affected by the chronic water-borne and dietary nickel exposure. The data on average wet weight, fork and total length increments in treated and control mediums were recorded on weekly basis and the mean values are presented in Table 1. Significantly maximum weight increment of 1.31±0.31 g was observed during 11<sup>th</sup> week while the same was minimally recorded as 0.18±0.03 g during 2<sup>nd</sup> week of the study period. The three age groups showed maximum weight increment in control medium as compared to treated with nickel.

Chronic nickel stress to the fish exerted a significant ( $p < 0.01$ ) impact on its growth in terms of average weights, fork and total lengths increment. Treated fish showed significantly lower value of weight increment than that of the control fish. The 90-day fish showed significantly better weight, fork and total length gain followed by that of 65- and 45-day age groups. These results were in similar with the findings of Hollis *et al.* (2000) they examined the effect of long term sub-lethal cadmium exposure on rainbow trout (*Oncorhynchus mykiss*) which showed reduction in growth rates, swimming performance and oxygen utilization. Sub-lethal metal exposure to fish may result in enhanced metabolic costs and ultimately the storage of the lipids and productive process depressed ( Congdon *et al.*, 2001 ; Sherwood *et al.*, 2000) Ptashynski *et al.* (2001) conducted an experiment to study the effect of nickel uptake by the adult lake whitefish fed with sub-lethal dietary metal. High nickel concentrations were observed in the fish kidney, liver and other organs while low in intestine. Blood glucose and electrolytes of fish were badly affected along with decrease in their body weights.

Significantly maximum and minimum fork and total length increments were recorded during 12<sup>th</sup> and 1<sup>st</sup> weeks, respectively, of the study period. The three age groups followed the same pattern as weight increments for increase in fork and total length increments in control and treated medium. Fish showed decrease in fork and total length increments in treated medium as compared to the control medium throughout the study period. These results were similar with Hayat *et al.* (2007) they exposed the fingerlings of three major carps viz, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, for 30 days to sub-lethal concentrations of manganese. It was concluded that control fish showed significant increase in weight, fork and total lengths than stressed major carps, under sub-lethal concentrations of manganese when reared under semi-intensive culture system.

During the 1<sup>st</sup> week of study period, feed intake by the fish was minimum (0.13±0.04 g) that gives the weight increment of 0.21±0.13 g, showing the better feed conversion ratio during the 1<sup>st</sup> week. The feed

conversion ratio in treated and control mediums by 90-day fish showed statistically non-significant differences at  $P < 0.05$ . Fish showed significantly maximum feed intake in control medium as compare to treated medium during the 12 weeks of experiments. However, the feed conversion ratio was significantly better in control fish than treated medium. The results were similar with the work of Vincent *et al.* (2002) as they concluded that heavy metals contamination usually showed depletion in food utilization parameters. Murai *et al.* (2003) studied the effects of dietary copper on channel catfish. During the experimental period purified diets with 0, 2, 4, 8, 16 and 32 mg supplemental copper/kg were fed to channel catfish fingerlings. Reduced growth and feed conversion were noted in fish fed 16 and 32 mg copper/kg. Mohanty *et al.* (2009) investigated the effect of copper on survival, growth and feed intake of *Cirrhina mrigala* for 60 days. At 0.23 mg L<sup>-1</sup> of copper there was almost no growth and at all the copper treatments feed intake rates reduced significantly ( $p < 0.05$ ).

The condition factor (K) calculated throughout the study period showed statistically non-significant differences among the 12 weeks of the study period. Among the three age groups 45- and 65-day control fish showed significantly better condition factor in control medium as compare to the nickel exposed medium. The fish reared in unstressed medium showed better length and weight relationship as compare to fish stressed with sub-lethal waterborne and dietary concentrations. However, the condition factor for 90-day fish in treated and control mediums were statistically at par (Table, 1). These results were accordingly with Ali *et al.* (2003).

They reported significant effect of different sub-lethal concentrations of water-borne copper on fish. A reduction in weight, specific growth rate and condition factor (K) has been observed.

The treated and control fish showed positively significant correlation between increase in wet weight with total ammonia (NH<sub>3</sub>) and electrical conductivity of the test medium. The relationship between magnesium and calcium was negatively significant. The physico-chemical parameters showed almost statistically non-significant correlation among each other and with increase in wet weight. The fish showed the correlation between carbondioxide and dissolved oxygen significantly positive while the relationship between calcium and magnesium was negatively significant and the relationship between magnesium and calcium was negatively significant while all other parameters showed statistically non-significant relationship with each other (Tables 2-4). Hypoxic conditions such as increase in temperature and acidification usually render the fish more susceptible to intoxication while increase in mineral contents (hardness and salinity) reduces metal's toxicity to the fish (Witeska and Jeziarska, 2003). Datta and Das (2003) reported that for *Cyprinus carpio* and *Catla catla*, the raise in water hardness from 60 to 720 mg L<sup>-1</sup> CaCO<sub>3</sub>, total alkalinity from 32 to 376 mg L<sup>-1</sup> CaCO<sub>3</sub>, pH from 7.60 to 7.90 and chlorides from 28 to 350 mg L<sup>-1</sup>, showed increase in 96-hr LC<sub>50</sub> of lead in *Cyprinus carpio* from 8.20 to 1291 mg L<sup>-1</sup>, while 5.30 to 865 mg L<sup>-1</sup> for *Catla catla*. Lead toxicity also decreased with increase in pH from 6.30 to 11.30.

**Table 1: Analysis of variance on growth parameters of *Cirrhina mrigala* reared under chronic water-borne and dietary nickel exposure.**

Week #	Comparisons of means					
	Inc. wt. (g)	Inc. F.l (mm)	Inc. T.l (mm)	F. intake (g)	FCR	K
1	0.21±0.13 fg	0.82±0.13 d	0.75±0.13 d	0.13±0.04 f	1.04±0.35 c	1.45±0.07 a
2	0.18±0.03 g	0.94±0.10 d	1.14±0.13 cd	0.34±0.05 ef	1.78±0.15 ab	1.43±0.07 a
3	0.35±0.06 efg	1.34±0.15 cd	1.59±0.16 bcd	0.67±0.12 de	1.96±0.05 a	1.44±0.07 a
4	0.48±0.09 defg	1.58±0.14 bcd	1.58±0.28 bcd	0.90±0.16 cd	1.63±0.27 ab	1.45±0.07 a
5	0.68±0.09 cdef	1.57±0.27 bcd	1.76±0.32 bc	1.22±0.15 bc	1.57±0.28 ab	1.49±0.07 a
6	0.73±0.13 bcde	2.24±0.22 ab	1.90±0.34 bc	1.34±0.24 abc	1.84±0.11 ab	1.49±0.08 a
7	0.82±0.12 abcde	2.36±0.23 a	2.18±0.21 ab	1.38±0.22 abc	1.68±0.04 ab	1.51±0.07 a
8	0.91±0.151 abcd	2.12±0.26 abc	1.93±0.37 bc	1.50±0.23 ab	1.67±0.07 ab	1.54±0.07 a
9	1.10±0.25 abc	2.04±0.39 abc	2.09±0.47 ab	1.41±0.32 abc	1.38±0.15 bc	1.58±0.10 a
10	0.98±0.26 abc	2.37±0.29 a	2.79±0.36a	1.38±0.30 abc	1.49±0.10 abc	1.58±0.10 a
11	1.31±0.31 a	2.48±0.49 a	2.88±0.36 a	1.83±0.37 a	1.46±0.12 abc	1.61±0.10 a
12	1.20±0.38 ab	2.59±0.43 a	2.93±0.48 a	1.58±0.41ab	1.47±0.14 abc	1.62±0.12 a

## Treatments × Age Groups

Age groups	Inc. wt (g)		Inc. F.l. (mm)		Inc. T.l. (mm)		F. intake (g)		FCR		K	
	Ni Exp.	Control	Ni Exp.	Control	Ni Exp.	Control	Ni Exp.	Control	Ni Exp.	Control	Ni Exp.	Control
45-day	0.38± 0.01 <sup>b</sup>	0.72± 0.12 <sup>a</sup>	1.38± 0.15 <sup>b</sup>	1.70± 0.22 <sup>a</sup>	1.45± 0.15 <sup>b</sup>	1.83± 0.28 <sup>a</sup>	0.63± 0.12 <sup>b</sup>	0.99± 0.13 <sup>a</sup>	1.20± 0.16 <sup>b</sup>	1.72± 0.08 <sup>a</sup>	1.26± 0.04 <sup>b</sup>	1.69± 0.09 <sup>a</sup>
65-day	0.57± 0.11 <sup>b</sup>	0.73± 0.10 <sup>a</sup>	1.68± 0.19 <sup>b</sup>	1.96± 0.27 <sup>a</sup>	1.75± 0.23 <sup>b</sup>	1.99± 0.28 <sup>a</sup>	0.96± 0.13 <sup>b</sup>	1.15± 0.14 <sup>a</sup>	1.49± 0.13 <sup>b</sup>	1.91± 0.12 <sup>a</sup>	1.48± 0.02 <sup>b</sup>	1.64± 0.03 <sup>a</sup>
90-day	0.75± 0.08 <sup>b</sup>	1.32± 0.285 <sup>a</sup>	1.97± 0.27 <sup>b</sup>	2.52± 0.29 <sup>a</sup>	2.02± 0.29 <sup>b</sup>	2.73± 0.32 <sup>a</sup>	1.18± 0.17 <sup>b</sup>	1.93± 0.35 <sup>a</sup>	1.57± 0.08 <sup>a</sup>	1.59±0.14 <sup>a</sup>	1.50± 0.027 <sup>a</sup>	1.51± 0.01 <sup>a</sup>

Means sharing similar letter in a row for each parameter are statistically non-significant ( $p>0.05$ ).

Inc. wt.: Increase in weight

FCR :Feed conversion ratio

Inc. F. l : Increase in fork length

K : Condition factor

Inc. T. l : Increase in total length

Ni Exp: Nickel exposure

F. intake: Feed intake

**Table 2: Correlation coefficients between increase in weight and physico-chemical parameters of three age groups used during the sub lethal nickel exposure.**

	NH <sub>3</sub>	DO	CO <sub>2</sub>	EC	Na	K	Ca	Mg
<b>DO</b>	-0.066 1.687							
<b>CO<sub>2</sub></b>	-0.035 1.535	0.999* 0.605						
<b>EC</b>	0.404 2.063	-0.220 1.650	-0.345 2.187					
<b>Na</b>	0.084 2.642	1.265 0.645	0.083 2.307	-0.664 0.968				
<b>K</b>	0.280 2.108	-0.158 2.433	0.821 1.265	-0.232 1.799	-0.694 0.962			
<b>Ca</b>	-0.205 1.485	-0.052 2.400	0.143 1.934	-0.082 1.281	-0.452 1.846	-0.007 2.098		
<b>Mg</b>	0.220 1.520	0.063 2.272	-0.116 1.878	0.073 1.234	0.202 2.267	0.008 2.137	-0.999** 0.000	
<b>Inc. wt</b>	0.922** 0.261	0.102 0.971	-0.430 1.656	0.216 1.399	0.011 2.701	0.186 0.992	0.090 1.299	-0.128 1.287

Upper values indicated Pearson's correlation coefficient.

Lower values indicated level of significance at 5% probability.

\* = Significant ( $P<0.05$ ); \*\* = Highly significant ( $P<0.01$ )

NH<sub>3</sub> = Total ammonia (mg L<sup>-1</sup>); DO = Dissolved oxygen (mg L<sup>-1</sup>); CO<sub>2</sub> = carbon dioxide (mg L<sup>-1</sup>); EC = Electrical conductivity (mS cm<sup>-1</sup>); Na= Sodium (mg L<sup>-1</sup>); K = Potassium (mg L<sup>-1</sup>); Ca = Calcium (mg L<sup>-1</sup>); Mg =Magnesium (mg L<sup>-1</sup>); Inc. wt. = Increase in weight (g)

**Table 3: Correlation coefficients between increase in weight and physico-chemical parameters of three age groups used during the study period in control medium.**

	NH <sub>3</sub>	DO	CO <sub>2</sub>	EC	Na	K	Ca	Mg
DO	0.466 0.848							
CO <sub>2</sub>	0.293 1.832	0.279 1.579						
EC	0.996* 0.890	0.217 0.948	-0.377 2.129					
Na	0.049 2.304	0.703 1.505	0.139 1.591	-0.993* 0.664				
K	0.707 0.940	-0.114 1.541	-0.016 1.172	0.999* 1.014	-0.820* 0.680			
Ca	-0.281 2.284	0.877 1.080	-0.156 1.378	-0.148 2.319	0.086 1.927	-0.237 2.423		
Mg	0.682 1.491	-0.660 1.545	0.058 1.246	0.034 2.588	-0.167 2.015	0.248 2.399	-0.999** 0.001	
Inc. wt	0.999** 0.000	0.397 0.912	0.467 1.744	0.982* 0.893	0.080 2.468	0.783 1.378	-0.322 2.240	0.654 1.568

Upper values indicated Pearson's correlation coefficient.

Lower values indicated level of significance at 5% probability.

\* = Significant (P&lt;0.05); \*\* = Highly significant (P&lt;0.01)

NH<sub>3</sub> = Total ammonia (mg L<sup>-1</sup>); DO = Dissolved oxygen (mg L<sup>-1</sup>); CO<sub>2</sub> = carbon dioxide (mg L<sup>-1</sup>); E.C = Electrical conductivity (mS cm<sup>-1</sup>); Na= Sodium (mg L<sup>-1</sup>); K = Potassium (mg L<sup>-1</sup>); Ca = Calcium (mg L<sup>-1</sup>); Mg =Magnesium (mg L<sup>-1</sup>); Inc. wt. = Increase in weight (g)**Table 4: Physico-chemistry of treated and control water medium for 45-day, 65-day and 90-day *Cirrhina mrigala* at constant water temperature, pH and total hardness.**

	Age Groups					
	45-day		65-day		90-day	
	Treated	Control	Treated	Control	Treated	Control
Temperature (°C)	29.41±0.63	29.98±1.06	29.90±1.06	30.50±1.10	29.54±2.90	30.68±0.91
pH	7.01±0.01	7.00±0.01	7.02±0.03	7.00±0.02	7.00±0.01	7.00±0.01
Total hardness (mg L <sup>-1</sup> )	200.75±0.79	201.15±0.69	201.10±0.91	201.32±0.47	201.47±0.42	206.47±6.47
Total Ammonia (mg L <sup>-1</sup> )	2.21±0.15	1.22±0.10	2.55±0.28	1.29±0.13	2.32±0.15	1.22±0.10
Dissolved oxygen (mg L <sup>-1</sup> )	4.31±0.24	5.37±0.27	4.42±0.23	5.41±0.30	4.54±0.26	5.60±0.20
Carbon dioxide (mg L <sup>-1</sup> )	1.38±0.66	1.67±0.49	1.50±0.52	1.41±0.51	1.42±0.51	1.45±0.51
Electrical Conductivity (m Scm <sup>-1</sup> )	2.97±0.32	3.09±0.36	3.23±0.48	3.22±0.29	3.10±0.30	3.35±0.45
Sodium (mg L <sup>-1</sup> )	300.08±11.30	302.67±9.49	302.33±10.15	301.08±11.60	303.33±9.62	304.41±13.78
Potassium (mg L <sup>-1</sup> )	8.56±0.61	8.73±0.66	8.87±0.57	8.65±0.66	8.94±0.51	8.17±0.68
Calcium (mg L <sup>-1</sup> )	15.53±4.82	15.53±4.28	16.86±4.54	16.53±4.67	16.68±4.07	16.20±3.93
Magnesium (mg L <sup>-1</sup> )	40.49±2.58	40.58±2.68	39.71±2.82	40.00±2.88	39.94±2.54	41.94±3.01

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