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# Effect of Sublethal Doses of Copper on Growth Performance and Survival Rate of Grass Carp (*Ctenopharyngodon idella*)

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**Abstract:** An experiment was conducted to determine the $LC_{50}$/96h value of copper sulfate and assess the growth performance of grass carp) *Ctenopharyngodon idella* (juvenile during 60 days of exposure to sublethal copper concentrations (Cu). After acclimation period of two weeks, to determine the $LC_{50}$/96h value, total of 24 fiberglass tanks each stocked with 30 fishes were used in our experiments. 21 fiberglass tanks and seven concentrations of Cu composed the 24 treatments while three other fiberglass tanks were used as control. For each treatment, three replications were conducted and experiment to determine the growth performance; fish were transferred into fiberglass tank of 200 L water capacity for growth trials. The treated fish were kept in the tanks containing sublethal concentrations of Cu (0.86 and 0.17 mg $L^{-1}$) for 60 days, while control fish were placed in metal free water. The results indicated that median lethal concentration ($LC_{50}$) of copper to grass carp for 96h of exposure are 1.717 ppm. The chronic sublethal water-borne Cu exposure to the fish exerted that juvenile had significantly decreased final body weight in comparison to control treatment. The growth factors and survival rate of juvenile in control treatment were compared to those fishes were in the chronic sublethal of copper sulfate. The results showed that juvenile in control treatment had significantly increased in final body weight in comparison to other treatments. Cu also had significant negative effects on specific growth rate (SGR) and food conversion efficiency (FCE) in comparison to control treatment. The food conversion ratio (FCR) and condition factor (CF) were significantly increased in comparison with the control treatment ($P<0.05$). Also survival rate in experimental treatments in comparison with control treatment, was significantly decreased ($P<0.05$).

**Key words:** Copper Sulfate · $LC_{50}$ · Grass Carp · *Ctenopharyngodon idella* · Growth Performance · Survival Rate

## INTRODUCTION

The rapid increase in human population has escalated the demand for quality food, like fish, in the world. To fulfill the food requirements, fish assume greater importance because, it contains high quality proteins, fats and minerals. Another feature of fish is its ability to convert raw materials into high quality proteins more efficiently than other terrestrial animals such as sheep, goats, cows etc [1]. Fish is an indicator to measure the freshwater contamination by heavy metals because they occupy different trophic levels in the aquatic ecosystem. The trace metals are essential for normal physiological processes. However, their high concentrations can be toxic to aquatic organisms [2]. Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals [1&3]. All living organisms require several essential trace metals. During biological evolution of prokaryotes and eukaryotes, several metals become incorporated as essential factors in many biochemical functions more or less in accordance with the abundance of these metals on the planet. As a result, the biological importance of transition metals is marked roughly in the order: Fe, Zn, Cu, Mn, Co and Ni [4]. Inhalation of metals may occur as a consequence of industrial exposure or atmospheric contaminator [5]. The tremendous increase in the use of heavy metals over the past few decades has

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inevitably resulted in an increased flux of metallic substances in the aquatic environment [6]. The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal pollution in natural water [7]. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life [8&9]. Heavy metals have long been recognized as serious pollutants of the aquatic environment.

Heavy metals such as copper have gained wide interest in the scientific community in recent years due to its potential human health hazards. Copper in the form of copper sulfate is used as an algaecide and as a therapeutic chemical for various ectoparasitic and bacterial infections [10]. Copper is an essential metal for all organisms including fish. It has an important role in metabolism and its concentration is well regulated. However, Cu is one of the most toxic metals to fish and affects various blood parameters, growth, behavior, enzyme activity and reproduction [11]. Metals are unique among pollutants, which cause adverse health effects, in that they occur naturally and in many instances are ubiquitous in the environment. Heavy metals have long been recognized as serious pollutants of the aquatic environment. Chronic exposure of fish to water-borne Cu, Cd or Zn has been shown to cause a variety of physiological and behavioral changes including loss of appetite, reduced growth, ionic loss and increased fish mortality [12]. Heavy metal contamination usually causes depletion in food utilization in fish and such disturbance may result in reduced fish metabolic rate and hence causing reduction in their growth [13]. Growth is a sensitive and reliable endpoint in chronic toxicological investigations [14].

The present study was design to investigate acute toxicity of copper and toxic effect of Cu on the growth performance and survival rate of grass carp under chronic sub-lethal concentration to evaluate its potential to growth in contaminated water.

## MATERIALS AND METHODS

Uniform juveniles of grass carp were obtained from the Institute of Pond Fish Culture in Gorgan (Agh Ghala), Iran. They were weighted (initial weight 4.3±0.5 g). The total length of the fish was also measured accurately (8.2±0.44 cm). Fish were fed with aquatic plant food (*Lemna* sp.) at least twice a day before during the experiments and the fish were not fed during the experiments. Physicochemical parameters viz. temperature, pH, total hardness, dissolved $O_2$, total $NH_3$, Na, K and $CO_2$ of the treated and control media were monitored on daily basis by following the methods of [15]. However, water temperature (24±1°C) pH (7-7.5) and hardness (275±2.5mg$L^{-1}$) was kept constant throughout the study. Nutritional compositions of experimental diets (*Lemna* sp.) are given in Table 1. Proximate composition of diets was carried out using the Association of Analytical Chemists AOAC, 2000 [16] methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40-60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550°C.

Table 1: Nutrient composition of experimental diets (%)

| Ingredients | % |
|---|---|
| Protein | 28 |
| Lipid | 11.4 |
| Fiber | 2.7 |
| Ash | 6 |

To investigate acute toxicity of copper, all fiberglass tanks (60 L) capacity, were filled with 50 L of dechlorinated tap water. A total of 24 a fiberglass tank that each stocked with 30 fishes were used in our experiments for Cu. Stock solutions of copper sulphate were prepared by dissolving analytical grade copper sulphate ($CuSO_4.5H_2O$ from Merck) in double distilled water. 30 fish were used per concentration of Cu. Ninety-six hours acute bioassays were performed following in general OECD guidelines for fish acute bioassays (guideline OECD203, 92/69/EC, method C1) [17]. For determination of the $LC_{50}$/96h (lethal concentration) values, following a range finding test, eight Cu (0.5, 0.75, 1, 1.5, 2, 2.5 and 3 mg/l) concentrations were chosen for grass carp. For metal-treated and control three replications were conducted. Metal solutions were prepared by dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The number of dead fish was counted every 12 h and removed immediately from the fiberglass tanks. The mortality rate was determined at the end of 24, 48, 72 and 96 h. Acute Toxicity test was conducted in accordance with standard methods [15]. In this study the acute toxic effect of copper on the grass carp was determined by the use of Finney's Probit Analysis $LC_{50}$ Determination Method [16]. Confidential limits (Upper and Lower) were calculated and also used SPSS18 for $LC_{50}$

value of copper with the help of probit analysis. Thereafter to investigate the toxic effect of Cu on the growth performance of grass carp under chronic sublethal concentrations, this experiment was conducted in a completely randomized design with three treatments. Separate groups of 60 fish each served as control for copper. 5% and 10% of $LC_{50}$/96h concentration for copper sulfate (0.86 and 0.17 mg $L^{-1}$) was used as sublethal levels for grass carp. The fish were weighed individually at the beginning and at the end of the experiment. At the end of the experiment, total juveniles from each tank were sampled and the final weight and length of body were measured. Growth parameters of fish were calculated based on the data of biometry of grass carp juvenile. One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

## RESULTS AND DISCUSSION

**$LC_{50}$/96h of Copper for Grass Carp:** Acute toxicity of copper showed that mortality is directly proportional to the concentration of the copper sulphate while the percentage of mortality is virtually absent in control (Table 2). Table 2, shows the relation between the copper concentration and the mortality rate for 96h of grass carp. Results according to SPSS18 analysis showed that the median lethal concentration ($LC_{50}$) of copper sulphate to grass carp for 96h of exposure is 2.422ppm (Table 3). In this experiment behavioral changes of the fish before and after the application of the toxic compound were recorded.

Behavioral changes of the fish before and after the application of the toxic compound were monitored. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. The results clearly showed that the copper sulphate had harmful effects on the growth parameters of grass carp.

Table 2: Showing correlation between the copper sulphate concentrations and the mortality rate on time (96h) of grass carp

| Concentration(mg $l^{-1}$) | Fish | Mortality rate on time (96h) |
|---|---|---|
| 0.00 | 30 | 0 |
| 0.50 | 30 | 0 |
| 0.75 | 30 | 3 |
| 1.00 | 30 | 6 |
| 1.50 | 30 | 11 |
| 2.00 | 30 | 18 |
| 2.50 | 30 | 26 |
| 3.00 | 30 | 30 |

Table 3: Lethal concentration ($LC_{1-99}$) of copper sulphate on time (24-96h) for grass carp

| Point | Concentration(mg $l^{-1}$) | (95% confidence limits) |
|---|---|---|
| $LC_1$ | 0.184 | (0.217-0.460) |
| $LC_5$ | 0.633 | (0.336-0.845) |
| $LC_{10}$ | 0.873 | (0.626-1.055) |
| $LC_{15}$ | 1.034 | (0.818-1.200) |
| $LC_{50}$ | 1.717 | (1.571-1.873) |
| $LC_{85}$ | 2.399 | (2.206-2.666) |
| $LC_{90}$ | 2.561 | (2.347-2.862) |
| $LC_{95}$ | 2.800 | (2.553-3.156) |
| $LC_{99}$ | 3.249 | (2.934-3.713) |

The feeding and growth parameters of grass carp are presented in Table 4. The growth performance and survival rate in control treatment of grass carp was better and had significantly different than treated groups. ($P<0.05$), followed by treatment T1 in growth factors had significant difference from other treatments (control group and T3) ($P<0.05$).

Actually, both different treatments of copper sulphate (T1 and T2) were significantly different to each other in growth performance and survival rate ($P<0.05$). The maximum of final body weight (FBW) observed in control group (6.80±0.1g), followed by better FBW obtained in treatment T1 (6.3±0.12g), (5 percent concentration of $LC_{50}$ of copper sulphate, 0.86 mg $L^{-1}$) and the lowest FBW observed in T2 (5.9±0.14g), (10 percent concentration of $LC_{50}$ of copper sulphate, 0.17 mg $L^{-1}$) and had

Table 4: Growth parameters and survival rate of Grass carp (*Ctenopharyngodon idella*) in experimental treatments (trial 1and 2) and control group

| | Treatments | | |
|---|---|---|---|
| Growth Indices | Control | T1 0.86 mg $L^{-1}$ copper sulphate | T2 0.17 mg $L^{-1}$ copper sulphate |
| Initial weight (g) | 4.30±0.1 | 4.33±0.06 | 4.30±0.1 |
| Final body weight (g) | 6.80±0.1$^a$ | 6.3±0.12$^b$ | 5.9±0.14$^c$ |
| Body weight Gain (g) | 2.50±0.17$^a$ | 1.97±0.1$^b$ | 1.6±0.17$^c$ |
| Specific growth rate for weight (% BW $day^{-1}$) | 0.76±0.05$^a$ | 0.64±0.03$^b$ | 0.53±0.05$^c$ |
| Feed Conversion Ratio (%) | 23.60±1.0$^c$ | 33.86±2.6$^b$ | 37.4±2.2$^a$ |
| Feed Conversion efficiency (%) | 0.042±0.0$^a$ | 0.035±0.0$^b$ | 0.027±0.0$^c$ |
| Daily Growth Rate (DGR) | 0.97±0.08$^a$ | 0.77±0.0$^b$ | 0.62±0.07$^c$ |
| Survival rate (%) | 93.22±3.28$^a$ | 79.55±3.11$^b$ | 64.13±3.19$^c$ |

Groups with different alphabetic superscripts in the same row differ significantly at $p<0.05$ (ANOVA) significantly different to other treatments (P<0.05). This is particularly true for specific growth rate (SGR), where the highest value was obtained in the control treatment (0.76±0.05) and showed significantly different to T1 (0.64±0.03) and T2 (0.53±0.05) (P<0.05). Also, for any of growth performance, that among the tow different concentrations of copper sulphate to grass carps, the greatest effect appeared to be obtained in treatments T1 (concentration 0.86 mg $L^{-1}$ of copper sulphate). The food conversion ratio (FCR) in the experimental treatments was significantly increased in comparison with control treatment (p<0.05) that the highest FCR was obtained in T2 (37.4±2.2), followed by treatment T1 (33.86±2.6). Also the greatest (lowest) FCR observed in control group (23.60±1.0) and had significantly different to each other (P<0.05). The highest survival was rate observed in control group (93.22±3.28) and had significantly different to T1 (79.55±3.11) and T2 (64.13±3.19), also T1 showed better result than T2 in this comparison and significantly different to each other (P<0.05).

Many metals apparently stimulate the activities in fishes by probably acting as a physical irritant to a potentially wide assortment of external tissues, causing an elevated metabolic rate [18]. Fish respond immediately to the presence of copper and show hyperactivity and reduced exploratory behavior [19&20]. Drummond *et al.* [21] reported that copper at low sublethal concentrations stimulated the locomotor activity in brook trout, *Salvelinus fontinalis*. The behavioral changes observed during this study are in close conformity with these findings. Al-Akel [22] and Al-Kahem [23] also reported similar types of behavioral changes in *O. niloticus* when subjected to different copper concentrations. Rising of the treated fish to the upper surface of the water with wide-open mouths indicate that they might be short of oxygen when exposed to different copper concentrations.

Metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem [24] and this is attributed to bioaccumulation, whereby metal ions are taken up from the environment by the organism and accumulated in various organs and tissues. Metals also become increasingly concentrated at higher trophic levels, possibly due to food-chain magnification [25]. The toxicity reported by other studies differs from this study probably due to different species used, aged, size of the organism, test methods and water quality such as water hardness, as this can affect toxicity [26&27]. Toxicity of metals may vary depending upon their permeability and detoxification mechanisms [28].

And toxic effect of Cu on the growth performance of grass carp under chronic sub-lethal concentration showed that the copper sulphate had harmful effects on the growth parameters on grass carp.

These results are in accordance with the findings of Kim and Kang [29] that reported a reduction in growth rate of rockfish (*Sebartes schlegeli*) due to Cu stress and there was an inverse relationship between growth and Cu exposure. Hayat *et al.*[30] exposed the fingerlings of three major carps viz. *Catla Catla, Labeo rohita* and *Cirrhina mrigala*, to sublethal concentrations of manganese for 30 days. During this exposure period, all the fish species showed negative growth. Ali *et al*. [31], observed reduction in growth of *Oreochromis niloticus* under different (0, 0.5, 0.3, & 0.5 ppm) water-borne Cu levels.

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