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# Short and Long-Term Effects of Waterborne Cadmium on Growth and its Muscle Accumulation in Common Carp Fish (*Cyprinus carpiol*.), an Experimental Study

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**Abstract**

Common carp fish (*Cyprinus carpio* Linnaeus, 1758) were exposed to varying concentrations of waterborne cadmium (10, 50 and 100 ppb) during 30, 60 and 90 days, respectively. At the end of each experiment time, the influence of cadmium on fish growth parameters including total length (TL) and weight (W) were measured and its accumulation in muscles was also determined. Data obtained showed that although growth rate of fish in all cadmium treatments were lower than control group, this differences was not significant statistically (P>0.05). Our finding related to cadmium accumulation indicated that exposure to cadmium as cadmium chloride for 30, 60 and 90 days lead to a significant increase (P<0.05) in cadmium concentrations in the fish muscles. Accumulation of cadmium in muscles has been elevated with increasing in concentration and duration of cadmium exposures. Therefore the maximum accumulation has been observed in third treatment (i.e. 100 ppb for dissolved cadmium) after 90 days of exposure. Bioconcentration factors (BF) of cadmium for fish muscles were also calculated for understanding the rate of cadmium accumulation. Inverse relationships were therefore observed between BF and water cadmium concentration. In other word, the maximum accumulation rate has been detected at lower cadmium concentration.

*Keywords*: Cadmium, common carp, growth, accumulation, bioconcentration factor.

**Su Kaynaklı Kadmiyumun Sazan Balığının (*Cyprinus carpiol.*) Gelişimine ve Kaslarda Birikimine Kısa ve Uzun Vadede Etkisi, Deneysel Bir Çalışma**

**Özet**

Sazan Balıkları (*Cyprinus carpio* Linnaeus, 1758) çeşitli konsantrasyonlarda (10, 50 ve 100 ppb) sırasıyla 30, 60 ve 90 gün boyunca su kaynaklı kadmiyuma maruz bırakılmıştır. Her deney sonunda, kadmiyumun boy (TL) ve ağırlık (W) gibi büyüme parametreleri ile kas birikimine etkileri tespit edilmiştir. Elde edilen sonuçlara göre, kadmiyum muamelesi görmüş balıkların büyüme oranlarının kontrol grubuna göre daha düşük olmasına rağmen istatistiki açıdan önemli bir fark (P>0.05) bulunamamıştır. Bulgularımıza göre kadmiyumu kadmiyum klorid olarak muamele ettiğimiz balıkların kasta kadmiyum birikimi zamana göre 30, 60 ve 90 günde öenmli bir (P<0.05) artış göstermiştir. Kaslarda kadmiyum birikimi kadmiyum muamelesinin konsantrasyon ve süre artışına göre parallel bir artış göstermiştir. Bundan dolayı maksimum birikim, muamelenin 90. gününün sonunda 3. örnekte gözlemlenmiştir. (Örneğin; 100 ppb çözünmüş kadmiyum için) Balık kasları için Biyokonsantrasyon faktörleri (BF) de kadmiyum birikim oranını anlamak için hesaplanmıştır. Bundan dolayı BF ve su kaynaklı kadmiyum konsantrasyonunda zıt bir iliki gözlemlenmiştir. Diğer bir deyimle maksimum birikim oranı en düşük kadmiyum konsantrasyonunda tespit edilmiştir.

***Anahtar Kelimeler:*** Kadmiyum, sazan balığı, gelişme, birikim, biyokonsantrasyon faktörü.

## Introduction

Cadmium is a non–essential toxic element, which can be entered in the aquatic environment from industrial process, chemical agricultural manuring and mining activities (Sorensen, 1991; Wood, 2001). Cadmium is also well documented as a highly toxic metal for human and animals' health (Ball, 1967; Moshtaghie *et al*., 1997; Bressler *et al*., 2004; Zaki *et al*., 2009).

Generally, heavy metals such as cadmium presents in the aquatic environment as ionic form ($Cd^{2+}$); it can simply enters to aquatic organisms' body, enters blood circulation and bind to sulfhydryl groups of protein, which can be accumulated in their body (Hadson, 1988; Kargın, 1996; Quig, 1998).

Usually considerable amount of cadmium will be accumulated in different tissue and it is based on pattern of exposure, diet or waterborne (Kraal *et al*., 1995), uptake and elimination rates (Ruangsomboon and Wongrat, 2006). Cadmium accumulation in gills, liver and kidney has been reported in several literatures (Giles, 1988; Bentley, 1991; Kock *et al*., 1996; De Conto Cinier *et al*., 1999; Hollis *et al*., 2000; Zohouri *et al*., 2001; Hans *et al*., 2006) whereas accumulation of cadmium in fish muscles was reported in a few articles (Hilmy *et al*., 1985; Suresh *et al*., 1993; De Conto Cinier *et al*., 1999). Due to biomagnifications of cadmium in food chain and its consumption by human, this could be considered as the most important subject for aquatic science (Kraal *et al*., 1995; Mansour and Sidky, 2002).

Cadmium exposure may lead to the results of some pathophysiological damages (Thomas, 1985); including growth rate reduction in fish (Kaviraj and Ghosal, 1997; Hansen *et al*., 2002) and also in other aquatic organisms (Das and Khangarot, 2010).

The $LC_{50}$ 96 hr for cadmium is dependent on water quality parameters (Alabaster and Lloyd, 1982) and fish characteristics (Witeska *et al*., 1995; De Conto Cinier *et al*., 1999). Consequently this index has been reported for fry and fingerling carp 4.3 and 17.1 ppm respectively (Suresh *et al*., 1993) and 22 ppm for adults carp (Wase and Forster, 1997).

Consideration of prolonged toxicity studies is clearly important because in the aquatic environment, organisms usually expose to low pollutant concentration with long period of time due to their long biological half-life (Kargin and Coğun, 1999; Silvestre *et al*., 2006). In this respect, the main aim of this study was to investigate the relationship between low cadmium concentration in water and its accumulation in common carp muscles as a long experimentally study. Furthermore, the influence of low cadmium concentrations on fish growth performance in the long-term exposure was also studied.

## Materials and Methods

### Materials and Instruments

All the chemicals used for this study were analytically grade. $CdCl_2$, 2.5 $H_2O$ was obtained from BDH Chemical Company and used for this study. HCl (with cadmium concentration less than 1 ppb) was provided from ROMIL Chemical Company (UK) and used for sample preparing test. Distilled deionized water were used for preparing stock solution of cadmium with cadmium concentration below than 1 μg/L. laboratory glass–ware were prewashed with $HNO_3$ 10% (analytically grade) and then washed with distilled deionized water. Plastic–ware were washed with formalin solution 4% and water initially for disinfection purpose. They were then washed with 10 mmol EDTA and three washing cycles to minimized cadmium contamination. Each tank was then filled with 200 L of water and containing 45 fish. Common carp fish (*Cyprinus carpio*) 4.75±1.02 g in weight (W) and 6.95±0.54 cm in total length (TL) were obtained from a fish farming (Karaskan, Isfahan, Iran). Fish were fed with normal diet (3% of body mass) daily for two times. During this study the water quality parameters including electrical conductivity (EC), total dissolved solid (TDS), pH, nitrate ($NO_3^-$), phosphate ($PO_4^{3-}$), dissolved oxygen (DO), total hardness, and temperature were measured by Conductivity meter, Jenwey 4310, England; metler Toledo, CheckMate 90, USA; Hanna Combo set, Italy; Ion Analyzer, Jenway 3040, England; Spectrophotometer, Jenway 6400, England; WTW, DXI 325, Germany; chemical titration method and Thermometer respectively. Water not changed during each period of experiment but aerated with air pump and circulated by water pump.

### Treatment of Animals

Initially, three groups of cadmium treatment and one control group were designed based on experimental purposes. Fish were exposed to three concentrations of cadmium (10, 50 and 100 ppb) for short and long-terms. To do this, cadmium chloride stoke solution was prepared by dissolving of 1.95 g cadmium chloride salt in 1 L of distilled water with final concentration1 g/L of cadmium. Aliquots of 2, 10 and 20 ml of stock solution were then added to each individual tank in order to obtain desired concentrations of cadmium. These concentrations were below than 1% of $LC_{50}$ 96 hr for this species (Suresh *et al*., 1993; Wase and Forster, 1997).

In order to investigate the effect of waterborne cadmium on growth rates of common carp fish, initially, all fish were anesthetized with clove oil and then W and TL of each fish were measured. These measurements were repeated at the end of each period of times (30, 60 and 90 days).

Five random samples were withdrawn from each experimental treatments tank and were then killed. Then 1 g of dorsal muscle of them were separated and covered by aluminum foil and froze at –20°C. Remained fish in the tanks were anesthetized by 100 ppm of clove oil (Coyle *et al*., 2004) and then the growth rate of them was also measured. After biometrical achievement in fish, the water of each tank was changed with freshwater and treated as mentioned above, the same as beginning of experiment.

A few mortality was observed in this project that may be due to siphon action, exchange water, biometry and not related to cadmium treatments.

Laboratory measurements were carried out with drying samples in the oven at 50ºC for 48 hr. Samples of muscle were then ashed at 550-600°C and then dissolved in HCl (cadmium concentration <1 ppb). Then cadmium concentrations in this solution were determined by Potentiometric Stripping Analyzer Ion. Finally bioconcentration factor (BF) according to Taylor (1983) was also calculated in this experiment.

### Statistical Analysis

SPSS software (version 13) was used for statistical analysis. One-Way analysis of variance (ANOVA) with LSD complementary test with 95% confidence limit was also applied for comparing variables and evaluates the statistical differences (Zar, 1999).

## Results

### Study of Water Quality Parameters

Daily water characteristics parameters including DO, pH, T, EC and TDS were measured; other chemical parameters were measured weekly. These analytical parameters were EC: 309–357 µs/m$^2$; TDS: 154–177 mg/L; pH: 7.3–7.61; $NO_3^-$: 25.98–44.6 mg/L; $PO_4^{3-}$: <0.6 mg/L; DO: 7.8–10.9 ppm; Total Hardness: 173–189 mg $CaCo_3$/L and T: 15.7–17.2°C.

### Cadmium Accumulation in Fish Muscle

This experiment was performed to investigate muscle accumulation of cadmium during short and long period exposures to waterborne cadmium. To approach this, five random samples were chosen from each tank following 30, 60 and 90 days. After preparing of sample as mentioned in methods, the amount of cadmium that accumulated in muscles were then measured. The obtained results show significant differences in all treatments in comparison to control groups (P<0.05) (Table 1). Cadmium contents of fish muscle following daily treatment with 10, 50 and 100 ppb for 30 days were 30.82±5.88, 70.67±5.42 and 112.06±10.12 µg per kg of dry weigh respectively. The level of cadmium that accumulated in fish muscle has been shown in Table 1. Maximum cadmium bioaccumulation for each treatment was detected on day 90.

### Bioconcentration Factor (Bf) Measurement

BF was calculated as a supplementary index to find out the amount of cadmium accumulation in fish muscles in comparison to water cadmium contamination. This index for carp muscle was calculated for three treatments. The BF for cadmium treatments of 10, 50 and 100 ppb after 30 days was 3.08±0.58, 1.41±0.11 and 1.12±0.1 respectively. This index in 60 days was 7.5±0.82, 5.45±0.1 and 4.85±0.24 for cadmium treatments, respectively. And at the end of experiment, calculated BF's were 14.53±0.6, 13.95±0.18 and 12.44±0.17. It was found that BF for muscle accumulation was decreasingly (Figure 1).

**Table 1.** Accumulation of cadmium (µg/kg dry W) in common carp muscles

| Exposure Time (Days) | Control (Mean±SD) | Treatment 1 (Mean±SD) | Treatment 2 (Mean±SD) | Treatment 3 (Mean±SD) |
|---|---|---|---|---|
| 30 | 0.09±0.07 | 30.82±5.88* | 70.67±5.42* | 112.06±10.12* |
| 60 | 0.06±0.03 | 74.99±7.58* | 272.59±5.04* | 485.47±23.98* |
| 90 | 0.07±0.04 | 145.34±5.97* | 697.32±9.27* | 1243.71±15.38* |

(Treatment1: 10 ppb, treatment2: 50 ppb and treatment3; 100 ppb). Each data calculated from 5 random samples. Significant differences from control group were expressed by (*) mark (P<0.05).

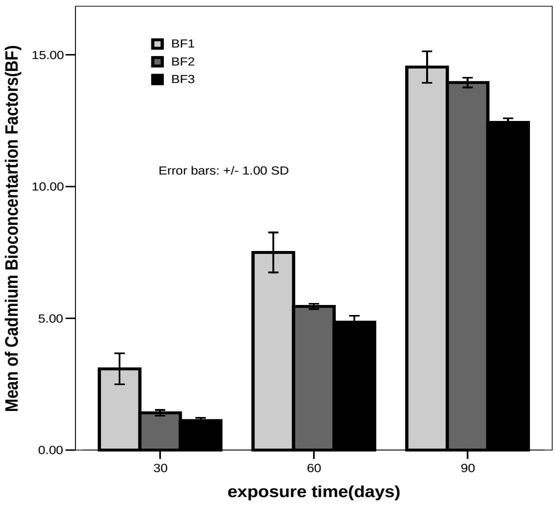


**Figure1.** Means ±SD for BF data in common carp fish muscles are shown. $BF_1$, $BF_2$ and $BF_3$ are related to cadmium treatments 0.01, 0.05 and 0.1ppm respectively. Cadmium accumulation graphs have also been shown.

### Effect of Cadmium on Fish Growth Rate

For understanding fish growth rate, W and TL of all fish were measured at the start and at the end of each experiment time. The changes of W and TL for all treatments were also described as relative changes in W and TL from the beginning of experiment (Table 2). Significant difference ($P<0.05$) for W was only observed in treatment 50 ppb in day 90. This reduction was not significant for other treatments (Table 2). The fish size at the beginning of experiment was different, but this difference was not significant for W; treatment 50 ppb had only significant difference ($P<0.05$) with treatment 100 ppb for TL (Table 2). Generally the maximum W following 90 days exposure was observed in control group. Relative change for control group was generally more than all cadmium treatments. The increase pattern for fish growth was nearly similar for all cadmium treatments (Figure 2), however significant reduction ($P<0.05$) was only observed in treatment 50 ppb (Table 2). The TL for fish, which has been treated with 50 ppb cadmium for 90 days, was lower than other treatments, whereas this difference not significant statistically (Table 2).

## Discussion

Hans *et al.* (2006) reported that accumulation of cadmium in some organs of common carp fish is dependent on time of exposure and doses of cadmium. The data, which has been presented in this study show that waterborne cadmium can produce a significant accumulation in fish muscle. Cadmium exposure as 50 ppb (treatment 2) resulted in elevation of cadmium in muscle following 60 days by 3.85–fold in comparison with the 30 days of cadmium treatment; whereas 2.5–fold elevation in cadmium accumulated was seen following 90 days of exposure in comparison with 60 days of exposure. As shown in Table 1, similar elevation was seen for other treatments. The accumulated cadmium in muscle suggested that cadmium content in fish muscle has elevated by time of exposure, but this increase is not linear with time of exposure. In agreement to our results, Suresh *et al.* (1993) reported that cadmium accumulation in common carp fingerling fish has a declining dependence with duration of exposure. According to our present results, the cadmium accumulation in fish muscle is correlated to the amount of cadmium in ambient water. Kim *et al.*

**Table2.** Effect of varying concentrations of cadmium (10-100 ppb) on fish growth rate

| Exposure Time (Days) | Parameters | Control (Mean±SD) | Treatment1 (Mean±SD) | Treatment2 (Mean±SD) | Treatment3 (Mean±SD) | Relative change ($M_{90}/M_0$) |
|---|---|---|---|---|---|---|
| 0 | W | 4.82±1.07 $^{a}$ | 4.83±1.28 $^{a}$ | 4.59±0.76 $^{a}$ | 4.88±0.77 $^{a}$ | +3.85 |
| | TL | 6.96±0.65 $^{ab}$ | 7.03±0.63 $^{ab}$ | 6.82±0.46 $^{a}$ | 7.15±0.37 $^{b}$ | +1.53 |
| 30 | W | 6.76±1.59 $^{a}$ | 6.76±1.69 $^{a}$ | 6.46±1.26 $^{a}$ | 6.90±1.04 $^{a}$ | +3.57 |
| | TL | 7.58±0.66 $^{ab}$ | 7.52±0.62 $^{ab}$ | 7.37±0.39 $^{a}$ | 7.74±0.41 $^{b}$ | +1.47 |
| 60 | W | 11.42±2.13 $^{a}$ | 11.18±1.47 $^{a}$ | 10.94±2.50 $^{a}$ | 11.52±1.97 $^{a}$ | +3.61 |
| | TL | 8.91±0.97 $^{a}$ | 8.70±0.92 $^{a}$ | 8.53±0.74 $^{a}$ | 8.96±0.92 $^{a}$ | +1.49 |
| 90 | W | 18.58±3.01 $^{b}$ | 17.26±2.09 $^{ab}$ | 16.57±3.17 $^{a}$ | 17.35±3.74 $^{ab}$ | +3.55 |
| | TL | 10.67±1.02 $^{a}$ | 10.36±0.82 $^{a}$ | 10.15±0.96 $^{a}$ | 10.41±0.98 $^{a}$ | +1.45 |

The data have been shown as Mean±SD for W (g) and TL (cm). Significant differences ($P<0.05$) were indicated with alphabetic letters. Relative changes were also represented in last column.

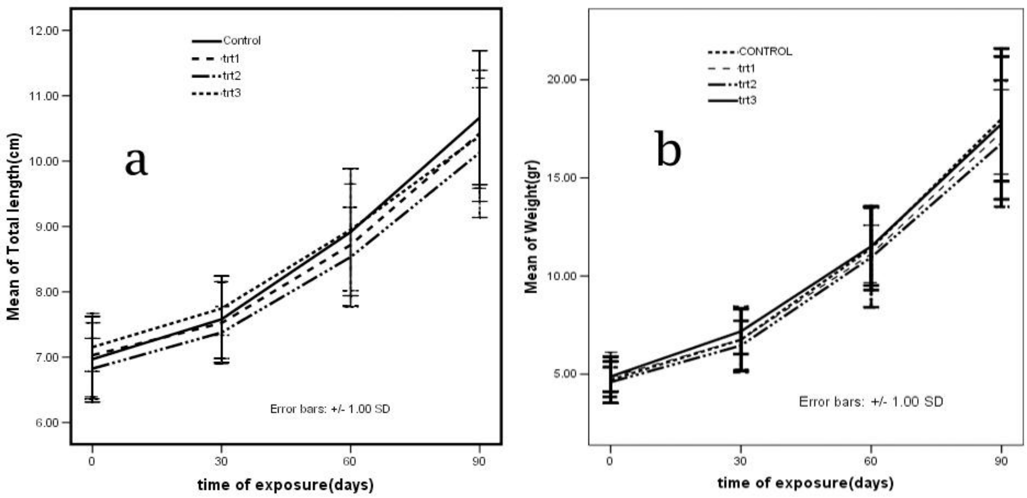


**Figure2.** Increasing pattern for common carp TL (a) and W (b) were represented as mean±SD as a result of cadmium treatments (0.01- 0.1 ppm) and also control group.

(2004) were also shown a similar increasing pattern in cadmium accumulation of olive flounder muscle. The muscle accumulation data have been presented in Table 3 for probable comparison to present investigation.

Similar results could be derived from Bioconcentration factor. As shown in Figure 1, BF for any individual will be increased with increasing duration time of exposure. It is obvious that the amount of increased for treatment with 10 ppb for 60 and 90 days became 2.44-fold and 3.23-fold higher than 30 days of exposure. Same elevation was seen for other treatment in our investigation (Figure 1). It has been therefore, found that association between BF and duration of exposure was increasingly but the inverted relationship between BF and water cadmium concentration was observed (Figure 1). In consistent to our results similar relationship between BF and exposure time, and also between BF and cadmium administration dose was also reported by De Conto Cinier *et al.* (1999).

As mentioned earlier, it seems that cadmium may reduce the growth rate in common carp fish, although due to differences in fish size at the beginning of experiment and also treatment with very low level of cadmium no statistical result was seen in the reduction of growth. However the fish growth rate in control group was higher than all cadmium treated at the end of experiment (Table 2). Some reports in the literature were also confirming our results. No significant effect of several lethal and sublethal doses of cadmium on growth rate in different fish species has been reported (Hilmy *et al*., 1985; Hollis *et al*., 1999; 2000; Zohouri *et al.*, 2001; Ossana *et al*., 2009). Reduction of growth rate in common carp fish (*Cyprinus carpio*) after cadmium exposure was reported in several literatures (Kaviraj and Ghosal, 1997; Hans *et al*., 2006). Reduction of weight in Bull trout (*Salvelinus confluenteus*) was detected at 0.786 ppb cadmium treatment; but after 55 days exposure to lower level of waterborne cadmium, smaller fish compared to control group was also seen (Hansen *et al.* 2002). Dietary cadmium can also reduces fish growth rate as 10 mg Cd per g food in Golden fish (*Carassius auratus gibelio*), but in lower dose, the deceasing effect has not been reported (Szczerbik *et al*., 2006). Adversely, low cadmium concentration may lead to elevation of growth in some fish species (Sloman *et al*., 2003). Reports on this subject is varying according to fish species, cadmium administrations period, exposure way and also water quality parameters.

In general, it can be concluded that cadmium is a deleterious agent for metabolism of anabolic hormones and can delay growth hormone expression in fish (Jones *et al*., 2005). Cadmium as a toxic element might be a stressor agent for fish. Stress has several effects on fish growth; first, the stress can increase cortisol level (Pratap and Wendelaar Bongaf, 1990; Gill *et al*., 1993) and as a result of this hormone the fish growth might be postponed (Jentoft *et al*., 2005). Secondly, stress can also reduce food intake and assimilation in administrated fish (Borgmann and Ralph, 1986). The interaction of cadmium with other micro and macro elements such as zinc, calcium, which are essential for growth has been also demonstrated previously (Fairbanks, 1982; Moshtaghie *et al*., 1997; Jezierska and Witeska, 2001). According to our pervious study, cadmium can also disturb bone metabolism in common carp fish (Malekpouri *et al*., 2011) and caused to diminution of exposed fish growth. Cadmium may cause to damage the beta cells in pancreas and so lead to decline the insulin hormone, this hormone is responsible for cytoplasmic growth (Heath, 1987). Cadmium can probably inhibit the absorption of some essential amino acid for growth and other essential nutrient for fish growth.

Low existence of cadmium in ambient water can produce significant accumulation of cadmium in fish muscle and its subsequent consumption by human may cause some pathophysiological disturbances in human body. Regarding that, further investigation needs to be done in order to elucidate the exact of muscle cadmium toxicity on human.

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**Table3.** The muscle accumulation of waterborne cadmium in some fish species

| Fish Species | Cadmium Concentration (ppb) | Exposure Time (days) | Muscle Accumulation (µg/kg) | Reference |
|---|---|---|---|---|
| *Cyprinus carpio* | 53 | 50 | 1000> | De Conto Cinier *et al*., 1999 |
| *Cyprinus carpio* | 53 | ~90 | ~500 | De Conto Cinier *et al*., 1999 |
| *Paralichthys olivaceus* | 10 | 30, 50 | >400, >400 | Kim *et al*., 2004 |
| *Paralichthys olivaceus* | 50 | 30, 50 | <600, >700 | Kim *et al*., 2004 |
| *Paralichthys olivaceus* | 100 | 30, 50 | 600, >800 | Kim *et al*., 2004 |

The presented data in this Table are comparable with data, which is exhibited in Table 1. The exact amounts of accumulation in all mentioned studies were not available because the authors provided their results as graphs.

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