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RECORDED CHANGES IN SOME BIOCHEMICAL INDICATORS OF GRASSCARP *CTENOPHARYNGODON IDELLA* EXPOSED TO MERCURY AND ZINC

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ABSTRACT

Grass carp *Ctenopharyngodon idella,* fingerlings were exposed to lethal and sublethal concentrations of mercury and zinc. The changes in haemoglobin (Hb) haematocrit (Ht), osmolality, branchial Na^+-K^+-ATPase, acetylcholinesterase (AchE), sodium (Na^+), potassium (K^+) and water content were recorded. Blood Hb and Ht showed a general trend of significant decrease in fish exposed to lethal and sublethal concentrations of mercury and zinc. The serum osmolality showed a significant increase on exposure of fish to lethal and sublethal concentrations of mercury and zinc. In fishes exposed to lethal concentration of mercury and sublethal concentration of mercury and zinc, the activity of branchial Na^+-K^+-ATPase was increased, while in case of lethal exposure to zinc, the enzyme activity was reduced. Serum AchE activity decreased in fish exposed to lethal concentration of mercury and sublethal concentration of zinc. On the other hand, the enzymatic activity was increased in fishes exposed to lethal concentration of zinc and sublethal concentration of mercury. In case of brain, the AchE activity increased in fishes exposed to lethal concentration of mercury and zinc and decreased in fishes exposed to sublethal concentration of mercury and zinc. The concentrations of (Na^+, K^+) in serum, liver and muscle were significantly increased when fish were exposed to either lethal or sublethal treatments. The water content of liver

and muscle increased generally, on exposure to lethal and sublethal concentrations of mercury and zinc.

INTRODUCTION

Pollution of aquatic habitats is an inevitable problem aquaculturists face. The intrusion of heavy metals and their salts in aquatic environment and their accumulation in biotic system induced several structural and functional alterations in the biota (Abel, 1998).

Many workers have attempted to identify certain biochemical and physiological parameters as indices of pollutants stress on aquatic animals (Heath, 1987 & Sorensen, 1991). The value of biochemical indices of stress lies in the fact that they may be early warning signs, signaling a possible detrimental effect prior to whole scale changes in population and community structure and function potential risks to human health from (Hg) accumulated in fish and other aquatic organisms has been a major concern since the massive poisoning following methyl mercury discharged into Menameta Bay, Japan in the 1950s. More concerns have arisen about the impact of Hg on health of indigenous people who rely on fish as a dietary staple in areas including the Amazon basin (Barbosa *et al.*, 1995 and Fleming *et al.*, 1995). Mercury has neurotoxic and teratogenic effects. Many studies involved exposing fish to high level of inorganic mercury (Hg) or methyl mercury (Me Hg), caused severe gill damage (Paulose, 1987) and interfered with physiological processes involving the gill, including gas exchange and ion regulation (Lock *et al.*, 1981; Stinson and Mallat, 1989).

Zinc is a potential toxicant to fish with water hardness and pH constituting the principal modifying factors of zinc toxicity (Everall *et al.*, 1989). Major toxic effects of elevated concentrations of water borne Zn are disturbances of acid-base and ion regulation (e.g. impairment of the branchial uptake of Ca^{2+}) disruption of gill tissue and hypoxia (Hogstrand *et al.*, 1994). Chronically, toxic concentrations do not affect the gills, but cause general enfeeblement and extensive deterioration of the liver, kidneys, heart, skeletal muscles, gonads and spleen (Holcombe *et al.*, 1979).

The objective of the present work was to study and evaluate some physiological and biochemical changes in the grass carp in relation to lethal and sublethal exposure to mercury and zinc.

MATERIAL AND METHODS

Experimental animal and design:

Grass carp fingerlings samples with average weight 20 ±2.0 g used in the present work were obtained from Delta Breeding Station (DBS), Cairo. Fish were acclimated to laboratory conditions for 2 weeks before experimental work. Mercuric chloride ($HgCl_2$) and zinc chloride ($ZnCl_2$), the toxicants were obtained from Merck Company (Reagent grade) and were mixed into solutions to provide the required concentrations.

Groups of Fish:

Ten fish per group were exposed to the 96-hLC50 (0.62 mg Hg++/l or 11.46 mg Zn++/l, lethal exposure) determined according to Salah El-Deen *et al.* (1996) and scarificed after 3, 6, 12, 24, 48 and 96 hours of exposure. Other groups were exposed to 1/10 of the 96-hLC_{50} (0.062 mg Hg^{2+}/l or 1.146 mg Zn^{2+}/l, sublethal exposure) and sacrificed after 1, 2, 4, 8, 16, 32 and 64 days of exposure. Control groups with no toxicant were also included in both experiments.

Analytical Techniques:

Eight fish from each group at every interval time after exposure were randomly selected and anesthetized in 120 mg/l tricaine methane sulfonate (MS222) solution. Blood was obtained by direct puncture of the heart using glass micropipette. Serum was collected by centrifugation (8000 rpm) and was stored in a deep freezer (-20°C) for further analysis. After decapitation of fish, piece of white epaxial muscle and liver were taken for further biochemical studies. Haemoglobin content (Hb) was estimated using cyanmethemoglobin method (Van Kampen and Zijlstra, 1961). Haematocrit percentage (Ht) was determined according to Britton (1963).

Serum osmolality was measured with freezing point depression osmometer. The branchial Na^+-K^+-ATPase activity

was measured at 37°C and calculated as the differences between the rate of inorganic phosphate liberated in the present and absence of ouabain (Johanson *et al.*, 1977). The released inorganic phosphorous was measured by the method of Fiske and Subbarow (1925). The serum and brain AchE activities were estimated the calorimetric method according to Ellman *et al.* (1961) using Boheringer Mannheium Kit.

Sodium and potassium concentrations in serum, muscle and liver were determined according to Loenn and Ockari (1982) and measured with a flame photometer (Corning 410). Muscle and liver water contents were determined through difference between fresh and dry tissue weights.

Statistical Analysis:

The results were expressed as mean ± S.E.M. Data were statistically analyzed using analysis of Variance and Newman-Kelus test to evaluate the comparisons between means at $P < 0.05$ (Steel and Torrie, 1980).

RESULTS

Blood Hb in the grass carp exposed to lethal and sublethal concentration of mercury and zinc (Table 1), showed a significant decrease ($P < 0.05$). On lethal exposure to mercury, the minimum concentration (3.58 ± 0.87 g/dl) was recorded after 96 hours of exposure. The same was observed in case of lethal exposure to zinc, the lowest value (3.37 ± 0.29 g/dl) was recorded after 96 hours. On sublethal exposure to mercury, the lowest concentration (3.09 ± 0.26 g/dl) was recorded after 32 days, also a significant decrease in Hb concentration was recorded. On sublethal zinc exposure, the minimum value (2.81 ± 0.23 g//dl) was recorded after 64 days of exposure.

In Table (1), the blood Ht % in fish exposed to lethal concentration of Hg exhibited a significant decrease and the lowest value was (23.46 ± 1.97%) after 96 hours of exposure. The same was observed in case of lethal zinc exposure and the minimum (%) was (20.40 ± 1.89%) after 96 hours of exposure. The same pattern of response was observed in sublethal exposure to mercury and zinc, the corresponding values were [1723 ± 1.56 (Hg) and 15.70 ± 1.15 (Zn) %] after 32 and 64 days, respectively.

As shown in Table (2), the serum osmolality of fish exposed to lethal concentration of mercury and zinc exhibited a significant increase and the maximum recorded levels were [343.50 ± 5..94 (Hg) and 360.43 ± 7.07 (Zn) mosm] after 96 hours in both experiments. Also sublethal exposure exhibited a significant increase in serum osmolality and the highest recorded levels were [366.95 ± 7.41 (Hg) and 385.65 ± 3.92 (Zn) mosm] after 32 and 64 days of exposure, respectively.

In Table (3), fish exposed to lethal concentration of mercury and sublethal concentration of mercury and zinc exhibited elevation in the activity of branchial Na+-K+-ATPase, the corresponding activities were (4.21 ± 0.05, 3.90 ± 0.14 and 4.28 ± 0.05 μ mol/Pi/mg protein / hr) after 96 hours, 32 and 64 days of exposure, respectively. In case of lethal exposure to zinc, a non significant decrease ($P > 0.05$) was observed in the enzyme activity.

Serum AchE activity (Table 4) decreased in fishes exposed to lethal concentration of mercury and sublethal concentration of zinc. Also, the brain AchE was decreased in fishes exposed to sublethal concentration of mercury and zinc. On the other hand, the serum enzymatic activity was increased in fishes exposed to lethal concentration of zinc and sublethal concentration of mercury and also increased in brain of fishes exposed to lethal concentrations of mercury and zinc.

The concentration of Na+ in serum, liver and muscle of the grass carp exposed to either lethal or sublethal concentration of mercury and zinc (Table 5) showed a general trend of significant increase ($P < 0.05$) compared to the control values. Regarding K+ concentration, it showed a general pattern of significant increase in serum, liver and muscle after lethal and sublethal exposure to mercury and zinc (Table 6).

The water content of liver and muscle of grass carp, increased significantly on exposure to lethal and sublethal concentration of mercury and zinc as shown in Table (7).

DISCUSSION

Physiological and haematological measurements have been used as indicators of the state of fish health condition and as a biochemical method for assessing the possible mode of action

of stressors (Heath, 1987 & Adams, 1990). The changes in Ht and Hb values in fish exposed to different environmental stressors and as a result of chemical treatment have been reported by Salah El-Deen (1991) when exposed *Ctenopharyngodon idella* to diquat; and Allen (1994) in case of *Oreochromis aureus* exposed to mercury.

Mazher *et al.* (1987), studied the effects of exposure of the Nile catfish *Clarias lazera*, to sublethal concentration of mercury (0.4, 0.6, 0.8 and 1.0 mg Hg^{2+} / l) and found a progressive fall in Hb content and Ht value, and an increase in WBCs count. The authers attributed such decrease to the reduction of RBCs production in the bone marrow under the action of mercury poisoning as well as to intrahepatic and intrasplenic haemorrhage.

Panigrahi and Misra (1980) exposed *Tilapia mossambica* to a sublethal concentration of 0.5 mg/l as mercuric nitrate [$Hg(NO_3)_2$] and found that there was a decrease in Hb content and Ht value as a result of haemolysis and vaculization of RBCs caused by mercury. In addition, Goel and Gupta (1985) reported a decrease in RBCs count; Hb content and Ht value of *Heteropneustes fossilis* in response to environmental exposure to zinc for 30 days. The observed decrease in red cell indices reflects a severe anemic state closely related to prolonged zinc exposure.

Abo-Hegab *et al.* (1989) pointed out that the Hb level in grass carp, *Ctenopharyngodon idella* exposed to urea decreased throughout the experiment. They owed such decrease to disturbances of the osmotic pressure inside and outside the cells due to gain of water in the extracellular fluid.

In the present study, *Ctenopharyngodon idella* was exposed to lethal and sublethal concentrations, of mercury and zinc showed a significant decrease in Hb content and Ht values. This decrease could be attributed to many features such as impairment of gas exchange by the gills, disequilibrium of the osmotic pressure, haemolysis of erythrocytes and/or dysfunction of the spleen and destruction of large number of erythrocytes. An alternative explanation of the reduction of Hb and Ht is the shrinkage of RBCs, and/or reduction in RBCs production in the haematopoietic organs under the action of heavy metal

concentrations as reported by Kumari and Banerjee (1993) and Abbas (1998).

Changes in plasma osmolality have long been considered as an indicator of stress. In fresh water fishes, an increase in osmolality is supposed to indicate stress (Heath, 1987). If the concentration of heavy metal in the water is high enough, there is supposed to be a disruptive influence on the structural organization of the gill tissue. This will influence osmotic and ionic regulation in fish (Allen, 1993).

Stagg *et al.* (1992) reported that plasma osmolality and chloride concentrations are considered as an indicator of gross osmoregulatory dysfunction. Changes in plasma solute concentrations will be determined not just by the ability of the fish to maintain osmoregulatory homeostasis but also by the osmotic and ionic gradients imposed on the fish by the changes in environmental stressors.

In addition, Stagg *et al.* (1992) showed that flounder, *Platichthys flesus* at Port Edgar had higher mercury levels and a higher plasma osmolality than those from Eden. This strongly suggests that the inhibitory effects of contaminants, such as mercury, present at Port Edgar may cause osmoregulatory dysfunction in flounder at this site.

In the present study, lethal and sublethal concentrations of mercury and zinc caused a significant elevation in serum osmolality. These changes could be attributed to either loss of serum water or/and increase of the internal inorganic and organic osmolytes, as a result of osmoregulatory dysfunction caused by the toxicant. This assumption is supported by the results of Stagg *et al.* (1992).

Branchial Na^+, K^+ ATPase is a key enzyme in fish osmoregulation being central to the transport of monovalent ions in the gills of both marine and fresh water teleost (Evans, 1987). The enzyme has been shown to be sensitive to a wide range of contaminants following acute and chronic exposure.

Sastry and Sharma (1980) had reported that Na^+, K^+ activated adenosine triphosphatase elevated by the acute exposure to mercuric chloride, while inhibited by the chronic exposure.

Kozik *et al.* (1977) observed a decrease in adenosine

triphosphatase activity in the wall of capillaries of cerebral cortex and assumed that the phenomenon may be indicative of an injury to the blood-brain barrier. Similarly, Chang and Hartmann (1972) observed injury to the endothelium of the capillaries as well as to the surrounding galial membrane after mercuric chloride intoxication.

In the present investigation, the branchial Na^+, K^+ - ATPase activity, in the grass carp showed a non significant alteration after lethal zinc exposure. On the contrary, Na^+, K^+ - ATPase in the gills of the grass carp is clearly inhibited by the lethal and sublethal exposure to mercury and sublethal exposure to zinc intoxication. The reduction in Na^+, K^+ -ATPase activity observed in the present study may be due to an increased demand for energy supply in the gill as ATPase breakdown ATP and liberates energy. This assumption is highly supported by the work of Lock *et al.* (1981) and Jagoe *et al.* (1996).

The mechanism of osmoregulatory disruption and alteration of Na^+ and K^+ concentrations have been studied by a number of investigators (Eddy, 1982 and Heath, 1987). Christensen *et al.* (1977), reported an increase in plasma Na^+ concentration of brook trout after being exposed to lead nitrate and methyl mercury chloride at different concentrations for 2 and 8 weeks. Methyl mercury injected into flounders, *Pesudopleuromectes americanus;* on a daily basis for 13 days caused accumulation of mercury in the gills up to 24 ppm, but this treatment showed no effect on either intracellular or extracellular electrolytes (Schmidt - Nielsen *et al.*, 1977). Bukley *et al.* (1979) observed an increase in plasma K^+ concentrations of Coho salmon, after being exposed to different concentration of ammonia for 91 days. Exposure of sheephead in sea water to a massive dose of copper (exceeding the 48-hLC_{50}) was accompanied by a large increase in all plasma electrolytes (Cardeilhac *et al.*, 1979). The latter authers suggested that the greatly elevated plasma potassium concentration might be the cause of death as a result of exposure to copper in sea water.

In normally functioning kidneys, sodium and potassium ions are reabsorbed from the glomerular filtrate and passes through the kidney tubules (Smith *et al.*, 1976). Since, heavy metals are known to damage renal tubules and induce renal

failure (Rojik *et al.*, 1983), the increased levels of sodium and potassium might be due to the renal dysfunction (Larrson, *et al.*, 1985).

Tulasi *et al.* (1990) reported a severe alteration and disturbance in the ionic balance in *Barytelphusa guerini* exposed to lead nitrate and lead acetate. The author attributed these changes to the alteration in the active transport of ions. Moreover, Haux and Larrson (1982) attributed the ionic disturbance to the outward leakage of intercellular ions, especially potassium, caused by lead ions toxications.

In the present study, grass carp exposed to lethal and sublethal concentration of mercury and zinc exhibited a gradual increase in Na^+ and K^+ ions in serum, liver and muscle. This increase could be considered as a result of electrolyte balance disturbance and may be attributed to the outward leakage of intercellular ions and/or to renal failure caused by accumulation of mercury and zinc in kidney, which may contribute to increasing in the Na^+ and K^+ as postulated by Zaghloul (1997) and Abbas (1998).

The activity of acetylcholinesterase, an enzyme that modulates the amount of the neurotransmitter substance (acetylcholine) at the nerve cell junction, was reported to vary in different organs in response to different environmental stressors (Coppage, 1971 and Adams, 1990). Pham and Plancade (1971) found that, after acute cadmium intoxication, the activity of brain AchE was increased, while chronic exposure did significantly change the enzyme activity. Tejendra *et al.* (1991), observed increase in brain AchE activity of rosy barb, *Barbus conchonius* exposed to 12.6 mg/L cadmium chloride. A similar increase in brain AchE activity was found in the sheephead minnow, *Ciprinodon variegatus* exposed to lower concentration of organophosphorous insecticide diazinon (Goodman *et al.*, 1979). Such increase of the enzyme activity was attributed by the authers to represent the alarm reaction to the presence of the pollutants and can be interpreted as an influence of the stressors on cholinergic function. On the other hand, mercuric chloride was found to produce inhibition in AchE activity in brain and spinal cord of rats (Kozik *et al.*, 1977). Olson and Christersen (1980) reported that the *in vitro* effect of Cd in the rosy barb resemble

those described in the fathead minnow, *Pimephales promelas* which also showed AchE inhibition in presence of Cd, Hg, Cu and Pb. These effects seem to be due to inhibition of Ca^{2+} function at presynaptic nerve terminals (Tejendra *et al.*, 1991).

In the present investigation, the AchE activity in grass carp exposed to lethal and sublethal concentrations of mercury and zinc showed different patterns of responses (biphasic response), while there was a general increase in AchE in brain exposed to lethal concentration of zinc, there was an inhibition in serum enzyme exposed to lethal concentration of mercury. On the other hand, inhibition of AchE activity in serum (exposed to lethal zinc concentration) and in brain (exposed to lethal mercury and zinc concentrations) was observed. In addition, there was an increase in AchE activity in serum exposed to sublethal concentration of mercury.

The increase in the AchE activity could be attributed to the alarm reaction to the presence of mercury or zinc and during which more nerve impulses transfer may be needed (Hanke *et al.*, 1983 and Assem, 1985). However, the decrease in AchE activity could be due to the binding of mercury or zinc ions to lipid – rich structural component of mitochondria and subsequently affect the activities of the enzymes like AchE which associate directly with lipid-rich fractions, specially where integrity of the structural components is necessary for maximum catalytic activity. Furthermore, the decrease in AchE activity could be due to the decrease in synthesis of the enzyme by the inhibitory nature of toxicants and also to asphyxiation. Such explanation is high supported by the work of Suresh *et al.* (1992) and Abu El-Ella (1996).

Water plays a vital role in the physiology and biochemistry of animals for maintaining pH and several others biochemical reactions. Zaghloul (1997) reported increase in liver and muscle water content in *Oreochromis niloticus* after exposure to mercury and zinc.

Moreover, Abbas (1998), reported increase in liver and muscle water content of *Oreochromis aureus* and *Clarias gariepinus* under the effect of lethal and sublethal exposure to copper and lead. This elevation in liver and muscle water content is in agreement with Wheatherly and Gill (1987) who reported

that, the depletion of muscle total protein (as observed in this study) result in tissue hydration and an inverse dynamic relationships between protein and water content in the liver and muscle. This assumption agrees with the results of the present investigation (increase liver and muscle water content after lethal and sublethal exposure to mercury and zinc) and might be attributed to reduction in metabolite activity under toxicant stress conditions as reported by Verma and Tonk (1983).

In conclusion, many workers have stressed the need for the establishment of normal physiological and hematological values in connection with pollution and its effect. However, the possibility to set a standard as a diagnostic tool is still not definitive.

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Table (1): Changes in blood haemoglobin (Hb, g/dl) and haematocrit (Ht, %) of grass carp; *Ctenopharyn godon idella*, after lethal and sublethal exposure to mercury and zinc.

Lethal exposure				
Parameter	Hb		Ht	
Metal	Hg	Zn	Hg	Zn
Conc. Time	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l
Control	4.60±0.19 a	4.32±0.18 a	35.61±1.72 a	33.10±1.48 a
3 hours	5.16±0.32 ab	4.74±0.39 ab	31.53±2.47 ab	35.10±5.39 ab
6 hours	4.46±0.45 abc	4.71±0.45 abc	32.31±1.88 abc	29.21±1.38 abc
12 hours	3.95±0.32 abcd	5.05±0.34 abc	37.31±2.55 abc	28.41±2.20 abcd
24 hours	3.24±0.61 de	3.32±0.43 e	28.65±3.12 bcd	28.46±1.25 abcde
48 hours	3.21±0.89 def	3.75±0.66 ef	24.48±2.35 de	25.47±1.58 def
96 hours	3.58±0.87 ef	3.37±0.29 ef	23.46±1.97 e	20.40±1.89 f

Sublethal exposure				
Parameter	Hb		Ht	
Metal	Hg	Zn	Hg	Zn
Conc. Time	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l
Control	4.80±0.22 a	4.51±0.33 a	31.11±2.41 a	27.83±2.78 a
1 day	5.36±0.32 ab	4.63±0.36 ab	28.05±1.48 ab	27.11±2.39 ab
2 days	4.90±0.30 abc	4.93±0.24 abc	28.33±2.05 abc	23.46±1.00 abc
4 days	5.01±0.23 abc	4.96±0.37 abcd	30.28±2.62 abcd	29.73±2.08 abcd
8 days	3.37±0.27 d	4.06±0.15 abcde	27.35±1.00 cd	25.88±1.35 abcde
16 days	3.37±0.32 de	3.59±0.31 ef	20.01±2.48 e	24.96±2.08 abcdef
32 days	3.09±0.26 de	2.71±0.25 fg	17.23±1.56 e	20.66±0.93 cf
64 days	*	2.81±0.23 fg	*	15.70±1.51

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05)
* Fish died.

Table (2): Changes in serum osmolality (mosm) of grass carp; *Ctenopharyngodon idella*, after lethal and sublethal exposure to mercury and zinc.

Lethal exposure			Sublethal exposure		
Metal	Hg	Zn	Metal	Hg	Zn
Conc. Time	0.62 mg/l	11.46 mg/l	Conc. Time	0.062 mg/l	1.146 mg/l
Control	248.00±5.39	243.03±4.28 a	Control	240.30±4.61 a	241.88±4.96 a
3 hours	286.66±8.27 a	253.90±2.61 ab	1 day	248.10±7.25 a	241.21±3.36 a
6 hours	316.68±5.86 b	255.75±4.20 abc	2 days	260.95±8.15 b	253.5±4.38
12 hours	329.41±10.69 bc	237.06±3.78 abcd	4 days	266.10±4.20 b	270.33±4.58
24 hours	334.86±8.39 bcd	319.05±8.68	8 days	289.91±9.32	283.76±4.12
48 hours	321.70±3.87 bcd	332.93±6.51 d	16 days	316.66±6.97	295.58±3.75
96 hours	343.50±5.94	360.43±7.07	32 days	366.95±7.41	315.55±3.65
			64 days	*	385.65±3.92

Table (3): Changes in branchial Na^+- K^+- ATPase activity (µmol Pi /mg protein/hr) of grass carp; *Ctenopharyngodon idella*, after lethal and sublethal exposure to mercury and zinc.

Lethal exposure			Sublethal exposure		
Metal	Hg	Zn	Metal	Hg	Zn
Conc. Time	0.62 mg/l	11.46 mg/l	Conc. Time	0.062 mg/l	1.146 mg/l
Control	5.22±0.09 a	5.14±0.10 a	Control	5.09±0.17	5.22±0.12 a
3 hours	5.26±0.07 ab	5.09±0.06 ab	1 day	4.56±0.13 a	5.39±0.04 ab
6 hours	4.59±0.09 c	5.11±0.05 abc	2 days	4.60±0.07 ab	5.46±0.03 ab
12 hours	5.04±0.08 ab	5.25±0.05 abcd	4 days	4.38±0.09 abc	5.09±0.09 abc
24 hours	4.28±0.12 c	5.16±0.12 abcde	8 days	4.22±0.17 abcd	5.01±0.12 ac
48 hours	4.30±0.08 d	5.19±0.09 abcdef	16 days	3.99±0.16 cde	4.74±0.09 d
96 hours	4.21±0.05 d	5.11±0.11 abcdef	32 days	3.90±0.14 de	4.45±0.06 d
			64 days	*	4.28±0.05 d

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05) * Fish died.

Table (4): Changes in acetylecholinesterase activity in serum (U/l) and brain (U/g fresh tissue) of grass carp, *Ctenopharyngodon idella* after lethal and sublethal exposure of mercury and zinc

Lethal exposure					Sublethal exposure				
Tissue	Serum		Brain		Tissue	Serum		Brain	
Metal	Hg	Zn	Hg	Zn	Metal	Hg	Zn	Hg	Zn
Conc. Time	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	Conc. Time	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l
Control	225.20±23.06 a	175.35±18.93 a	26.33±0.90 a	26.83±0.87 a	Control	171.32±13.07 a	181.66±13.89 a	25.93±0.89 a	26.01±0.71 a
3 hours	133.80±16.24 b	164.94±5.06 ab	24.47±0.66 b	27.49±0.74 ab	1 day	158.90±12.03 ab	137.23±5.64 ab	14.36±0.62 a	19.38±0.30 a
6 hours	115.71±9.23 bc	115.55±15.04 ab	24.13±0.63 b	28.92±0.83 abc	2 days	180.77±13.26 abc	128.06±9.54 abc	17.86±0.84 b	19.69±0.39 ab
12 hours	134.04±13.93 bcd	215.59±25.72 ad	27.89±0.38 a	28.98±1.13 abc	4 days	193.33±16.18 abcd	143.17±14.63 abcd	19.24±0.72 bc	19.93±0.36 abc
24 hours	141.53±13.13 bde	219.17±27.55 ad	30.46±0.30 c	32.47±0.48 d	8 days	181.11±13.73 abcd	128.44±6.16 abcde	20.75±0.85 c	18.38±0.49 abcd
48 hours	194.41±18.98 abcdef	246.15±13.33 c	32.28±0.63 c	38.46±1.10 d	16 days	228.59±11.52 e	163.08±12.11 abcde	17.31±0.63 bc	16.21±0.77 e
96 hours	179.45±17.13 abcdef	119.72±8.44 ab	39.76±0.61 e	36.38±0.72 d	32 days	261.32±16.50 e	111.62±13.92 abcd	14.84±0.42 a	17.83±0.78 abcde
					64 days	*	100.16±10.68 abc	*	14.58±0.57 a

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05)
* Fish died.

Table (5): Changes in Sodium concentrations in serum (mmol / l), liver and muscle (mmol/kg fresh tissue) of grass carp, *Ctenopharyngodon idella* after lethal and sublethal exposure to mercury and zinc.

Lethal exposure							Sublethal exposure						
Tissue	Serum		Liver		Muscle		Tissue	Serum		Liver		Muscle	
Metal	Hg	Zn	Hg	Zn	Hg	Zn	Metal	Hg	Zn	Hg	Zn	Hg	Zn
Conc.	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	Conc.	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l
Time							Time						
Control	131.50±8.07 a	133.00±4.02 a	144.92±3.10 a	143.04±4.84 a	51.01± 2.74 a	50.75± 1.88 a	Control	125.83±4.40 a	129.50±7.14 a	144.33±4.68 a	143.76±5.43 a	51.07±2.77 a	50.90± 2.04 a
3 hours	134.00±5.81 ab	136.83±8.45 ab	148.93±3.01 ab	147.11±4.59 ab	50.56± 2.22 a	48.25± 1.91 ab	1 day	125.66±4.51 a	133.16±5.73 a	145.39±4.67 ab	145.07±4.95 ab	59.16± 2.17 ab	55.67± 2.29 a
6 hours	135.16±4.52 abc	139.83±4.38 abc	149.61±4.00 abc	149.25±5.70 ab	54.89± 1.57 b	50.29± 1.27 abc	2 days	134.16±4.36 b	144.33±6.46	150.56±4.67 bc	149.00±6.71 abc	56.65± 4.72 ab	58.03± 2.12 ab
12 hours	134.66±5.99 abc	137.66±7.94 ab	154.88±4.65 d	150.38±3.00 bc	56.58± 1.42 b	51.11± 2.29 abcd	4 days	137.33±6.43 b	154.83±5.27 b	153.98±4.63 c	150.15±5.89 bc	65.06± 4.31	61.71± 4.17 b
24 hours	147.66±5.35 d	142.66±6.97 c	151.41±6.82 bcd	150.13±2.99 bc	62.18± 1.34 c	51.48± 1.71 abcd	8 days	150.66±6.97	151.83±6.24 b	162.10±3.52	154.03±4.83	80.20± 5.08	70.35± 5.28
48 hours	150.50±4.36 dd	154.00±5.62 d	159.79±9.68 e	154.03±4.62 c	64.85± 2.77 c	61.36± 1.32	16 days	159.33±6.73	151.66±5.30 b	174.71±5.74	160.88±8.02	93.27± 5.15	77.32± 5.01
96 hours	157.33±7.07 e	151.66±4.97 d	157.95±9.81 e	159.92±4.23	72.10± 3.34	65.31± 1.99	32 days	171.83±5.41	172.85±8.17	180.70±6.43	165.66±8.65	103.92±9.57	92.89± 5.81
							64 days	*	178.13±6.29	*	177.73±9.53	*	96.83± 5.82

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05)
* Fish died.

Table (6): Changes in potassium concentrations in serum (mmol/l), liver and muscle (mmol/kg fresh tissue) of grass carp, *Ctenopharyngodon idella* after lethal and sublethal exposure to mercury and zinc.

Lethal exposure							Sublethal exposure						
Tissue	Serum		Liver		Muscle		Tissue	Serum		Liver		Muscle	
Metal	Hg	Zn	Hg	Zn	Hg	Zn	Metal	Hg	Zn	Hg	Zn	Hg	Zn
Conc.	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	Conc.	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l
Time							Time						
Control	4.91± 0.26 a	4.85± 0.20 a	132.16±6.86	132.75±6.69	143.93±7.78	145.05±8.09 a	Control	5.51±0.23 a	4.76±0.22 a	132.25±5.74 a	132.94±5.47 a	145.94±9.83 a	145.37±9.1
3 hours	5.06± 0.21 ab	5.86± 0.26 ab	132.47±8.34 a	134.13±5.22 a	133.09±8.21 a	145.86±9.31 ab	1 day	4.85±0.38 ab	4.75±0.44 ab	131.08±4.84 a	129.69±8.88 ab	137.09±11.77 a	159.54±8.13
6 hours	5.73± 0.27 abc	6.35± 0.37 abc	145.98±7.93	116.02±5.57 a	152.26±7.54	133.72±10.54 c	2 days	4.98±0.34 abc	4.76±0.41 abc	147.8± 7.51 b	130.10±9.22 ab	131.07±11.30 ab	141.26±7.01
12 hours	4.53± 0.51 abc	5.95± 0.44 bc	137.12±9.23 b	137.36±8.46 a	169.74±7.55 a	149.86±10.96 abd	4 days	5.26±0.51 abc	5.18±0.33 abcd	153.24±8.52 b	133.77±7.44 ab	134.06±9.51 ab	178.09±11.61
24 hours	6.26± 0.39 d	6.75± 0.18 d	160.52±10.17 bc	142.08±5.46 a	177.06±6.34 a	136.50±8.18 abcd	8 days	6.11±0.16 abc	4.35±0.38 abcd	170.79±6.83 c	137.66±8.13 a	202.62±11.33 cd	150.69±8.82
48 hours	6.76± 0.34 de	6.93± 0.44 de	163.90±11.78 c	156.90±3.54 a	186.31±9.25 ab	160.54±11.94 a	16 days	6.71±0.30 d	6.25±0.45 c	178.15±5.45 c	170.71±6.79 a	197.41±10.63 bc	191.36±7.87
96 hours	6.41± 0.35 de	6.95± 0.38 de	159.41±9.69 c	160.34±6.16 a	190.15±13.26 b	167.81±8.21 a	32 days	6.83±0.31 d	6.66±0.41 cf	192.72±10.33	194.01±10.47 a	195.59±10.45 cd	190.22±10.31
							64 days	*	7.11±0.22 cf	*	213.10±10.75	*	203.78±11.92

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05)
* Fish died.

Table (7): Changes in liver and muscle water contents (%) of grass carp, *Ctenopharyngodon idella* after lethal and sublethal exposure to mercury and zinc

Lethal exposure					Sublethal exposure				
Tissue	Liver		Muscle		Tissue	Liver		Muscle	
Metal	Hg	Zn	Hg	Zn	Metal	Hg	Zn	Hg	Zn
Conc. Time	0.62 mg/l	11.46 mg/l	0.62 mg/l	11.46 mg/l	Conc. Time	0.062 mg/l	1.146 mg/l	0.062 mg/l	1.146 mg/l
Control	71.58±4.64 a	72.41±1.02 a	81.02±0.58 a	80.93±0.32 a	Control	75.45±3.56 a	74.76±2.00 a	80.91±0.61 a	80.84±0.36 a
3 hours	73.60±2.59 a	71.02±1.53 ab	82.60±0.71 ab	80.55±0.19 ab	1 day	76.46±2.14 ab	73.70±1.25 ab	80.56±0.28 ab	82.38±0.50 ab
6 hours	77.59±2.44 ab	72.40±1.33 abc	82.72±0.95 abc	80.90±0.81 abc	2 days	78.20±1.91 bc	73.95±0.84 abc	81.65±1.89 abc	81.93±0.42 abc
12 hours	80.78±2.30 abc	71.96±1.41 abcd	82.21±0.60 abcd	81.66±0.37 abcd	4 days	71.11±1.71	71.30±2.85 abc	81.85±0.41 abcd	81.26±0.43 abcd
24 hours	74.39±1.05 abcd	75.75±1.06 acd	81.27±0.32 abcd	80.90±0.71 abcd	8 days	78.40±1.59 b	75.23±0.23 bcd	83.21±0.92 abd	82.23±0.23 abcd
48 hours	78.44±2.17 bd	79.50±1.48 e	83.82±0.48	84.88±0.47 e	16 days	81.55±2.64	77.83±2.81 de	86.25±0.79	85.10±0.41 e
96 hours	81.45±1.43 cd	78.75±1.48 e	86.60±0.48	85.45±0.33 e	32 days	85.35±1.11	78.36±2.7 e	89.31±0.24	85.45±1.74 e
					64 days	*	82.33±1.45	*	89.64±0.56

- Data are represented as mean ± SE.
- Means with the same letters in the same column are not significantly different (P>0.05)
* Fish died.