Research Article

Sensitivity of Common Carp, *Cyprinus carpio* (Linnaeus, 1758) to the Grey List Metal, Zinc under Laboratory Condition

Kishore Dhara[1], Shubhajit Saha[2], Nimai Chandra Saha[3,*]

[1]Directorate of Fisheries, Government of West Bengal, Meen Bhaban, Kolkata, West Bengal, INDIA.
[2]Department of Zoology, Sundarban Hazi Desarat College, South 24 Parganas, West Bengal, INDIA.
[3]Vice-Chancellor, The University of Burdwan, Bardhhaman, West Bengal, INDIA.

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ABSTRACT

In the present investigation, acute toxicity of the grey list metal, Zinc was carried out under laboratory condition to *Cyprinus carpio* (Linnaeus, 1758). The 96 h LC_{50} value of zinc with 95% confidence limits to *C. carpio* was recorded as 15.804 (15.083-16.721)mg/l. None of the unexposed fish died during the bioassay. Mortality rate of the exposed fish to the toxicant significantly ($p<0.05$) varied over the control at all concentrations during every 24h time interval. Significant relationship ($p<0.05$) was also observed between mortality rate and exposure times (24, 48, 72 and 96 h) at all concentrations of the toxicant except 13.5, 14.5 and 15.0 mg/l. Toxicity factor for the toxicant to the organism increased with the time of exposure. The behavioral responses in respect of activeness, body balance, swimming and mucous secretion were also changed in the exposed fish. The intensity of behavior altered with the increasing doses and progress of time of exposure. The opercular movement of the fish increased significantly ($p<0.05$) over the control with increasing concentration but decreased significantly ($p<0.05$) with the progress of time of exposure at all concentrations.

Key words: *Cyprinus carpio*, Zinc, Acute toxicity, Toxicity factor, Safe level, Behavioral responses, Opercular movement.

Correspondence:
Prof. NC Saha
Vice-Chancellor, The University of Burdwan, Bardhhaman-713 104, West Bengal, INDIA.

Phone no: +91 9051342474
Email: prof.ncsahavcbu@rediffmail.com

INTRODUCTION

Heavy metal contamination in natural waters has become inevitable presently due to intense activity in industrial and agricultural sectors on a global scale.[1,2] Heavy metals are considered as a serious threat not only to aquatic organisms, especially to fishes but also to aqua-ecosystem in a whole because of their long biological half-lives, environmental persistence and ability to be accumulated by aquatic organism.[3-5] The heavy metals bind to sediments initially and then they gradually become available to organisms of aquatic ecosystem.[6,7] This phenomenon accelerates the process of biomagnification of heavy metals from the bottom sediments to the successive level of food chain due to their high bioaccumulative potentiality.[8]

Zinc is the second most abundant trace element after iron,[9] but it is one of the most toxic heavy metals included in the grey list of the international convention.[10] Because of its non-biodegradability and tendency to accumulate in the animal tissues, zinc is considered as the very serious environmental threat.[11] It is ubiquitous aquatic pollutant, which has also been detected in higher concentration in the aquatic environment.[12] Like many other heavy metals, the source of zinc in natural water bodies is from geological rock weathering or from anthropogenic activities such as industrial and domestic wastes water discharge.[13] It is a major effluent from the industries such as soft drink flavoring, fur dressing and dyeing, fish processing and laundry.[14]

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Zinc can be a potential toxicant in higher concentration to the aquatic organisms by interfering with the internal dynamics of the aquatic ecosystem into irreversible and inflexible condition leading to serious damage and even death of aqutic animals.[15,16] In polluted environments, aquatic organisms are continuously exposed to ambient zinc and it enters through body surface, gills and food.[17] Chandra (1984)[18] reported that excess zinc interacts with free thiol groups on macromolecules and also blocks the active sites of enzymes, co-enzymes and membrane receptors like other heavy metals and thus causes physiological toxic effects. Very high levels of zinc can disturb the protein metabolism and lipid profile.[19,20] It also results in a lower ATP production.[21] Dineley *et al.* (2000)[22] reported that increased intracellular free zinc (Zn^{2+}) is toxic to neurons and It also induce cell death either through apoptosis or by necrosis.[23] Some workers[24,25] also revealed that zinc induce neuronal death.

The present investigation was undertaken to estimate the sensitivity of fresh water fish, *Cyprinus carpio* on the basis of 24, 48, 72 and 96h lethal toxicity and to estimate the possible safe concentration of zinc in aquatic environment. During bioassay their behavioral changes were also assessed. The study further showed the alteration in their opercular movement which determined the degree of toxicity.

MATERIALS AND METHODS

Fresh water exotic major carp, *Cyprinus carpio* (mean length 72.51±3.23 mm, mean weight 16.50±3.45g) belonging to Class Actinopterygii and Family Cyprinidae was used in the bioassays as the test organism. The fish were collected from the local unpolluted private fish farm, treated with 0.1% $KMnO_4$ solution to avoid any pathogen infection and allowed to acclimatize to the test condition prior to 96h of the experiment. While acclimatization the test organisms were kept in the cement tanks of 1000 litre capacity filled with unchlorinated, well aerated water (pH 7.50 ± 0.35; temperature 26.50 ± 1.32°C) under 12 h each dark and light cycle. During this period, food was supplied to the fish in the form of commercial pellets with 40% crude protein.

Analytical grade zinc sulphate, $ZnSO_4$, $7H_2O$ (purity 98%, molecular weight 161.47 g/mol; E. Merck (India) Ltd., Mumbai) was used as the test chemical in this study.

Static replacement bioassay test with the healthy, disease free fish (irrespective of sex) was conducted in 15l glass aquaria containing 10l un-chlorinated water following the methods outlined in American Public Health Association.[26] The physico-chemical values of different parameters of water used in the experiment were as follows: temperature 27.5 ± 0.25°C, pH 7.8 ± 0.35, free CO_2 10.8 ± 0.43 mg/l, DO 5.79 ± 0.23 mg/l, total alkalinity 140 ± 10.71 mg/l as $CaCO_3$, total hardness 119 ± 5.17 mg/l as $CaCO_3$. Each bioassay was accompanied by four replicates with control. Each replicate was provided with ten fish randomly. They were not fed for 24h prior to commencement of the experiment.

Rough range finding experiments were conducted initially to determine range of concentrations at which the mortality of fish may occur. The selected test doses of zinc finally used to determine the 24, 48, 72 and 96h median lethal (LC_{50}) values were 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5 and 18.0 mg/l. During the experiment, the dead fish were removed quickly from the aquaria to avoid any microbial decomposition causing decrease the level of dissolved oxygen and the number of dead fish was recorded at every 24h interval. The 10% of the test water was replaced by newly prepared test water at every 24h interval to maintain a fixed concentration.

Toxicity factors of the tested organism to zinc at different time of exposure was assessed after Ayoola *et al.* (2011)[27] by multiplying LC_{50} value at 24h with LC_{50} at any other exposure time.

The safe level estimation for *C. carpio* was calculated by multiplying the 96h LC_{50} with different application factors (AF) based on Edwards and Brown (1966),[28] Burdick (1967),[29] Sprague (1971),[30] Committee on Water Quality Criteria (CWQC, 1972),[31] International Joint Commission (IJC, 1977),[32] European Inland Fisheries Advisory Commission (EIFAC, 1983)[33] and Canadian Council of Resources and Environmental Ministry (CCREM, 1991)[34] and also on the basis of formula developed by Hart *et al.* (1948).[35]

Mean mortality of *C. carpio* after 24, 48, 72 and 96h of bioassay was used to calculate the LC_{50} values (with 95% confidence limit) through the statistical software Probit program version 1.5.[36] The lethal concentration (LC_{50}) was determined in MS Excel by plotting the test doses against the fish mortality within 24 hr, 48 hr, 72hr and 96h after the experiment.[37] The values of percentile mortality of the fish were subjected to analysis of variance (ANOVA) using R-software (2012)[38] succeeded by Duncan's Multiple Range Test (DMRT) to find out the significant difference within the mean values at different doses of Zinc at 24, 48, 72 and 96h of exposure. The relation between mortality rate with exposure time and doses was evaluated using correlation analysis.[36,39]

The behavioral alterations in respect of activeness, body balance, rate of swimming and mucus secretion in the exposed fish were also recorded systematically by naked eye observation during the experiment following the method of Rand (1985).[40] Scoring of behavioral changes was performed independently by two observers and the scores were collated, and calculated average was quantified and plotted graphically in terms Normal (= 0), None (= -3), Strongly decreased (= -2), Mildly decreased (= -1), Mildly increased (= +1), Moderately increased (= +2) and Strongly increased (= +3).[41] Changes in the opercular movements to determine respiratory rates of the fish exposed to different concentrations of the toxicant were also recorded during 96h bioassay condition. Opercular movements of the fish per minute for both control and exposed were counted twice a day at every 24 h during the bioassay and their mean values per concentration were plotted graphically.

RESULTS

No test organism died during the acclimatization period. The acute toxicity values of zinc ($LC_{1,5,10,15,50,85,90,95,99}$) with 95% confidence limit to *C. carpio* during the exposure period of 24, 48, 72 and 96 h are given in Table 1. No mortality was also observed in the control group during the test.

Significant relationship ($p<0.05$) between mortality rate of *C. carpio* was observed at all concentrations over the control during every 24h time interval. The mortality rate of the exposed fish significantly ($p<0.05$) varied over the exposure times (24, 48, 72 and 96h) at all doses of the toxicant except 13.5, 14.5 and 15.0 mg/l concentrations of the toxicant. The relation between concentration of zinc and fish mortality at 24 h was, $y = 18.11\ln(x) - 8.361$, $R^2 = 0.767$ (Figure 1); it was $y = 24.38\ln(x) - 9.713$, $R^2 = 0.837$ at 48h (Figure 2); at 72 h it was $y = 27.26\ln(x) - 3.382$, $R^2 = 0.887$ (Figure 3) and at 96 h was $y = 28.00\ln(x) + 1.344$, $R^2 = 0.918$ (Figure 4).

The toxicity factors as calculated from the medial lethal toxicity (LC_{50}) values at different time of exposure are tabulated in Table 2. With the progress of time the toxicity factor values for the tested fish species to zinc were increased gradually.

The estimated possible safe level of zinc for the fish as calculated by multiplying their 96h LC_{50} values with different application factors and formula are recorded in Table 3. In the present study, the safe level was estimated for the toxicant at 0.158 – 6.322 mg/l**.**

The behavioral alterations in respect of activeness, body balance, rate of swimming and mucus secretion observed in the test organisms exposed to various lethal concentrations of zinc are graphically plotted in Figure 5-8. The intensity of activeness, body balance and rate

Table 1: Acute lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of Zinc to *C. carpio* at 24, 48, 72 and 96h (Control group theoretical spontaneous response rate = 0.000).

Lethal Concentration parameters	Concentration values with 95% confidence limits (mg/l)			
	24h	**48h**	**72h**	**96h**
LC_1	12.745 (9.737-13.941)	11.126 (7.457-12.646)	10.739 (7.634-12.184)	10.389 (7.338-11.852)
LC_5	14.132 (12.035-15.003)	12.684 (9.810-13.831)	12.148 (9.614-13.284)	11.748 (9.192-12.925)
LC_{10}	14.933 (13.401-15.667)	13.602 (11.327-14.541)	12.973 (10.857-13.929)	12.543 (10.356-13.547)
LC_{15}	15.499 (14.338-16.310)	14.259 (12.450-15.080)	13.561 (11.772-14.398)	13.110 (11.214-13.996)
LC_{50}	18.138 (17.174-20.876)	17.404 (16.474-19.816)	16.357 (15.629-17.565)	15.804 (15.083-16.721)
LC_{85}	21.243 (19.025-29.837)	21.226 (19.204-28.769)	19.729 (18.146-24.501)	19.052 (17.679-22.922)
LC_{90}	22.269 (19.642-32.941)	22.030 (19.694-31.075)	20.624 (18.728-26.610)	19.914 (18.254-24.836)
LC_{95}	23.881 (20.582-38.160)	23.278 (20.439-34.846)	22.025 (19.611-30.091)	21.262 (19.14-27.994)
LC_{99}	27.225 (22.458-50.320)	25.813 (21.900-43.223)	24.913 (21.359-37.934)	24.042 (20.842-35.082)
Slope ± SE	15.179±4.047	11.972±3.228	12.730±3.765	12.768± 2.998
Intercept ±SE	14.104±4.895	9.853±3.872	10.451 ±3.661	10.306±3.557

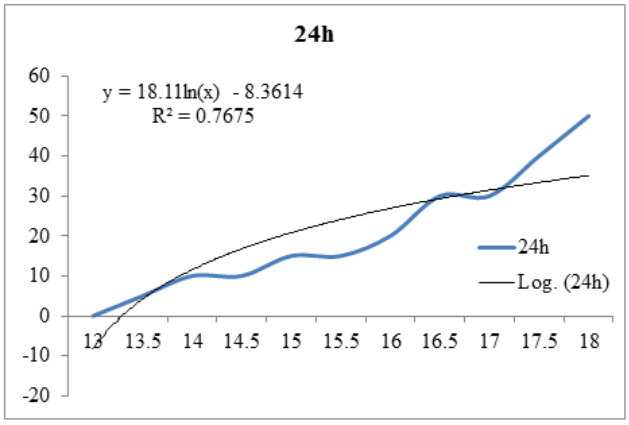

Figure 1: Relationship between the concentrations of Zinc and mortality of *C. carpio* during 24h.

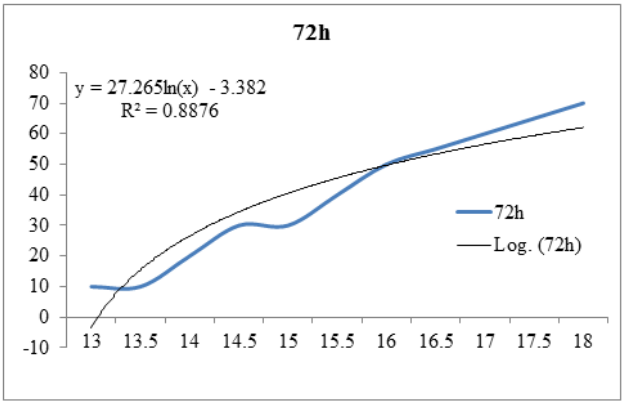

Figure 3: Relationship between the concentrations of Zinc and mortality of *C. carpio* during 72h.

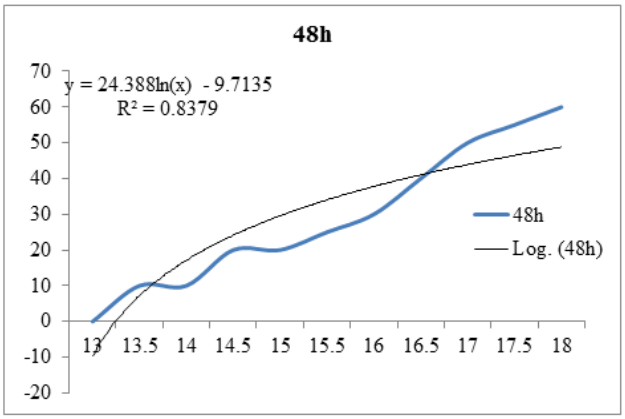

Figure 2: Relationship between the concentrations of Zinc and mortality of *C. carpio* during 48h.

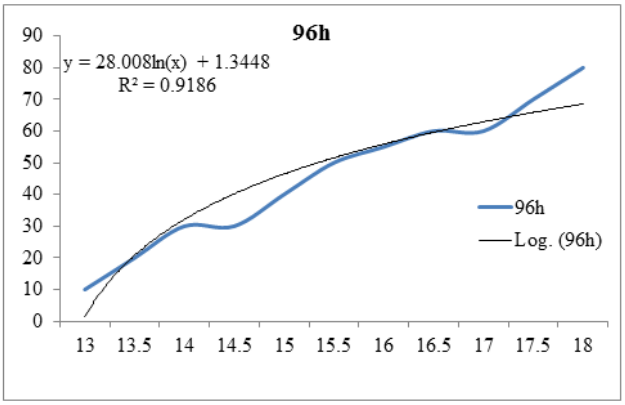

Figure 4: Relationship between the concentrations of Zinc and mortality of *C. carpio* during 96h.

of swimming were found decreased but mucus secretion increased in the exposed fish with the increasing doses and progress of time of exposure (Figure 5-8).

Significant (p<0.05) time and dose dependent relationship in respect of opercular movement in the exposed *C. carpio* over their control group was observed (Figure 9). The opercular movement of the fish increased significantly (p<0.05) over the control with increasing concentrations at all exposure time. Similarly, it was significantly (p<0.05) increased with progress of time at all treatments.

Table 2: Toxicity factors for *C. carpio* exposed to Zinc at different time scale (24, 48, 72 and 96h).

Exposed time (h)	Toxicity factor value
24	1.000
48	1.042
72	1.109
96	1.148

DISCUSSION

The present study on the zinc lethality to *Cyprinus carpio* confirms that the metal is a potent toxic to the fish. The toxicity of zinc in the present study to the fish was probably due to the deposition of precipitated toxicant on the gill surface resulting in a formation of insoluble metal-mucus layer.[42,43] Such precipitation may cause damage of gill tissues which leads to respiratory failure followed by anoxia.[44] Impairment of gill function may also in turn causes disturbances of acid-base regulation, inhibition of major uptake of major ions (Na^+, Ca^{2+}, Mg^{2+}, Cl^-), osmoregulatory failure, acidosis and low oxygen tensions in arterial blood.[45-48] Furthermore, Skidmore (1970)[49] suggest that epithelial damage decreased the permeability of the gills to oxygen. As a result exposed fish died due to hypoxia when maximum gill ventilation was no longer sufficient to supply the oxygen needs of the fish. Sheline *et al.* (2000)[50] and Strydom *et al.* (2006)[21] recorded that acute exposure of zinc induced ATP depletion preceded cortical neuronal death which may be linked to the present study. The sensitivity of freshwater fish to zinc is very

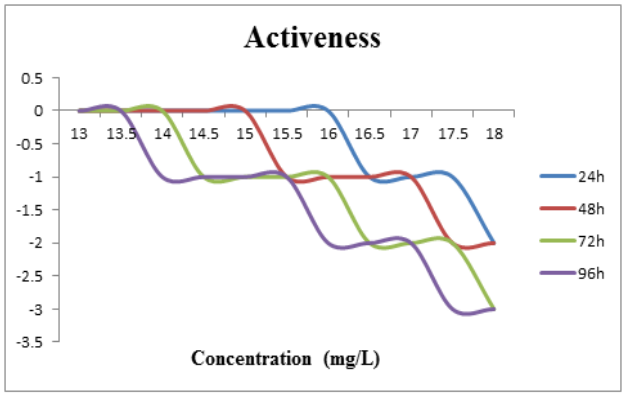

Figure 5: Changes in activeness in fishes exposed to different concentration of zinc.

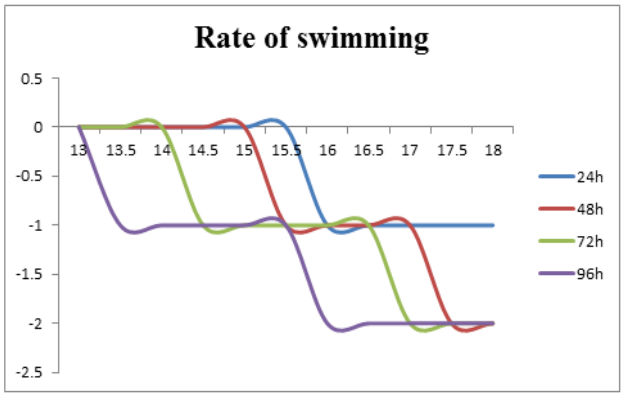

Figure 7: Changes in rate of swimming in fishes exposed to different concentration of zinc

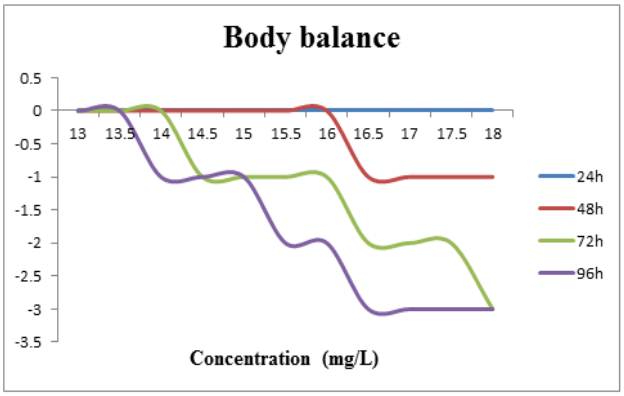

Figure 6: Changes in body balance in fishes exposed to different concentration of zinc.

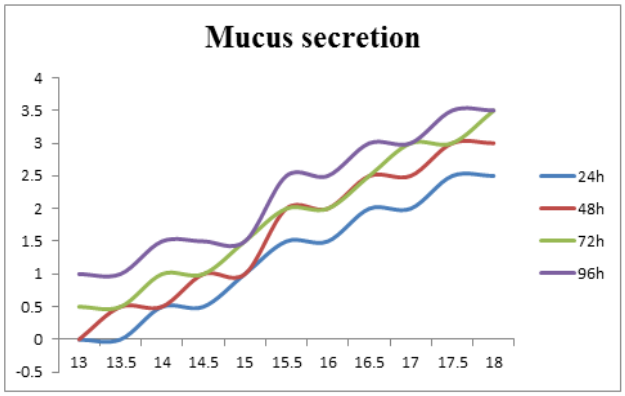

Figure 8: Changes in mucus secretion in fishes exposed to different concentration of zinc.

Table 3: Estimate of safe levels of Zinc to *Cyprinus carpio* at 96h of exposure time.

Name of the test organism	96h LC_{50} value (mg/l)	Method	Application factor (AF)	Safe level (mg/l)
Cyprinus carpio	15.804	Hart *et al.* (1948)*	-	0.481
		Edwards and Brown (1966)	0.4	6.322
		Burdick (1967), Sprague (1971) and EIFAC (1983)	0.1	1.580
		CWQC (1972)	0.01	0.158
		IJC (1977)	5% of 96h LC_{50}	0.790
		CCREM (1991)	0.05	0.790

(*C = 48h LC_{50} X 0.03/S^2, where C is the presumable harmless concentration and S = 24h LC_{50}/48h LC_{50})

much variable. In the present observation the 96h LC_{50} value of zinc to *C. carpio* is 15.804 mg/l. It nearly corresponds with the median lethal values of *Clarias lazera* (12.25 mg/l), *Cnesterodon decemmaculatus* (12.40 mg/l), *Parophrys vetulus* (14.50 mg/l) and *Clarias batrachus* (17.22 mg/l).[51-54] Hedayati *et al.* (2013)[55] recorded 96h LC_{50} value of zinc to *C. carpio* as 41.10 mg/l while it was 42.0 mg/l to the fertilized eggs of the species.[56] Such variation in the median lethal toxicity among the different studies may be due to the variations in kinetic variables that may play a role in explaining these differences. Salanki and v-Balogh (1985, 1989)[57,58] suggested that the variety in the degree of toxicity is related to kind of species, its sensitivity and physiological responses to zinc and their uptake and depuration rate. Moreover, species variation, their age, sex, weight and size differences, physical state of the treated organisms, presence or absence of enzyme system that can degrade the pollutant may also influence on the acute toxicity values for a particular metal to the same or different

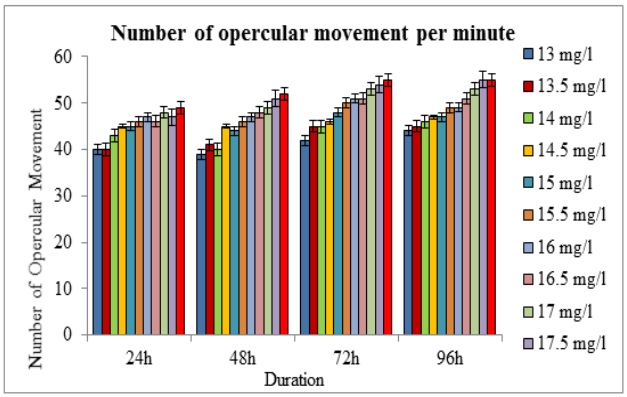

Figure 9: Mean opercular movement (no. /minute) of *C. carpio* exposed to Zinc.

organisms.[59-64] Many workers like Weatherley *et al.* (1980)[65] and Wood (2001)[66] also stated that zinc toxicity to aquatic organisms is affected by various limnological parameters like pH, temperature, dissolved oxygen, total alkalinity and hardness.[59,65-69]

In the present investigation lethality of zinc to *C. carpio* was dose and duration dependent. There was a negative relationship between the mortality time and concentration level; when the concentration level increased, the mortality time decreased. This result collaborates with the findings of Rao and Patnaik (1997)[70] on *Mystus vittatus*, Sveceviėius (1999)[71] on rainbow trout (*Oncorhynchus mykiss*), three-spined stickleback (*Gasterosteus aculeatus*), roach (*Rutilus rutilus*), perch (*Perca fluviatilis*) and dace (*Leuciscus leuciscus*), Gündoğdu (2008)[72] on *Onchorhyncus mykiss*, Shwetha and Hosetti (2009)[73] on *Cirrhinus mrigala*, Hedayati *et al.* (2013)[74] on *Cyprinus carpio.*

Tolerance is an important mechanism of the organism to react upon their surrounding adverse environment.[74] In the present study, the degree of tolerance of *C. carpio* to zinc determined by the toxicity factor (TF) at different time of exposure (Table 2) gradually increases with the progress of time probably in accordance with the degree of decreased uptake, increased excretion or redistribution of the metal to less sensitive target sites.[74]

The estimated possible safe level for zinc based on the application factor (AF) recorded in the present study (Table 3) showed large variation (0.158-6.322 mg/l) and thus made controversy over its acceptability.[75,76] So it is difficult to interpret laboratory data to the field as acceptable concentration as "safe" for the toxicant.[77,78] Several behavioral changes in *C. carpio* to zinc toxicity recorded in the present study were probably an early indication of their avoidance reaction from the test chemical.[79] Internal disturbances of the body functions such as inhibition of enzyme functions, impairment in neural transmission, and nervous impairment due to blockage of nervous transmission between the nervous system and various effecter sites, and disturbances in metabolic pathways may be attributed to these behavioral changes in fish.[40] Annune *et al.* (1994)[80] reported that sub-acute dose of zinc can cause behavioral changes in *Clarias lazera* and *Oreochromis niloticus*, which included lose of balance, agitated swimming, air gulping and death. Similarly, zinc toxicity results erratic opercular movement and swimming pattern, copious mucous secretion in *Channa punctatus*.[81] The behavioral changes like vertical and downward swimming patterns and sudden movements, capsizing in water, loss of balance and finally motionless condition were observed by Gül *et al.* (2009)[82] in guppy, *Poecilia reticulata* exposed to zinc. Again, Joshi (2011) recorded erratic and darting movements with imbalanced swimming activity followed by hyper and hypo opercula activity, loss of equilibrium, and mucus secretion all over the body in *Clarias batrachus* exposed to zinc. Some of these behaviors were consistent with the findings as seen in the present study in *C. carpio*. Similarly, the treated fish exhibited gradually decreasing swimming rate with the increasing dose of zinc over time exposure. Loss of balance during swimming in exposed fish as observed during the study might be due to some neurological impairment in central nervous system as evident by inhibition of acetyl cholinesterase by zinc.[83,84] Activeness gradually decreased in the exposed fish in the present study was probably due to the effects of zinc on central nervous system.[50,85] Excess mucus secretion in the fish exposed to zinc may was probably to prevent the metal ions from entering the body as the coagulated mucus all over the body might be acting as a protective ion trap by the –SH groups present in the mucus.[86]

Opercula movements are directly associated with respiratory process and exposure to the toxicant adversely effects on it.[87] Zinc produced respiratory distress in *C. carpio* in the present study as reflected by the increased opercula movements. The overall increase in opercula beats as observed in present study was probably to compensate hypoxia caused by degeneration of gill filament, pynotic nuclei of epithelial cells and hypertrophy of gill.[88,89] Zinc causes gill epithelial damage or gill necrosis resulting in a decrease in the oxygen consumption because of ionregulatory and acid-base balance disturbances.[46,90] This was reflected

through the increased opercula movement in *C. carpio* exposed to lethal concentrations of zinc.

CONCLUSION

This experimental work reveals that Zinc is a potent toxicant and may cause mortality in *C. carpio* even at short period of exposure. The acute toxicity value of zinc to *C. carpio* may be used for avoiding its toxic effects and for determining the safe dose of the toxicant prior to release to the aquatic environment. Further, alteration in fish behavior and changes pattern in their opercula movement can also be used as an indicator of acute changes in the in aquatic environment.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

pH: power of hydrogen ions; **LC_{50}:** lethal concentration 50; **Mg:** Magnesium; **Na:** Sodium; **WQI:** water quality index.

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