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# Effect of Cadmium on Fin Regeneration in the Freshwater Fish, *Oreochromis mossambicus*

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Metals are unique environmental and industrial pollutants in the sense that they are neither created nor destroyed by human beings but are only transported and transformed to various products (Shukla and Pandey 1985). Cadmium has been recognized as one of the potent aquatic pollutants and most common contaminant of industrial effluents (Sprague 1987). Cadmium is known to cause a wide spectrum of toxic effects to fish viz., vertebral deformities (Bengston et al. 1975), retarded larval growth (Woodworth and Pascoe 1982), teratogenicity (Eaton 1974), hematological alterations (Ruparelia et al. 1990), reproductive impairment (Karels et al. 2003), genotoxicity in terms of development of micronuclei (Zhu et al. 2004) and adreno-toxic effect with higher endocrine disruptive potential (Lacroix and Hontela 2004). Besides abundant literature on cadmium toxicity to fish little is known about the effect of cadmium on fin regeneration (Weis and Weis 1976).

Fishes are able to regenerate their fin after amputation/ablation or being lost (Wallace 1981). On one hand the fish loses its prey capture capacity and on other hand easily becomes the victim to predators, making their life hard for survival (Weis and Khan 1991). The regeneration capacity of fish allows them to restore their morphology. Morphological regeneration is effective in adopting these populations to changing/competitive local environment, and also protecting them from their enemies (Mari-Beffa et al. 1999). The absence of whole fin or part of fin puts fish at a disadvantage in nature. Thus, regrowth of fin is always beneficial to fish to restore the missing part. Kemp and Park (1970) first studied the regeneration of tail fin of the tilapia fish, *Oreochromis mossambicus.* Fin regeneration was chosen as an effect parameter because it is a model of differentiation and growth phenomena, which are sensitive to toxicants, and results can be obtained within two week as wound healing occurs faster in fish species than terrestrial vertebrates (Fontenot and Neiffer 2004). The purpose of the present investigation was to determine whether fin regeneration could be used as an animal model for aquatic toxicity test, and to assess the effect of cadmium on fin regeneration. Moreover, cadmium was chosen as a test compound because it is a known teratogen in developing embryos and thus would be expected to disrupt the process of fin regeneration.

## MATERIALS AND METHODS

The freshwater fish, *Oreochromis mossambicus* (Peters), used in this study were obtained from a local pond and acclimatized for 20 days in a PVC tank of 1100 L

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capacity. After that fish were transferred to different glass aquaria of 50 L capacity. Average weight and length of the test fish was (9.08±1.65 g) and (9.06±0.72 cm), respectively. The physical and chemical characteristic of dilution water used in this study was determined as per the standard methods (APHA 1985). The intended nominal concentration of cadmium in test water was 1.0, 2.0 and 4.0 ppm. Control set of experiment was run side by side. The test water was renewed and re-dosed twice in a week in order to maintain proper concentration of cadmium and good water quality. Cadmium concentration in experimental medium was measured without any prior treatment on an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 373). The experimental protocol is summarized in Table 1.

**Table 1.** Summary of test protocol for fish bioassay.

| Name of the fish | *Oreochromis mossambicus* |
|---|---|
| Weight and length of fish | Average wt.9.08g; average length 9.0 cm |
| Test type | Static renewal |
| Temperature and Light | $27 \pm 2^{0}$ C, Ambient laboratory illumination |
| Test vessel | 50 liter glass aquarium |
| Test solution volume | 30 liter |
| Test organism/vessel | 10 |
| Test concentrations | 1.0, 2.0 and 4.0 ppm of cadmium |
| Feeding regime | Feeding continued during exposure period |
| Diluent water | Bore- well dilution water |
| Test duration | 17 days |
| Observation period | 7,12 and 17 days |
| Parameter | regeneration/regrowth |

Forty specimens of the fish have been studied for their capacity for morphological restoration following ablations/amputation. Fishes were divided into four equal groups of 10 each. Group I served as control. Fish of group II, group III and group IV were exposed to cadmium (as cadmium acetate) at the nominal concentration of 1.0, 2.0 and 4.0 ppm, respectively. Fish were fed standard fishmeal twice a day and supplemented with *Tubifex* worms thrice in a week. The lower half portion of the caudal fin was amputated just before its base line, with the help of sharp razor. Study was conducted for 17 days and caudal fin regenerated length was measured with the help of transparent scale in mm (Conant 1973) after $7^{th}$, $12^{th}$ and $17^{th}$ days of exposure and post amputation. Data were subjected to analysis of variance (ANOVA) followed by post-hoc test (Bonferroni and Tukey) between and within the various groups for the test of significance. A linear regression analysis was also performed for regression equation and coefficient of determination ($R^2$) value to find out correlation between exposure concentration and fin growth inhibition.

## RESULTS AND DISCUSSION

Results of the study are shown in Figures (1 & 2) and Tables (2-4). Physico-chemical characteristics of dilution water are given in Table 2. The chemical characteristics

**Table 2.** Physicochemical characteristic of dilution water.

| Parameters | Unit | Mean Value |
|---|---|---|
| Temperature | $^0C$ | 29.77 |
| pH | - | 7.97 |
| Total hardness (as Ca $CO_3$) | ppm | 220.00 |
| Alkalinity (as Ca $CO_3$) | ppm | 333.56 |
| Dissolved oxygen | ppm | 8.51 |
| Zn | ppb | 76.89 |
| Cr | ppb | 5.26 |
| Cd | ppb | ND |
| Pb | ppb | 12.42 |
| Cu | ppb | 26.75 |
| Mn | ppb | 2.71 |
| Ni | ppb | 9.02 |

ND= Not detected

showed that water used in this study was hard (220.0 ppm) and alkaline (333.56 ppm). The level of metals in dilution water showed that zinc had highest level (76.89 ppb) followed by copper (26.75 ppb), while cadmium concentration could not be detected. The measured concentration of cadmium in test water is given in Table 3. The level of measured cadmium ion concentration varied from 72% to 80% of nominal concentration in test medium. As a matter of fact the metal ions get precipitated with the hardness and alkalinity ($CaCO_3$) of water. Observations were also made during the whole experiment in order to detect the visual sign of cadmium poisoning. Fish showed slight excitement occasionally coupled with vigorous movement at the highest concentration (4.0 ppm). It is important to note that the fish remained otherwise healthy throughout the experimental period.

**Table 3.** Cadmium concentration in test medium.

| Group | Nominal concentration (ppm) | Measured concentration (ppm) |
|---|---|---|
| I | 0.0 | ND |
| II | 1.0 | $0.725 \pm 0.054$ |
| III | 2.0 | $1.634 \pm 0.144$ |
| IV | 4.0 | $3.167 \pm 0.213$ |

ND= Not detected

The pattern of regeneration/regrowth of caudal fin of the control and exposed fish is shown in Figure 1. In the present study all the three concentrations of cadmium retarded the fin regeneration in fish. In the fish of control group (Group I) steady regeneration of caudal fin was observed. The analysis of variance (ANOVA) showed significant effect of cadmium on retardation of regeneration between different doses of cadmium as well as days of exposure (Table 4). The post-hoc test both Bonferroni and Tukey, showed similar results. The retardation at 1.0 and 2.0 ppm did not show significant difference during early observation period (i.e. on $7^{th}$ day), while it was

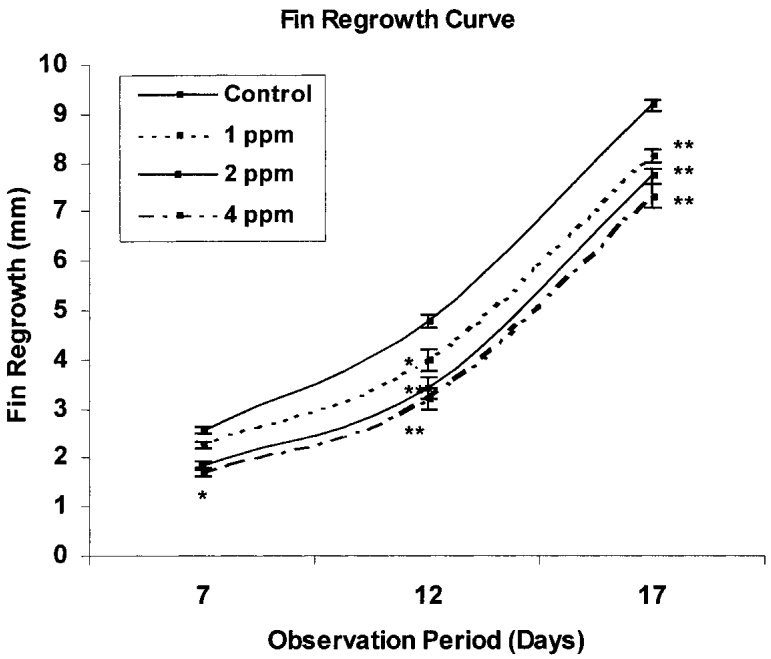


**Figure 1**. Caudal fin regrowth pattern in control and exposed group

significant at all time point to the higher concentrations (Figure 1). The maximum inhibition (28.0 and 31.0%) in fin growth was found during initial observation at 2.0 and 4.0 ppm of cadmium exposure.

**Table 4** Analysis of variance (ANOVA) on effect of cadmium on fin regrowth.

| Source of Variation | Sum of Square (SS) | Degree of freedom (DF) | Mean Square (MS) | F value | Significance (p) |
|---|---|---|---|---|---|
| Between Groups | 792.294 | 11 | 72.027 | 331.298 | $p<0.001$ |
| Within Groups | 23.480 | 108 | 0.217 | - | $p<0.001$ |
| Total | 815.774 | 119 | - | - | - |

A linear correlation was observed between the fin growth inhibition and exposure concentrations with a high degree of confidence. The regression equations were ($y = 10.392x + 3.5294$), ($y = 8.1522x + 6.8841$) and ($y = 4.3716x + 7.1038$) with a coefficient of determination ($R^2 = 0.8856$, $R^2 = 0.848$ and $R^2 = 1.0$) for $7^{th}$, $12^{th}$ and $17^{th}$ days, respectively (Figure 2).

The regrowth pattern during regeneration followed similar course in experimental fish and the control fish but for an initial lag in the former group. The fish of control group started regeneration sooner than the experimental fish (Figure 1)/or caudal fin regenerated in control group and exposed group at a comparable rate regardless of

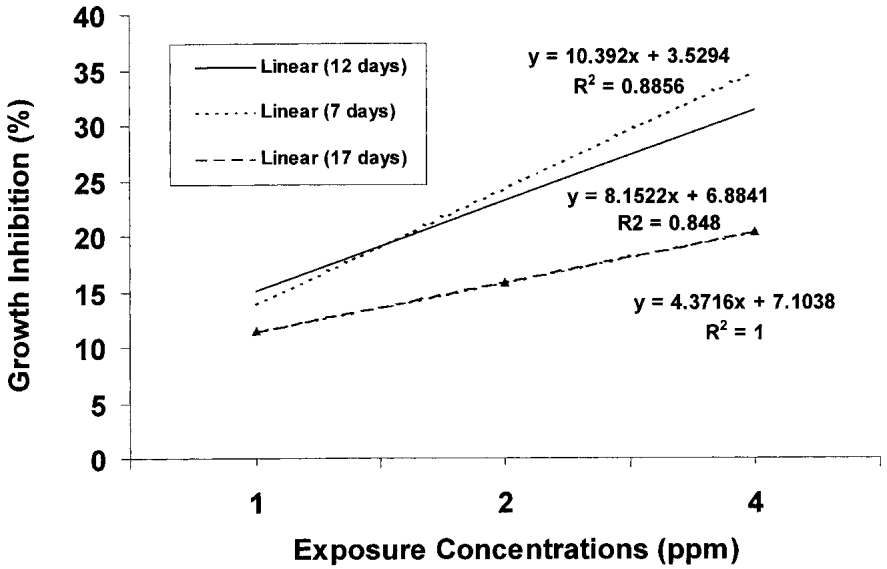


**Figure 2**. Relationship between fin growth inhibition and exposure concentrations.

exposure except for an initial lag in latter group. Thus retardation of fin regeneration was more marked in early stages of blastema formation and wound healing, particularly at 2.0 and 4.0 ppm of cadmium exposure. Hence, it is obvious that cadmium interferes with the process of blastema formation and wound healing which in turn affects rate of fin regeneration. Our finding is in accordance with the report of Weis and Weis (1976). They reported that cadmium at 0.1 to 1.0 ppm retarded the fin regeneration in fish *Fundulus heteroclitus* by interfering with the process of blastema formation not the rate of fin regeneration. Further, in another study with heavy metals, growth, frequency of molting and regeneration was found to be reduced by cadmium in fiddler crabs (Weis et al. 1992). Recently, Zodrow and Tanguay (2003) also noted similar effect in caudal fin regeneration of zebra fish *(Brachydanio rerio)* exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). They reported that more inhibition was observed during early days of blastema formation and healing in exposed group compared to the control group, and thereby retardation of fin regeneration. Similar inhibition of caudal fin regeneration by zinc in tilapia (*Oreochromis mossambicus)* was reported (Verma 2004).

Pioneer work done by Weis and Weis (1975,1976,1978) on fin regeneration showed that pesticides and metals retard the rate of fin regeneration in fish. The pesticides like malathion, sevin and DDT were found to retard the rate of fin regeneration in fish (Weis and Weis 1975). Heavy metals like, lead, cadmium (Weis and Weis 1976) and methyl mercury (Weis and Weis 1978) have also been reported to inhibit the rate of fin regeneration. The earlier studies on regeneration of caudal fin showed that fish inhabiting polluted areas exhibited reduced growth and reduced fin regeneration compared to the fish of unpolluted areas (Weis and Khan 1991). Thus, retardation of fin regeneration by the chemicals is another detrimental effect to the fish. Contrary to these findings cadmium has been reported to accelerate the rate of fin regeneration.

Acceleration has also been observed in other cases also when the exposure level of toxicant is below the inhibitory concentrations (Weis and Weis 1986), such phenomenon has been termed as 'hormesis'.

Regeneration of caudal fin in tilapia fish (*Oreochromis mossambicus)* was first reported by Kemp and Park (1970), that involve several stages including epithelial cap formation, differentiation of mesenchyma cells, blastema formation and finally out growth of blastema and regeneration of fin. Caudal fins regenerate immediately by forming blastema at cut ends of fin rays (lepidotrichia) and then new lepidotrichia attach to the remnants old ones. The wound healing (epithelial cap formation) and blastema (regeneration bud) formation must occur before regeneration starts properly, and if they are retarded by a toxicant, initiation of regeneration will be delayed. The recent breakthroughs that have been made in the past decade with the arrival of a new model, the zebra fish, *Danio rerio*, now offers the possibility to combine cytological, molecular and genetic analysis and has opened new perspectives in understanding the mechanism of fin regeneration (Akimenko et al. 2003).

The exact mechanism involved in retardation of fin regeneration is not yet known. However, one of the possible hypotheses involved in retardation of fin growth may be the lowering of metabolic activity caused by exposure to toxicants (Jodrow and Tanguay 2003; Weis and Weis 1976). Earlier studies of Woodworth and Pascoe (1982) found retarded growth rate in larvae of rainbow trout, *Salmo gairdneri* exposed to cadmium. Kaviraj (1983) reported reduction in the growth of cadmium treated tilapia fish *Oreochromis mossambicus.* Dalal and Bhattacharya (1994) and Jakim et al. (1970) in a bid to explore the metabolic aspects of cadmium treated fishes reported reduced enzyme activities. Thurberg and Dawson (1974) reported reduced oxygen consumption in the cunner (*Tautogolrbus adspersus)* exposed to 3.0 ppm of cadmium. Similar effect of cadmium was also noted in tilapia fish *Oreochromis mossambicus* (Kaviraj 1983). Thus an overall lowering of metabolic activity induced by cadmium probably resulted in retardation in fin regeneration.

In this study author determined that fin regeneration is significantly affected by cadmium. This study also demonstrates that fish caudal fin regeneration is simple, sensitive and easy to perform, and can serve an unique model in determining the toxicity of the pollutants in aquatic environment, and may be helpful in characterizing the chemicals as developmental toxicants as tissue regeneration is a process of growth and differentiation. Further, this assay is also helpful in understanding the mechanism of toxicity, and at which stages the regeneration is impaired.

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## REFERENCES

APHA, AWWA, ASTM (1985) Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC

Akimenko MA, Mari-Beffa M, Beccera J, Geraudie J (2003) Old question, new tools, and some answer to mystery of fin regeneration. Dev Dyn 226: 190–201

Bengston BE, Carlin CH, Larson A, Svanberg O (1975) Vertebral damage in minnow, *Phoxinus phoxinus*, exposed to cadmium. Ambio 4: 166–168
Conant EB (1973) Regeneration in African lungfish, *Protopterus.* Biol Bull 144: 248–249
Dalal R, Bhattacharya S (1994) Effect of cadmium, mercury and zinc on the hepatic microsomal enzymes of *Channa punctatus.* Bull Environ Contam Toxicol 52: 893–897.
Eaton JG (1974) Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus*, Rafinesque). Trans American Fish Soc 103: 729–735
Fontenot DK, Neiffer DL (2004) Wound management in teleost fish: biology of the healing process, evaluation, and treatment. Vet Clin North American Exot Anim Pract 7: 57–87
Jackim E, Hamlin M, Soni S (1970) Effect of metal poisioning on five liver enzymes in killifish, *Fundulus hetroclitus.* J Fish Res Bd Canada 27: 383–390
Kaviraj A (1983) Chronic effect of cadmiumon behaviour, survival, growth and reproduction of a fish in aquatic ecosystem. Environ Ecol 1: 65–70
Karels AA, Manning S, Brouwer TH, Brouwer M (2003) Reproductive effect of estrogenic and antiestrogenic chemicals on sheephead minnows *(Cyprinodon varigatus).* Environ Toxicol Chem 22: 855–865
Kemp NE, Park JH (1970) Regeneration of the lepidotrichia and actonotrichia in the tail fin of the teleost , *Tilapia mossambica.* Dev Biol 22: 321–342
Lacroix A, Hontela A (2004) A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens.* Aquat Toxicol 67: 13–21
Mari-Beffa M, Palmquist FM, Gron F, Montese GS, Becrra J (1999) Morphometric study of the regeneration of individual teleost tail fin. J Anatomy 195: 393–405
Ruparelia SG, Verma Y, Saiyed SR, Rawal UM (1990) Effect of cadmium on blood of tilapia, *Oreochromis mossambicus* (Peters) during prolong exposure. Bull Environ Contam Toxicol 45: 305–312
Sprague JB (1987) Effect of cadmium on freshwater fish. In: Niriagu JO, Sprague JB (eds) Cadmium in the Aquatic Environment. John Wiley and Sons, Inc. p 139
Shukla NP, Pandey GN (1985) Effect of heavy metals on fish- a review. Rev Environ Health 5: 87–99
Thurberg FP, Dowson MA (1974) Physiological responses of the cunner, *Tautogolrbus adspersus* to cadmium. III. Change in the osmoregulation and oxygen concentration. In: Physiological Responses of the Cunner, *Tautogolrbus adspersus* to Cadmium. NOAA Tec Rpt NHFS SSRF 681, p11
Verma Y (2004) Retardation of fin regeneration in freshwater fish *Oreochromis mossambicus* by zinc. Acta Hydrochim Hydrobiol 32: (In Press)
Weis JS, Weis P (1975) Retardation of fin regeneration in *Fundulus* by several insecticides. Trans American Fish Society 104: 135–137
Weis P, Weis JS (1976) Effects of heavy metals on fin regeneration in the killifish, *Fundulus heteroclitus.* Bull Environ Contam Toxicol 16: 197–202
Weis P, Weis JS (1978) Methylmercury inhibition of fin regeneration in fishes and its interaction with salinity and cadmium. Estuarine Coastal Mar Sci 6: 327–334
Weis P, Weis JS (1986) Cadmium acclimation and hormesis in *Fundulus heteroclitus* during fin regeneration. Environ Res 39: 356–363
Weis JS, Khan AA (1991) Reductions in prey capture ability and condition of mummichogs from a polluted habitat. Trans American Fish Soc 120: 127–129
Weis JS, Cristini A, Ranga Rao K (1992) Effect of pollutant on molting and

regeneration in crustacea. American Zool 32: 495–500
Wallace H (1981) Vertebrate limb regeneration. John Wiley and Sons, New York
Woodworth J, Pascoe D (1982) Cadmium toxicity to rainbow trout, *Salmon gairdneri*, Richardson : a study of eggs and alevins. J Fish Biol 21: 47–57
Zodrow JM, Tanguay RL (2003) 2,3,7,8–Tetrachlorodibenzo p–dioxin inhibits zebra fish caudal fin regeneration. Toxicol Sci 76: 151–161
Zhu Y, Wang J, Bai Y, Zhang R (2004) Cadmium, chromium, and copper induce polychromatocyte micronuclei in carp (*Cyprinus carpio* L.). Bull Environ Contam Toxicol 72: 78–86