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# LOW DOSE EFFECTS OF CADMIUM AND LEAD ON GROWTH IN FINGERLINGS OF A VEGETARIAN FISH, GRASS CARP (*CTENOPHARYNGODON IDELLA*).

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## ABSTRACT

A study was conducted to evaluate the effects of cadmium and lead on growth in the fingerlings of a truly vegetarian fish, grass carp (*Ctenopharyngodon idella*). Single breed fingerlings (12.0 ± 1.25 cm) of grass carp were obtained from a commercial fish seed hatchery recommended for good quality of fish seeds. Five treatments groups (T1-T5) in triplicate each containing 50 fingerlings of grass carp were maintained at 24 ± 0.25°C, DO 7.18 ± 0.24 mg/l, pH 6.7 ± 0.22 and hardness 140.7 ± 3.8 mg/l in 90 liters of water in glass tanks. A zero dose treatment (T 1) was maintained as control. While other fingerlings were exposed to 5.0 µg/l (permissible) and 500 µg/l (sublethal) of waterborne cadmium (Cd) as treatment 2 and 3 respectively and 50 µg/l (permissible) and 1000 µg/l (sublethal) of waterborne lead (Pb) as treatment 4 and 5 respectively. Permissible (according to European Union standards for Cd and Pb in drinking water) and sub-lethal concentrations of Cd and Pb were prepared by dissolving cadmium chloride and lead acetate in deionized water. All fish were fed with pelleted green leaf feed (Cd, 0.05 – 0.08 µg/g and Pb, 0.08 – 0.11 µg/g dry weight) to an equivalent of 3 % wet body weight twice daily. Uneaten food and the feces were removed at 30 minutes after feeding from all tanks daily. It was observed that cadmium tiureatments caused significant ($p<0.05$) reduction in growth as compared to lead treatments and control group. The present studies has revealed that even very low concentrations (permissible) of heavy metals like Cd and Pb, that are considered as safe, do cause effects on growth of a freshwater fish meant for human consumption. It is therefore recommended to take particular precautionary measure to safeguard our water bodies from being contaminated with industrial effluents containing various heavy metal ions.

**Key words:** fish growth**,** grass carp, heavy metal toxicity, permissible dose, sub lethal dose, non-essential heavy metals, Cd & Pb.

## INTRODUCTION

Generally in fishes, the toxic effect of heavy metal influence physiological functions, individual growth and mortality (Rose, *et al*., 2006). Fish can be used as an indicator of fresh water contamination by heavy metals because they occupy different trophic level in an aquatic ecosystem. High exposure concentration of heavy metals is known to be toxic for aquatic organisms but the low metal concentration also cause toxicity when they are introduced into the environment by a wide spectrum of natural and anthropogenic source. Heavy metals are non biodegradable and once they enter the aquatic environment their bio-accumulation may occur in fish tissue by means of metabolic activities and bio-absorption process (Ahmed and Bibi, 2010; Ahmed *et al*., 2011).

Cadmium is an "end of the road" heavy metal pollutant, bioavailable and toxicologically active. It is one of the most toxic metals and constitutes a threat for fish because of its widespread occurrence in the aquatic environment (Bervoets and Blust, 2003). Even at very low environmental concentrations, fish can accumulate Cd ions that eventually cause deleterious effects (Hensen *et al*., 2002). When exposed to water-borne Cd, fish often show a decreased energy uptake and retarded growth. Consequently, both decreased growth and reproduction have been used as sub-lethal endpoints in ecotoxicological tests for Cd

Most studies on the effects of Cd in fishes have focused on its lethal toxicity. Information about the long-term exposure effects of very low i.e permissible (US-EPA-issued permissible level of Cd in drinking water is 5 ppb) and sub-lethal concentrations of Cd on growth in fishes is very limited. A study on amphibian tadpoles revealed that low concentrations of dietary Cd have bimodal effects on growth (Sharma and Patino, 2008). Inhibition of growth was found at 0.1-10 ppb, no effect was observed at 100 ppb, and inhibition was again reported at 1000 ppb. This complex pattern of contaminant effects at low concentrations has been termed as "low-dose effect" and has important implications for the development of environmental and human health risk assessments. A study on goldfish revealed that dietary Cd at relatively high doses (10 mg/g dry wt. in the diet) inhibited growth and impaired behavior whereas relatively low doses (1 mg/g) Cd stimulated growth (Szczerbik *et al*., 2006). Dietary Cd at doses as high as 100 mg/kg slowed the growth and altered the behavior of tilapia (*Oreochromis niloticus*) after long-term exposure (16 months) (Nogami *et al.*, 2000).

Lead released into water as a result of weathering of soil, sewage and industrial effluents which leads to bioaccumulation through topical absorption and food ingestion in various body tissues of aquatic animals like fishes (Ahmed and Bibi, 2010). Kasthuri and Chandaran (1997) conducted sublethal lead exposure on a catfish, *Mystus gulio* and found decreasing trends in feeding energetic and growth rates in young fish as compared to adults. Zaki *et al.* (2010) assessed the hematological, biochemical and immunological damage leading to altered growth in *Tilapia zilli* because of lead poisoning. In another study conducted by Alkahmal-Balawi *et al.* (2011) on African catfish, *Clarias gariepinus* exposed to sublethal concentration of lead acetate (140 mg/l) revealed reduced growth, altered hematological parameters and reproduction. Lead acetate is also been found causing histological damage in gills and digestive system leading to reduced growth in silver sailfin, *Poecilia latipinna* (Mobarak *et al*., 2011). Nursita *et al*. (2005) found some selected heavy metals (Cd, Cu, Pb & Zn) has lowering effects on growth rate in *Proisotoma minuta* because of been accumulated in the body and affecting metabolism. While in a review study about three metals (Cd, Pb & Hg), Mobarak (2008) revealed developmental toxicity and tertogenicity in fishes.

Grass carp is an herbivorous fish having habit of continuously consuming aquatic vegetations (phytoplankton, algae and submerged plant). Because of its high intake of aquatic food as well as water (ingested alongwith food during feeding), takes-up high contents of waterborne metal ions associated with food. Juvenile grass carp is a good model for metal exposures and can easily be maintained in the laboratory conditions. It is an equally important fish among other commercial fishes meant for human consumption. While being in natural water bodies (those receiving metal pollutants even at lower concentration), until they reach the marketable size (> 2 years) would already have been under the influence of toxic effects of heavy metal that could alter / retard growth. Lower fish production, under the influence of toxicants will lead to significant economic losses on one hand and availability of contaminated fish on the other hand that may hamper human health. Keeping in view the economic importance of grass carp and low dose toxicity of Cd and Pb, present study was carried out to determine the effects caused by intoxification of permissible and sub-lethal concentration of cadmium and lead (nonessential heavy metals) on growth in fingerlings of grass carp.

# MATERIALS AND METHODS

**Experimental fish model:** Single breed fingerlings measuring 6-8 ± 1.2 cm were purchased from a commercial fish seed hatchery. All fish were given 0.1% methylene blue bath and acclimatized to 12:12-hrs light:dark regime in 90 liters glass tanks for two week in a flow through system prior to metals exposure. Fifteen glass tanks each containing 50 fingerlings (healthy and uniform in size) were maintained at 24 ± 0.25 °C, DO 7.18 ±0.24 mg/l, pH 6.7 ± 0.22 and hardness 140.7 ± 3.8 mg/l (table 1). All fish were fed with pelleted green leaf feed (Cd, 0.05 – 0.09 µg/g and Pb, 0.08 – 0.16 µg/g dry weight) to an equivalent of 3 % wet body weight twice daily. Uneaten food and the feces were removed at 30 minutes of feeding from all tanks daily.

**Metal concentrations and exposure:** Fish fingerlings were exposed to various concentrations of Cd and Pb for 8 weeks. For Cd, 5.0 µg/l as treatment 2 (permissible) and 500 µg/l treatment 3 (sublethal concentration). Exposure concentrations of lead were 50 µg/l as treatment 4 (permissible) and 1000 µg/l as treatment 5 (sublethal). A zero treatment group (T1) was also paralleled to these exposure groups for comparison. Permissible (according to European Union standards for Cd and Pb in drinking water) and sub-lethal concentrations of Cd and Pb were prepared by dissolving cadmium chloride and lead acetate in deionized water. All concentrations were maintained in glass tank (in triplicate for all treatments). All tanks were continuously aerated using electric aerators. Cadmium and Pb levels in exposure media were monitored through the entire duration of the study. A pilot trial indicated that their levels in the present test system are stable for at least 7 days (M. S. Ahmed, unpublished observations). Fifty percent of the concentration solution exchange was done weekly as to stabilize exposure solution concentrations.

**Fish and exposure water sampling:** Five fish from each tank (15 per treatment) were sampled on day zero and weekly later on. Fish were euthanized by immersing in 3-aminobenzoic acid ethyl ester (MS-222, Sigma-Aldrich Chemical, St Louis, MO; at 1.0 g/l), until the cessation of opercular movement. The fingerlings were washed in clean water, paper dry, weighed (g) and measured for total length (cm). Physicochemical parameters like temperature, dissolved oxygen (DO), pH and hardness were measured directly from the tanks using YSI 85 meter (Yellow Springs, OH).

**Statistical analysis:** One-way nested ANOVA was used for assessing the effects on growth in all groups. Mean separations was conducted using Tukey's multiple comparison tests. An overall α value of 0.05 was used to assess significant differences. Most statistical analyses were conducted with STATISTICA data mining software, version 8 (StatSoft, Inc., Tulsa, OK). Bartlett's test was used to assess homogeneity of variances. Data are expressed as standard error of means (± SEM)

## RESULTS

**Effect of cadmium (Cd) on growth:** The average increase in total length in the fingerlings of grass carp, *C. idella* at permissible concentration of Cd was similar as at zero concentration. At sublethal concentration of Cd, a non-significant increase in total length was observed from day 0-21 p.e (post exposure) but later on no change in length was observed until day 56 p.e. While a significant ($p<0.05$) increase in total length was observed from day 42-56 in control group as compared to both exposure concentrations (figure 2). The average increase in wet body weight was similar at zero and permissible concentration. At sublethal concentration the average increase in wet body weight was not different until day 28 but the weight of fish started decreasing thereafter and become significantly different ($p<0.05$) from day 42-56 p.e. from zero and permissible concentrations (figure 3).

**Effect of Lead (Pb) on growth:** A non-significant difference in total length was noticed from day 7 – 56 p.e. at permissible concentration of Pb when compared with zero concentration while at sublethal concentration of Pb, the increase in total length was similar as in zero concentration until day 21 p.e. but thereafter no change in length was observed until day 56 which was significantly lower ($p<0.05$) from zero as well as permissible concentrations (figure 4). There was a non-significant difference in increase in wet body weight at zero and permissible exposure concentrations until day 35 and a significant difference was observed on day 56 p.e. At sublethal exposure concentration there was no significant increase in wet body weight until day 21 thereafter a rapid decrease was observed and it become significant ($p<0.05$) from day 35 until day 56 p.e. when compared with zero and permissible concentrations (figure 5).

**Table 1: Physico-chemical parameters maintained in the water for fingerlings of grass carp during Cd and Pb exposure. Data is presented as ±SEM, n=15**

| Weeks | Temperature (C°) | pH | Hardness (mg/l) | Dissolved oxygen (mg/l) |
|---|---|---|---|---|
| 0 | 23.5 | 6.9 | 140 | 7.4 |
| 1 | 24.1 | 7.0 | 134 | 6.9 |
| 2 | 24.0 | 6.8 | 145 | 7.5 |
| 3 | 24.3 | 6.3 | 138 | 7.1 |
| 4 | 24.1 | 6.5 | 139 | 7.3 |
| 5 | 24.1 | 6.6 | 144 | 7.3 |
| 6 | 23.9 | 6.8 | 145 | 6.8 |
| Mean | 24.0 ±0.25 | 6.7 ±0.22 | 140.7 ±3.8 | 7.18 ±0.24 |

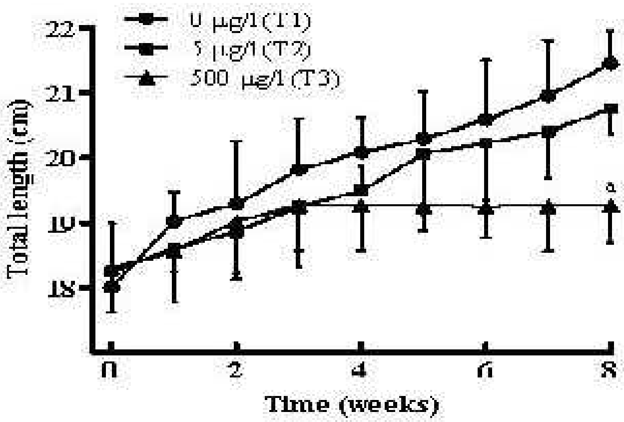

**Fig. 1: Effects of various concentrations (T1, 0.0 µg/l; T2, 5 µg/l and T3, 500 µg/l) of waterborne cadmium on length in the fingerlings of grass carp. The letter a on the bar represent significant (P<0.05) difference of T3 from T1.**

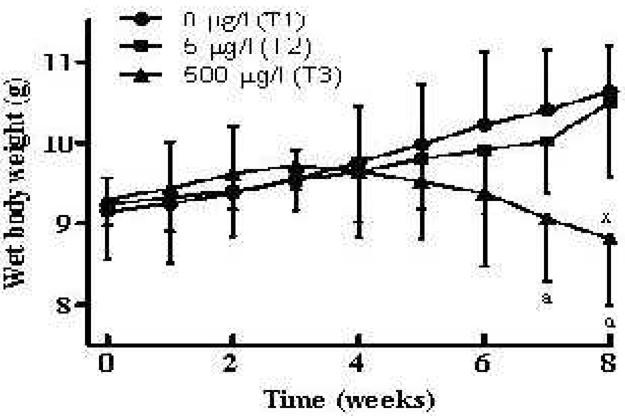

**Fig. 2: Effects of various concentrations (T1, 0.0 µg/l, T2, 5 µg/l and T3, 500 µg/l) of waterborne cadmium on weight in the fingerlings of grass carp. The letter a on the bar represent significant (P<0.05) difference of T3 from T1 and x from T2.**

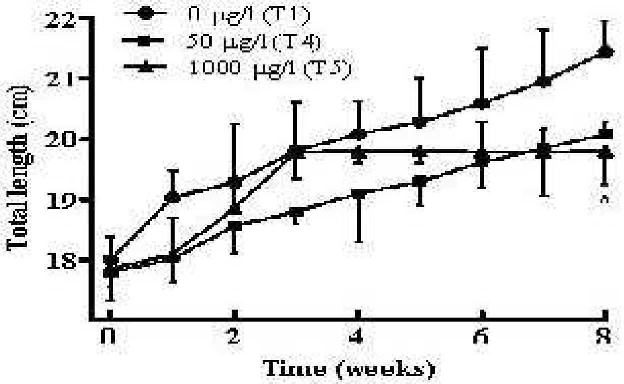

**Figure 3: Effects of various concentrations (T1, 0.0 µg/l, T4, 50 µg/l and T5, 1000 µg/l) of waterborne lead on length in the fingerlings of grass carp. The letter a on the bar represent significant (P<0.05) difference of T5 from T1.**

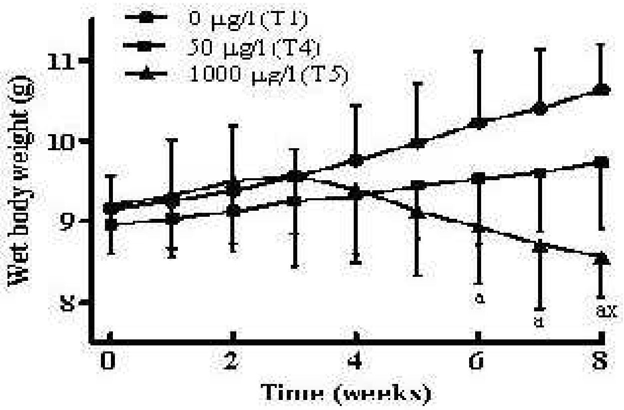

**Figure 4: Effects of various concentrations (T1, 0.0 µg/l, T4, 50 µg/l and T5, 1000 µg/l) of waterborne lead on weight in the fingerlings of grass carp. The letter a on the bar represent significant ($P<0.05$) difference of T5 from T1 and x from T4.**

## DISCUSSION

The most common endpoint used by earlier studies of toxicity of Cd in aquatic organism (fish, amphibians etc) is survival and growth. It is also known that Cd cause adverse effects on reproduction and osmoregulation in fishes (Verbost *et al*., 1989; Park *et al*., 2006; Sellin and Kolok *et al*., 2006; Pierron *et al*., 2007; Sofvan *et al*., 2007; van Dyke *et al*., 2007; Cao, *et al*., 2009, Mobarak *et al*., 2011). The changes in the wet body weight and total length at permissible concentration (5 µg $l^{-1}$) of cadmium showed some difference when compared with zero exposure concentration but not significantly different. While the changes at sub-lethal exposure concentration (500 µg $l^{-1}$) of waterborne cadmium has negative effects on growth during last few weeks of exposure experiment as compared to zero and permissible exposure concentration. This decrease in wet body weight and total length showed some increase during first three weeks and then no change in length was observed afterward eventually representing significant influence of Cd toxicity on fish growth. This reduction in growth is attributed as minimum utilization of food during exposure period. It was also observed that fish feeding behaviour was altered with the passage in time. Vincent *et al*., (2002) exposed *Catla catla* to sub-lethal concentration of waterborne cadmium and found that cadmium exhibited depletion in food utilization parameters in fish and it was concentration dependent. They concluded that metal ion intoxication was found to exhibit reduction in total biomass in fishes. In another study on white sucker larvae, *Ecitoslomus commersoni* and young common shiners, *Notropis commits* were exposed to cadmium chloride at concentrations ranging from 6.24 to 200 µg $l^{-1}$. The relative growth rate of fish was significantly reduced at Cd concentration 36 µg $l^{-1}$ or more in the case of white suckers and 63 µg $l^{-1}$ or more in the case of common shiners. Inhibitory effects of Cd on Xenopus tadpole growth were observed at 0.1µ g/l, a level that is below the current federal safety criterion of 0.35 µg Cd/l (at hardness of 170 mg/l; U.S. Environmental Protection Agency, 2006) for the protection of freshwater aquatic life. Stage of development and rate of metamorphic climax initiation seemed to show a slight trend for a biphasic response to Cd exposure, but these endpoints were significantly different (lower) only at 855 µg Cd/l. None of the animals exposed to 855 µg Cd/l was able to advance beyond NF 51 (hind limb emergence) by the end of the 47-day exposure (Sharma and Patino, 2008). They further added that thyroid follicle cell height was also reduced in tadpoles at exposure concentrations of 855 µg Cd/l, even when the comparison was made against the sub population of NF 49–51 animals pooled from the 0 to 84 µg Cd/l groups. These observations indicate that the thyroid gland not only failed to activate at 855 µg Cd/l, but also that its activity was suppressed resulting into retarded growth in Xinopus.

The change in average wet body weight was observed at permissible concentration of waterborne Pb but not at significant level when compared with zero concentration during first half of exposure duration which later on became significant on day 56 p.e. The changes in wet body weight and total length at sub-lethal exposure concentration of lead, negative growth was observed as compared to other two concentrations. There was decrease in average wet body weight but not significantly different until day 21 p.e but it was significantly different from day 35 until 56 p.e. when compared to zero and permissible exposure concentrations. Similarly there was some increase in total length during the first few weeks of exposure but no change was observed later on which means no increase in length and minimum increase in wet body weight of the exposed fish resulting minimum increase in biomass. Even at the lowest lead concentration (0.20 µmol $l^{-1}$), metabolic demand for the fish increased, challenging the carp with increased demand for food. It is also known that lead accumulation mainly occurred in various tissues (Ahmed and Bibi, 2010) particularly liver, reaching equilibrium between uptake and excretion after one month of exposure. Similar findings are documented by Kasthuri and Chandaran (1997) when they exposed three different size groups of the estuarine catfish (*Mystus gulio*) to sub-lethal concentration of lead. They found that there was a decreasing trend in feeding energetic and growth rate of the three size group fingerlings seemed to be more sensitive to Pb poisoning followed by immature and then mature fish. The metal ions like Pb and Hg not only influence growth but also accumulate in various tissues of exposed fishes (Ahmed and Bibi, 2010, Ahmed *et al*., 2011). Thus heavy metals like Cd and Pb intoxication even at very low concentration i.e. permissible and sublethal levels was found to exhibit reduction in biomass of aquatic organisms particularly in commercial fishes.

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