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Accumulation of Cadmium and its Effects on the Survival and Growth of Larvae of *Heteropneustes fossilis* (Bloch, 1794)

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Abstract: Cadmium (Cd) pollution continues to be a global problem. Early life stages of fish appear to be especially susceptible to this form of pollution. The fish *Heteropneustes fossilis* is an useful indicator species for freshwater pollution. Investigations were carried out on acute (96 h) and Chronic (21 day) mortality rate, accumulation of toxicant and growth of fish larvae under Cd exposure. Ten day old *H. fossilis* larvae were exposed to graded series of concentrations of Cd under static-renewal test conditions. Present results indicated that *H. fossilis* larvae were very sensitive even to low concentration of Cd. The mean NOEC, LOEC and LC_{50} values for survival were 500, 750 and 1921 μg L^{-1} for acute (96 h) and 90, 125 and 382 μg L^{-1} for chronic (21 day) exposure, respectively, while the mean NOEC, LOEC and IC_{25} values for growth were 60, 90 and 125 μg L^{-1} after 21 days of exposure, respectively. *H. fossilis* larvae rapidly bioaccumulated with Cd during prolonged period (21 days) of exposure. Cd accumulation in the larvae reached the level of 62.53 μg g^{-1} after 21 day of exposure, representing a two fold increase over the use of lowest test concentration of 30 μg L^{-1} in their exposure media. Also, the Cd accumulation in larvae was time and concentration dependent.

Key words: Fish, *H. fossilis* larvae, growth, survival, bioaccumulation, cadmium toxicity

INTRODUCTION

In many aquatic systems, metal concentrations are greater than natural background levels, due to a continuous release of metals from industrial, mineral mining and agricultural sources. Cadmium (Cd) is an environmental pollutant, particularly, the industrial sewage sludge as a major source of Cd on environment (Wilkinson *et al*., 2003). It is a persistent neurotoxic contaminant that has been one of the most commonly used heavy metals in the 70's (Moore and Ramamoorthy, 1984). This metal is not eliminated from water ecosystem, it accumulates in sediments and is released in water during heavy rainfall, snowmelt and run off episodes (Olsvik *et al*., 2000) over short periods, i.e., hours or days, such metallic concentrations reach levels that may cause physiological stress and even kill organisms (Spry and Wiener, 1991).

Cd may enter the food chain and concentrate within organisms but in fish, it may also penetrate through gills as attested by its rapid accumulation during waterborne exposure (McDonald and Wood, 1993). The accumulated Cd has been shown to induce heavy metal binding proteins such as metallothionein in several tissue (Olsvik *et al*., 2000) may participate to detoxification by sequestering the metal.

Many metal toxicity studies have been carried out with both juveniles and adult fish (Witeska *et al*., 1995) but due to their sensitivity to environmental toxicants, fish embryo and larvae are frequently favoured as bio-indicator for water quality (Lin and Hwang, 1998). Bio-assay allow the detection of this effects by measuring the biological response of aquatic organisms, particularly in

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their highly sensitive early life stages (His *et al*., 1999). Especially, toxicity test with early stages supply more information on mortality and numerous other significant endpoints such as malformations, growth inhibition and developmental process delays (Herkovits *et al*., 1997). In the context of regulatory environmental assessment the early life stage of fish as an experimental model (Hutchinson *et al*., 2003), because it is fairly rapid, low cost and comparable tool to screen test chemicals and effluents (Woltering, 1984).

Fish, *Heteropneustes fossilis* was selected as a test organism because of its economic importance in India and in other ASIAN countries and because of its tolerance to avoid range of water variations. The age of exposed animals should be taken into consideration; younger animals are more at risk of toxicity (Holsapple, 2003). Therefore studies on developmental toxicology may elucidate whether there is a relationship between survival rate and body growth with the accumulation process during exposure to Cd on the early larval life of fish. The result obtained from this study will be useful for the Pollution Control Department for setting up or developing water quality standard for aquatic life and human health protection.

In this regard, the present study was an investigation of Cd exposure during the early development period of *H. fossilis* larvae which includes survival rate, body growth and accumulation of Cd in whole body.

MATERIALS AND METHODS

Effects of cadmium on the *Heteropneustes fossilis* have been studied, by carrying out 'post larval test' at the Department of Zoology, Annamalai University during the year 2005. *H. fossilis* larvae (5.80±0.430 mg weight; and 3.53±0.338 mm length of 10 day old) were acclimated in dechlorinated tap water in the laboratory prior to the beginning of the test. The quality of the test water is reported in Table 1. The fish were fed with newly hatched *Artemia nauplii* (< 24 h old) during acclimation period. Such acclimated fish were exposed to Cd to assess the acute and chronic mortality rate, growth inhibition and Cd residues in whole body of fish under chronic test in the present investigation.

Treatments were made from 1000 mg Cd L^{-1} stock solution prepared by dissolving 0.00179 g of analytical grade Cadmium Chloride ($CdCl_2$) in 100 mL of distilled water. Tests were conducted in 2 L glass beakers containing 1 L of test solution. The experiments were conducted at ambient temperature (25-29°C) with 12:12 h light: dark photo period. Test solutions were not aerated during exposure period and were renewed daily. In the present investigation, two experiments were performed: In Experiment-I, assessment of acute (96 h) and chronic (21 days) mortality rate of 10 days old larvae to Cadmium toxicity; in Experiment-II, evaluation of growth inhibition and residue level of Cd for whole body of fish (10 days old larvae) under various concentrations of Cd during long-term test (21 days).

Experiment I

A series of LC_{50} (lethal concentration for 50% of the population) test were performed for 96 h (Acute) and 21 day (Chronic) using several different ranges of Cd concentrations. Briefly, groups of 25 larvae of *H. fossilis* (10 day old) were randomly placed into each of 2 L beakers in each test. The fish larvae were exposed under static and renewal (at every 24 h interval) conditions to various Cd

Table 1: Water quality parameters used in experiment

Parameters	Value
Temperature (°C)	22±1
pH	7.4-7.6
Dissolved oxygen (mg L^{-1})	6.8-7.5
Salinity (mg L^{-1})	0.3-0.35
Alkalinity as $CaCO_3$ (mg L^{-1})	162-168

concentrations (from 30 to 3000 μg L^{-1}) and a control. The fish were not fed in acute test (96 h) whereas in chronic test fed with *Artemia nauplii* twice a day. Cd was added to the exposure containers from concentrated stock solution of $CdCl_2$. Assessment of mortality as the end point for the test was done via visual observations of immobility and after gentle prodding of organism with a plastic pipette. The number of dead larvae were documented for every 24 h in both acute and chronic test. Median lethal Cd concentrations (LC_{50}'s) were calculated by the method of Finney (1971) for each test with mortality data from the Cd exposures.

Experiment II

Observation of these larvae allowed an evaluation of growth and Cd residues after they were exposed to obtained sublethal concentrations of Cd which was derived from chronic mortality test (21 days). Six replicates were prepared for each treatment. Fifty fish larvae (10 day old) were randomly distributed into each test containers. The fishes were fed with newly hatched *Artemia nauplii* (< 24 h old) twice a day on 0-20 and increasing the amount of food proportionally as the fish grew. *Artemia nauplii* was provided so that there was only a small quantity of uneaten food left in the test containers. The fish were not fed on the last day of the tests (day-21).

At 7, 14 and 21 days of exposure, two 10 larval fish sub samples were collected at random from each concentration of Cd exposure. One sub sample with 10 number of fish from each were used for quantify the whole body Cd residue. Total Cd levels were determined by an Atomic Absorption Spectrophotometer (Perkin Elmer) in an air acetylene flame. Another sub sample of 10 fish were used for body growth (total length and weight) measurement. For each test concentration, the collected 10 larvae were preserved in 10% formalin, blotted dry and weight and length was analyzed.

Data Analysis

The data was expressed as means±standard deviation (SD). Significant differences were calculated using the student's t-test and one way ANOVA. Significant levels were set at $p<0.05$ and 0.01. Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) were calculated by use of appropriate method (Fundamentals of aquatic toxicology: Methods and application, 1985). The maximum acceptable toxicant concentration was represented as the geometric mean of NOEC and LOEC.

RESULTS AND DISCUSSION

The results of acute (96 h) and chronic (21 days) toxicity of Cadmium on mortality rate of *H. fossilis* larvae (10 day old) are summarized in Table 2. The Cd was found to be acutely toxic to larvae of the test fish at high concentration ranging from 750 to 3000 μg L^{-1}. The calculated LC_{50} value was 1921 μg Cd L^{-1} for 96 h and 382 μg Cd L^{-1} for 21 days of exposure. The mortality response of *H. fossilis* larvae during chronic exposure was found to be 5 times more sensitive than those exposed to acute toxicity (96 h) of cadmium. Exposure duration evidently affected sensitivity of fish larvae and influenced the value of LC_{50}. The greater sensitivity of larvae to cadmium toxicity was also observed in test with *Salmo gairdneri* (Beattie and Pascoe, 1978).

The results of the present study closely related with the LC_{50} value reported for Cd to Seabass larvae (*Lates calcarifer*) is 6360 μg L^{-1} (Thongra-ar and Musika, 1997); *Parceutrotus lividus* larvae are 3800 μg L^{-1} (Heyvang, 1994); 3372-11,241 μg L^{-1} (Warnau *et al*., 1996). Similarly for *Arbacia punctulata*, the EC_{50} value for Cd is 7380 μg L^{-1} (Carr, 1996) and for *Dendraster excentricus*, 5200-10,800 μg L^{-1} (Dinnel *et al*., 1989) and 1921 μg L^{-1} for acute mortality in the present study. The present investigation lends support to the report of the Cd toxicity for fish seems more effective after hatching and may be in relation to considerable increase of Ca^{2+} uptake (Chen *et al*., 2003). Also, Eaton (1974) found that larvae of the bluegills (*Lepomis macrochirus*) were the most sensitive stage to Cd exposure.

Table 2: Cd concentration and mortality rate (%) of *H. fossilis* larvae

Cadmium concentration (μg L^{-1})	% of average mortality	
	Acute (96 h)	Chronic (21 days)
Control	8.0	15
30	8.4	19
60	8.6	21
90	9.0	23
125	9.5	28*
250	10.8	41*
500	13.6	67*
750	21.5*	100*
1000	26.5*	100*
1500	37.3*	100*
2000	52.5*	100*
3000	79.8*	100*
3500	100.0*	-
LC_{50}	1921	382
(95% confidence intervals) (μg L^{-1})	(1692-2136)	(216-489)

*Significantly different from the control ($p \leq 0.05$)

Table 3: Cd level in 10 day old *H. fossilis* larvae to various Cd concentration over 21 days of exposure

Exposure period (days)	Exposure concentration (μg L^{-1})				F-value (Between exposure groups)
	30	60	120	250	
7	22.30±0.44	36.70±0.34	48.55±0.47	53.29±0.51	35.76*
14	46.17±0.32	62.18±0.71	73.36±0.44	86.75±0.38	61.50*
21	62.53±0.61	83.27±0.56	98.11±0.72	111.16±0.57	145.61*

Mean±SD, (n = 6); values expressed in μg g^{-1} of dry weight *Significantly different between exposure groups at 0.05 level

Table 4: Summary of statistical endpoints on survival and growth rate of 10 day old *H. fossilis* larvae

Survival effect (μg L^{-1})			Growth effect (μg L^{-1}) (21 day exposure)	
Acute (96 h)	NOEC : 500	Weight	NOEC	: 60
	LOEC : 750		LOEC	: 90
	MATC : 625		MATC	: 75
	LC_{50} : 1921		IC_{25}	: 125
	(CI) : (1692-2136)			
Chronic (21 days)	NOEC : 90	Length	NOEC	: 60
	LOEC : 125		LOEC	: 90
	MATC : 107.5		MATC	: 75
	LC_{50} : 382		IC_{25}	: 125
	(CI) : (216-489)			

NOEC: No Observed Effect Concentration, LOEC: Lowest Observed Effect Concentration, MATC: Maximum Acceptable Toxicant Concentration, LC_{50}: Lethal Concentration to 50% mortality, CI: Confidence Interval, IC_{25}: 25% Inhibited growth concentration

A summary of statistical endpoints for each test (NOEC, LOEC, LC_{50}, MATC) is presented in Table 4. The mean NOEC, LOEC, MATC and LC_{50} values for survival were 500, 750, 625 and 1921 μg Cd L^{-1} for chronic test (21 day), respectively, while the mean NOEC, LOEC and MATC values for growth (weight and length) of larvae under chronic test were 60, 90 and 75 μg L^{-1}, respectively. From these results, the survival was significantly reduced at concentrations greater than 125 μg L^{-1}, while growth was significantly reduced at concentrations greater than 90 μg L^{-1} during long term test.

The survival of *H. fossilis* larvae were more sensitive to Cd toxicity than the observed 96 h LC_{50} value of 67.47 mg L^{-1} (96 h) for adult fish is previously reported (Kalaiselvi, 2005). This is because earlier life stages of aquatic organisms are generally more sensitive than adult (McKim, 1977; Wang, 1987). Similarly Middaught and Dean (1977) also found that larvae *fundulus* and *Menidia* were

more sensitive to Cd toxicity than their adults. Comparison to other freshwater fish larvae could not be made due to lack of the data. The mortality of Cd exposed larvae were found to be time and concentration dependent which may partly be due to gradual accumulation of Cd in the fish which was evidenced in the present findings (Table 3). Further, the noted variations of Cd toxicity range from the larvae of other fish species may be due to differences in phenotype and life experience that exhibit different mortality rates to a toxin (Rose *et al*., 1993).

The mortality of test fish larvae stage to Cd is closely agreed with the report of Gray and Metcalfe (1999) who have suggested that the mortality of a fish larvae to a toxin related with increased energy needs or the action of toxin on stored energy. The inhibited stored energy mobilization could be another possible cause of mortalities of fish larvae (McCormick and Jensen, 1989). The present results are clearly indicated that an increased Cd concentration which decreased the exposure time required to bring 50% mortality of test fish larvae. This trends were showing a regular mode of action due to accumulation and subsequent magnification as time passed upto dangerous level that lead to death.

Result of second set of experiments (chronic test) revealed that the mean Cd deposition in whole body of *H. fossilis* larvae, exposed to low sublethal Cd concentrations (30, 60, 120 and 250 μg Cd L^{-1}) were progressively increased towards the post exposure period of 21 day (Table 3). However, during exposure to different concentration of Cd, the maximum magnification was found in fish exposed to higher concentration at the final day of test periods. This observations are in close to the findings of Vincent and Ambrose (1994) and Sharma *et al*. (2003) who have also noted a significant time and concentration related accumulation of Cd in *Catla catla* and young *Labeo* fry, respectively. Similar findings have been reported by Eisier and Gardner (1973). They emphasized that Cd act additively in biocidal properties. Further, Jones and Walker (1979) suggested that as the animal gets older (grew) the amount of stored metal increases more rapidly.

Larvae were fed with *Artemia nauplii,* during the experimental periods. Thongra-ar *et al*. (2003) have reported that the *A. nauplii* can tolerate very high concentration of a heavy metal and also can absorb the metal from test solution. Therefore, the test fish may have a possibility to uptake of Cd from the solution via Artemia feeding. However, the direct uptake of Cd from water occurs almost entirely across the gills (Olson *et al*., 1973). In contrast to the above statement, the fish larvae have poorly developed gills and kidney (Hwang and Yang, 1997) therefore, appointing the skin as the functional site of metal uptake (Keinanen *et al*., 2000). The mechanism(s) of metal uptake by the cells has not yet been elucidated; the evidences indicate that metal across the cell membranes essentially by a passive transport process although endocytosis may also occur (George and Viarengo, 1984). After entering the Cd into the freshwater fish, it binds to albumin and erythrocyte in the blood then is transferred into tissue where it is bound to protein of low molecular mass producing metallothioneins (MTs) by induction of mRNA synthesis (George *et al*., 1996). The result of the present findings also to support the view of Liu and Klaassen (1996) who have suggested that the main role of the MTs in the Cd exposure is associated with higher retention of Cd in the tissues, resulting in a protective mechanisms of *H. fossilis* larvae.

Cd tends to accumulate in the gills and erythropoietic system of fish (Goering *et al*., 1995). A previous report on Cd treated cat fish species of adult showed a level of 70 μg g^{-1} Cd in muscle tissue (Jayakumar and Paul, 2006), which is well above the lethal level of the cat fish of *H. fossilis* larvae in the present finding.

Concentration and time dependent in growth (weight and length) inhibition was found in Cd exposed *H. fossilis* larvae (10 day old) for 21 days of exposure. Concentrations of 90, 125 and 250 μg L^{-1} of Cd caused a maximum reduction of 12.39, 25.42 and 37.41% larval weight and 6.17, 12.19 and 18.31% length, respectively at the end of 21 day exposure. In comparison with the control (42.44±0.32 mg weight and 19.93±0.47 mm length), a significant reduction in the mean body mass was

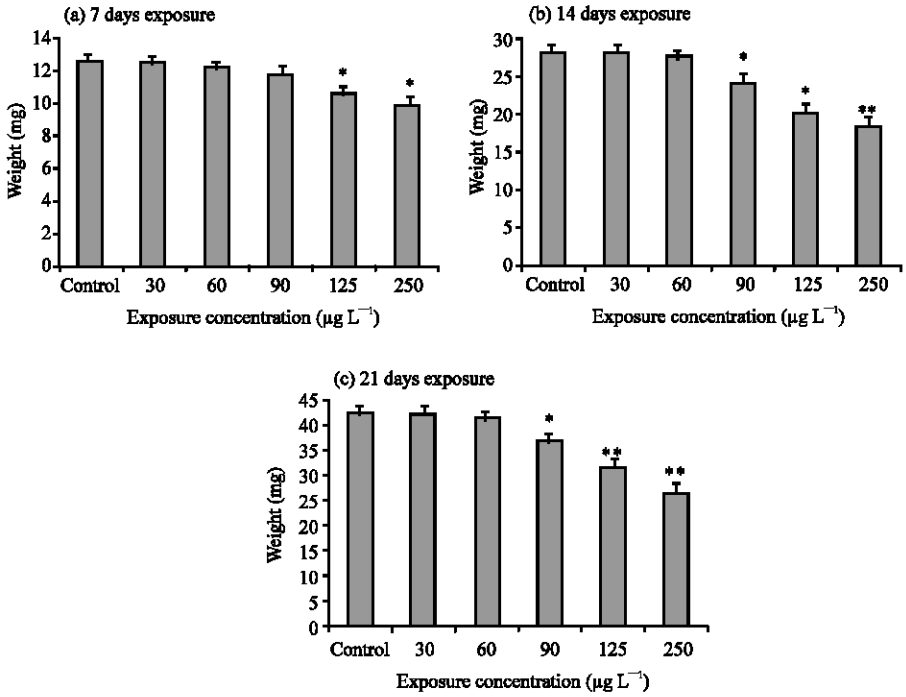

Fig. 1: Effect of Cd on mean individual weight of *H. fossilis* larvae (10 day old) to various concentrations during long term exposure, Mean±SD, (n = 10), *Differed significantly from control (*$p \leq 0.05$; **$p \leq 0.01$)

observed at the concentration of 90 µg L^{-1} of Cd and above (Fig. 1 and 2). Further this result noted that the larval growth was significantly reduced without any mortality in the same concentrations of Cd. The LOEC of chronic mortality test value (125 µg Cd L^{-1}) inhibiting the larval growth which was markedly lower than those causing 50% of larval mortality. The early larval growth is therefore a more sensitive response than survival success of *H. fossilis*. This investigation is closely agreement with the report of the retarded growth of fish larvae at a Cd concentration below those cause significant mortality (Eaton *et al*., 1978). The fish growth was the most sensitive indicator of Cd toxicity to long term (Rombough and Garsidae, 1980) and sublethal (Christensen, 1975) response. Similarly Fernandez and Beiras (2001) have reported that 50 µg L^{-1} of Cd reduced the mean larval length of *Paracentotus lividus* by 33.7% compared to the control values over 7 days of exposure. Also a retarded growth was recorded in Labeo fry exposed to 0.5 mg L^{-1} Cd for 35 days (Sharm *et al*., 2003) and in Tilapia larvae exposed to 200 µg L^{-1} for four days (Hwang *et al*., 1995). Christensen (1975) noted a significant of weight reduction in alevins of brook trout *Salvelinus fontinalis* held in 3.4 µg Cd L^{-1}.

The culmination of many biochemical phenomena that occur in a somewhat regulated pattern and reduced growth may occur when contaminant intoxication induces biochemical changes that interfere with normal growth process (Mehrle and Mayer, 1985). Cd is known to cause physiological and metabolic changes related with growth (Khan and Shinha, 1995). McKim and Benoid (1971) have also reported that the reduced growth to be indicative of suppressed metabolism; possibly on specific enzymes or hormones system. Thus it is unlikely that poor growth is the result of energetic deficiency brought on by the need to mobilize and eliminate toxicant (Moles *et al*., 1987). The present findings

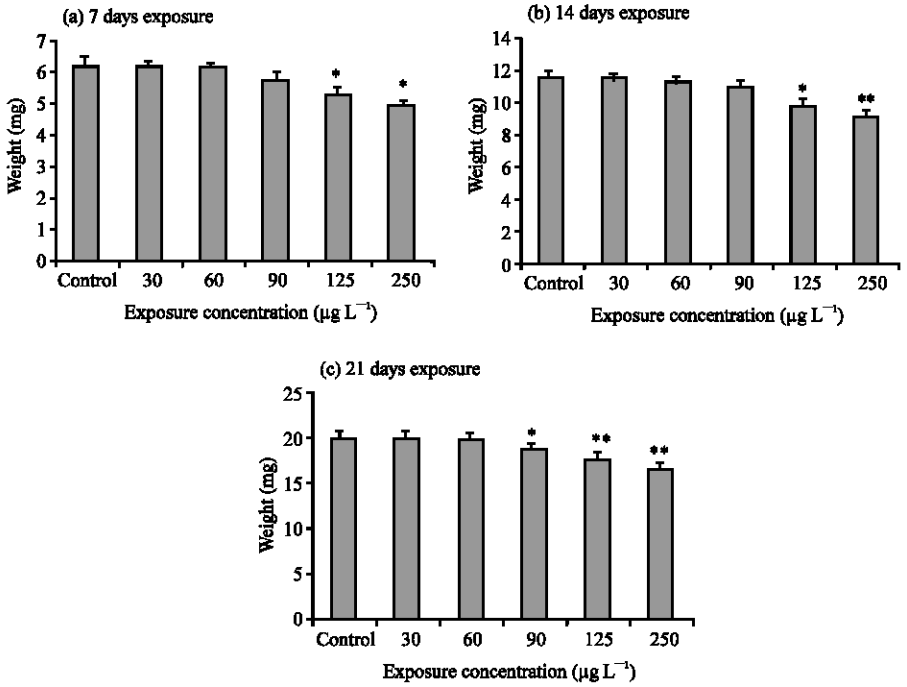

Fig. 2: Effect of Cd on mean individual length of *H. fossilis* larvae (10 day old) to various concentrations during long term exposure, Mean±SD, (n = 10), *Differed significantly from control (*p≤0.05; **p≤0.01)

is also closely consistent with the above statement. Changes in growth rates in fish are also associated with the relationship between Cd accumulation and growth responses. Similar result was observed in copper exposed larvae of Rainbow trout (Stasiunaite, 2005).

The inhibitory effect of Cd on growth of fish larvae was probably due in part to the impact of Cd on feeding ability. The reduction in feeding may be due to a loss of coordination and earlier satiation of hunger from neurotoxic effect by Cd (Von Westernhagen, 1988; Beauvais *et al*., 2001). Moreover, the Cd inhibits the intestinal absorption of nutrients such as amino acid and sugar in fish (Farman farmaian *et al*., 1989) which may cause reduction in growth of *H. fossilis* larvae. Khan *et al*. (1992) also observed a decrease in colorific content in tissue of *Garra mullya* on exposure to sublethal concentration of Cd. Further they have reported, the Cd significantly reduced the energy profile in tissues. Bio-accumulation and magnification of Cd in test fish of *H. fossilis* larvae in the present investigation may create stress and changes in tissue energy profile resulting in retarded growth and is coincided with the previous report of Heath (1995). The result of our investigations indicated that Cd might impose a considerable stress on early development of *H. fossilis* larvae. Decrease in growth rate of fish may limit the survival in future by decrease of its swimming capability (Cleveland *et al*., 1991). Successful early development of fish forms the basis for fish recruitment (Houde, 1994) therefore the data obtained in this study may provide useful additional information on the toxic effect of Cd on early development of *H. fossilis*.

CONCLUSIONS

In conclusion, the heavy metal Cadmium was very toxic to early life stage of *H. fossilis* larvae and the effects were determined on all the parameters studied. The larval growth was considerably more

sensitive to Cd toxicity than their survival rate. Further, at the young age of fish larvae, Cd toxicant rapidly accumulated from the medium, which correspondingly increased with the concentrations and time of exposure. Together with the wide spread geographical distribution and produced results in the present study of Cd effect on this species will be an extremely useful tool in any freshwater bio-monitoring laboratory.

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