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Toxicity of cypermethrin, effects on serum electrolytes (Ca^{+2}, Mg^{+2} and Pi) levels and recovery response in fresh water catfish *Heteropneustes fossilis* Bloch

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ABSTRACT

The study was aimed to determine the toxicity of cypermethrin, a synthetic pyrethroid pesticide on the freshwater catfish *Heteropneustes fossilis*. The experiment was conducted in two parts for a period of 96 h, under static conditions. In the first part, the LC_{50} value of cypermethrin for 24, 48, 72 and 96 h was estimated. Behavioral changes in fish such as increased frequency of surfacing and gulping of atmospheric air, decrease in opercular movement (OCM), alteration in swimming pattern, violent shaking and jerking of head and gradual loss of balance due to cypermethrin exposure were regularly monitored for 96 h. In the second part of the study, the fish were subjected to short term (96 h) exposure of 3.42 µg/l (75% of 96 h LC_{50}) cypermethrin. Thereafter, fish were released in the cypermethrin-free water in order to study the recovery patterns in serum electrolyte levels. The data obtained from the acute toxicity tests were evaluated using EPA Probit analysis, Version 1.5, software based on Finney's Probit Analysis method. The LC_{50} values for cypermethrin 24, 48, 72 and 96 h were 5.43, 5.12, 4.82 and 4.56 µg/l, respectively. The Ca^{+2} levels decreased in the exposed fishes up to 96 h, whereas, Mg^{+2} and Pi recorded an increase. During recovery period the serum electrolytes recorded a pattern towards normalcy when compared with 96 h exposed fishes.

Key words: LC_{50}, pyrethroids, exposure, normalcy.

INTRODUCTION

Pyrethroids are the most popular pesticides owing to their short environmental persistence and high effectiveness against target organisms. The average pesticide consumption in India is 288 g/ha which is quite low in comparison to global average of 900 g/ha (Agnihotri, 2000). India is currently the second largest manufacturer of pesticides next to Japan in Asia (URL1). More than 31 million kilograms of pesticide were applied to UK crops alone in 2005 (URL2). The increasing dependence on pesticides throughout recent decades has led to pest resistance, disease susceptibility, loss of biological control and reduced nutrient-cycling. India Infoline Sector Reports on Agrochemicals 2002 (URL3) that the demand of cypermethrin has increased from 1100 metric tons (1995-96) to 1300 metric tons (1998-99). A dramatic increase in use of pyrethroids has been observed worldwide in recent years. The Pesticide Usage Survey Report of U.K. Ministry of Agriculture and Food (U.K.MKFF 90, 1995) has reported that the application area of cypermethrin has increased from 2.16×10^5 ha in 1988 to 8.63×10^5 ha in 1994. The study of environmental hazards associated with contamination of water bodies through runoff after agricultural and farming applications is still in preliminary stage. The surface water pollution due to pesticides represents a problem of global importance.

Pyrethroids are synthetic analogs of pyrethrins belonging to non-systemic chemical group of insecticides. This group can be classified into two categories- Type I and Type II, depending on their structure, properties and mechanism of toxicity (URL4). Pyrethroids generally affect central and peripheral nervous system. The primary site of action of these pesticides is the sodium channel in the nerve membrane. They cause sudden and prolonged progressive increase in Na^+ permeability of the nerve membrane resulting in long lasting chain of impulses in the sense organs and frequency dependent depression of nerve impulses in nerve fibers (Roberts and Hudson, 1998; Soderlund et al., 2002). The pyrethroid insecticides are extremely toxic to fish with 96-hour LC_{50} values generally below 10 µg/l. Corresponding LD_{50} values in mammals and birds are higher (hundred to several thousand mg/kg). Sensitivity of fish to the pyrethroids is dependent on their relatively slow metabolism and delayed elimination (Bradbury and Coats, 1989).

Cypermethrin [CAS: 52315-07-8, Chemical Name: (R,S)-alpha-Cyano-3-phenoxybenzyl - 2,2-dimethyl(1R,1S) - cis, trans - 3 - (2,2 - dichlorovinyl)cyclopropane carboxylate] is a class II - moderately toxic, highly active and broad spectrum, non accumulative pyrethroid insecticide, which is effective in public health and animal husbandry, and targets a wide range of pests in agriculture. It was first synthesized in 1974 and marketed in 1977 (IPCS.EHC82, WHO, 1989). The products containing cypermethrin are classified as Restricted Use Pesticides (RUP) by the EPA because of their very high toxicity to fish, as 96 h LC_{50} generally ranges between 0.4-2.8 µg/l (URL 5). A negative temperature coefficient of pyrethroid toxicity (more toxic at low temperature) has been observed by Steven et al. (1998). However, their toxicity is little affected by pH or water hardness (Mauck et al., 1976).

Aquatic contamination of pesticides causes acute and chronic poisoning of fish and other organisms. The pesticides damage vital organs (Cengiz and Unlu, 2006; Yildirim et al., 2006; Velmurugan et al., 2007; Peebua et al., 2008), skeletal system (Singh et al., 1997a) and produce disturbances in serum electrolytes of the exposed fish (Singh et al., 1996, 1997b, 2002; Srivastav et al., 1997; Mishra et al., 2001, 2004, 2005; Logaswamy et al., 2007).

Heteropneustes fossilis has a wide range of distribution occurring in small ponds, ditches, lakes and large swampy paddy fields. Therefore, they are easily exposed to agricultural runoff. The fish owing to its air breathing habit is more tolerant to adverse environmental conditions than carps. The present investigation aimed to study 96 h toxicity of commonly used pyrethroid cypermethrin and its effect on serum calcium, magnesium and inorganic phosphate levels. The fish recovery in cypermethrin-free tap water was also assessed. Although there are reports on the effect of insecticides on serum electrolytes, but reports on the recovery pattern after exposure are scanty and hence, the significance of this study.

MATERIALS AND METHODS

Fish, *Heteropneustes fossilis* of both sexes and generally uniform body size (length 17-19 cm, weight 25-30 g) were procured from the local ponds and safely brought to laboratory and transferred to 500 L capacity plastic tank containing tap water. They were kept on 12 h natural photoperiod for 14 days to acclimatize under laboratory conditions. Fish were daily fed with a mixture of wheat flour, mustard cake, dried prawn powder and soya bean in a ratio of 3:1:1:1. Food was given in the evening and the tank was thoroughly cleaned and filled with fresh water in the subsequent morning. During acclimatization, the physicochemical properties of tap water were routinely analyzed. The bioassay was carried out in static conditions following APHA (2005). 15 L capacity troughs filled with 12 L tap water were used for experiment and control.

Cypermethrin 25% EC, technical grade (Rallis India Pvt. Ltd., Mumbai) was diluted in acetone to prepare the stock solution and test concentrations. The concentrations of cypermethrin in the short-term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which 100% mortality occurred in the range tests. The test solution was prepared in aged tap water. After determining the test range, 9 test concentrations; 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, and 6.50 µg/l were set in triplicate for bioassay and 10 acclimatized fish were introduced in each trough. Fish were not fed 24 h before and during the bioassay. A control was simultaneously run with equal number of fish and a measured quantity of acetone (1 ml) was added in it to avoid any discrepancy in bioassay as acetone was used to prepare the test solutions of cypermethrin. The progress of experiment was regularly monitored and mortality data were recorded for 24, 48, 72 and 96 h. Dead fish were immediately removed from the trough. Behavioral changes such as swimming pattern, surfacing and gulping, jerking and tremor in body, change in opercular movement (OCM) of fish due to cypermethrin exposure were observed regularly at 1 h interval for 12 h on the first day and thereafter, every 12 h during the 96 h of exposure. The fish mortality data were analyzed by Probit Analysis Program version 1.5 developed by US EPA (URL 6) based on Finney's (1971) method for LC_{50} determination.

After determining the toxicity, a group of 48 fishes (24 hours starved) were exposed to sublethal concentration of cypermethrin (75% LC_{50} = 3.42 µg/l). Six exposed fishes were randomly selected and anaesthetized with 1g/3L, MS-222 (tricaine methane sulfonate) and blotted with dry absorbent paper for collection of blood after 24, 48, 72 and 96 h. The blood was collected in citrated tuberculin syringe directly from the conus arteriosus to avoid any contamination. Remaining 24 fishes from this group were transferred to cypermethrin-free water to study the subsequent recovery pattern in serum Ca^{+2}, Mg^{+2} and Pi levels. Another group of 24 fish exposed to sub lethal concentration of cypermethrin (3.42 µg/l) was concurrently run as control to compare with the recovery.

Serum calcium was determined according to Trinder (1960) using calcium reagent and naphtholhydroxamic acid. Inorganic phosphate in the blood was estimated according to Fiske and Subbarow (1925) by adding 2.5% of molybdate I and II solutions along with 0.25% of amino naphthal sulphonic acid. Serum magnesium was determined after adding 0.05% polyvinyl alcohol and 0.05% titan yellow to the deproteinized blood according to the technique of Neil and Nelly (1956). All the biochemical analyses were performed on Spectronic 20 D (Milton Roy Company, New York) spectrophotometer and the values were expressed in mg/100ml. The difference between the mean values of control and exposed and also between control and recovery group was statistically evaluated by Student's t-test for significance ($P < 0.05$ being accepted as significant).

RESULTS

Toxicity

The physicochemical properties of water during bioassay were; temperature 24 ± 1 ^{0}C, pH 7.2 ± 0.15, dissolved oxygen 7.8 ± 0.76 mg/l and hardness as $CaCO_3$ 115.34 ± 1.45 mg/l. The bioassay results show no mortality at concentration 2.50 µg/l for 96 h, 30% mortality at 4.00 µg/l in 72 h, 60% mortality at 5.50 µg/l in 48 h and 100 % mortality at 6.50 µg/l in 24 h. No mortality was recorded in control. The LC_{50} values of cypermethrin for 24, 48, 72 and 96 h in *Heteropneustes fossilis* is 5.43, 5.12, 4.82 and 4.56 µg/l, respectively (Table 1).

Behavioral responses of fish in control group and at lowest concentration (2.50 µg/l) exhibited normal behavior. Similarly no apparent change in fish activity was observed at 3.00 µg/l and 3.50 µg/l concentrations during first 48 h of exposure, but afterwards, some abnormalities in locomotion and loss of equilibrium were observed. First sign of stress and alteration in behavior of fish were noticed after 15 min of exposure to cypermethrin at concentration 4.00 µg/l and above. Exposed fishes exhibited increased frequency of surfacing and gulping of atmospheric air compared to control groups. There was a change in swimming pattern of exposed fish, marked by sudden, short and swift movement followed by a brief period of calm settlement of fish at the bottom of trough during early exposure to the toxicant (within 2 hours). The opercular movement (OCM) of fish however, decreased at all exposure concentrations except control. As is evidenced in Figure 1, the OCM suddenly falls after the exposure (2 h) but shows some improvement up to 24 h and thereafter, gradually falls again throughout the test period. A two-way ANOVA shows significant ($P<0.05$) decline in OCM of fish with exposure time and concentration. A thick coat of mucus gradually appeared on the entire body surface of exposed fish which increased with time of exposure. Apparently the fish exhibit violent shaking and jerking of their heads while swimming. Eventually the fish showed gradual loss of balance before death.

Effects on serum electrolytes (Ca^{+2}, Mg^{+2} and Pi) levels and Recovery responses

Serum Ca^{+2} level recorded significant ($P<0.001$) decline gradually up to 96 h on cypermethrin exposure (Figure 2). However, on exposure to the pesticide-free water, the serum Ca^{+2} levels recorded a significant ($P<0.05$) increase towards normalcy (Figure 2). Serum Pi and Mg^{+2} levels exhibited a gradual increase at 48 h and 72 h ($P<0.05$) recording a significant rise ($P<0.001$) at 96 h in cypermethrin exposed fishes (Figures 3 and 4). When the fish were transferred to cypermethrin free water, they exhibited a decline in serum Pi and Mg^{+2} within 24 h ($P<0.05$) and thereafter, a significant ($P<0.001$) recovery within 96 h from the toxicant induced stress (Figures 3 and 4).

DISCUSSION

In the present study, 96 h LC_{50} value for *H. fossilis* is 4.56 µg/l. This value is very high when compared with the results of Saha and Kaviraj (2003), i.e. 1.27 µg/l (96 h). The difference in LC_{50} value may be due to difference in ambient temperature (Pandey et al., 2008). Saha and Kaviraj (2008) performed the test at 20 °C, whereas in present study, the temperature recorded was 24 ± 1 °C. Generally, the pyrethroids record lower LC_{50} value at low temperature (Steven et al., 1998). Moreover, Saha and Kaviraj (2003, 2008) reported that LC_{50} values of cypermethrin do not change from 72 h to 96 h because cypermethrin remains active in water for a maximum period of 72 h. However, in present study, cypermethrin induced mortality up to 96 h, indicating persistence of its toxicity (Table 1). The findings in this study are agreed, despite the variance in degree with those of Mishra et al. (2002) who reported low toxicity (LC_{50} 7.20 µg/L) at 96 h for the same species. The fish used by Mishra et al. (2002) appear much hardy, probably because of larger size and weight (37.42 g). The increased surfacing and gulping frequency may be an indication of respiratory trouble due to the damage caused by cypermethrin in fish gills. Yildirim et al. (2006) and Cengiz and Unlu (2006) have reported damage in the fish gill after deltamethrin (pyrethroid) exposure. Hughes and Singh (1971) have observed that under normal physico-chemical condition of water, *Heteropneustes fossilis* consumes more oxygen (59%) from the water than from air (41%). In the present study, a negative correlation was observed in OCM

Table 1: LC_{50} values of cypermethrin and its confidence limits in *Heteropneustes fossilis*.

Duration (h)	LC_{50} (µg/l)	Confidence limits Lower	Upper	Intercept	Slope functions
24	5.43	5.25	5.36	5.38 (±1.24 SE)	14.13 (±1.70 SE)
48	5.12	4.94	5.32	4.07 (±1.01 SE)	12.78 (±1.42 SE)
72	4.82	4.63	5.00	3.11 (±0.87 SE)	11.87 (±1.25 SE)
96	4.56	4.30	4.67	2.45 (±0.79 SE)	11.43 (±1.72 SE)

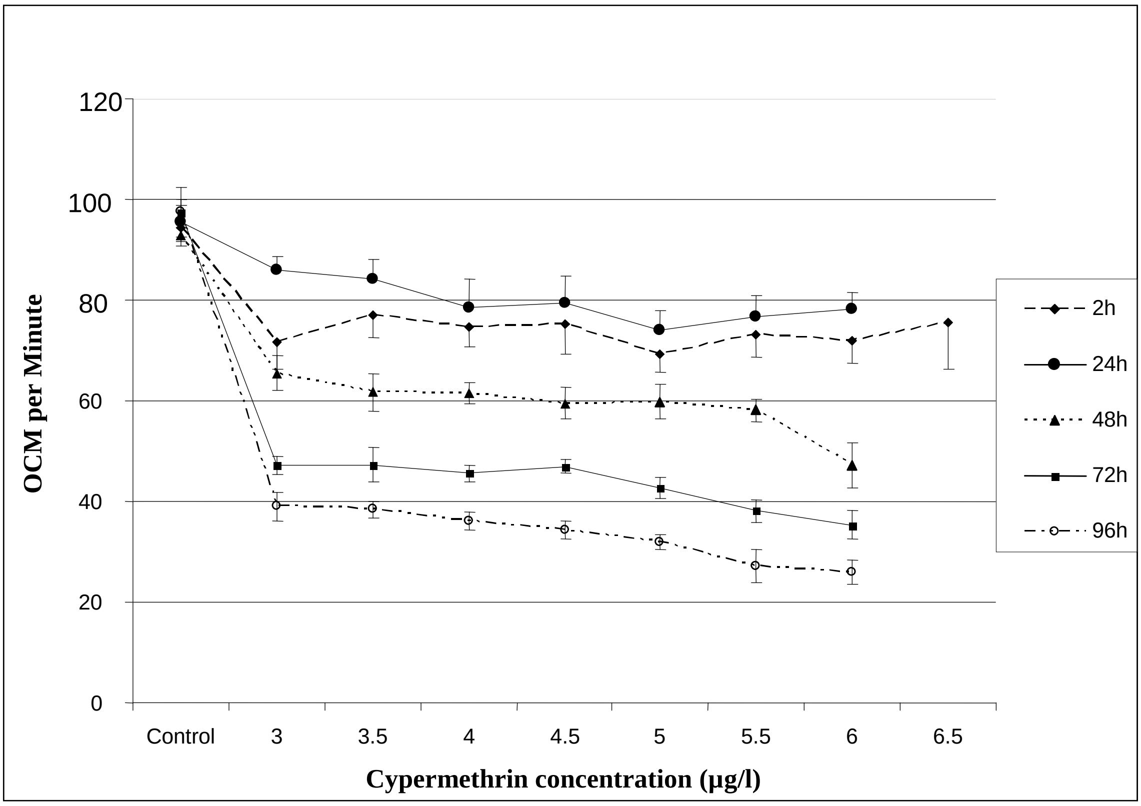
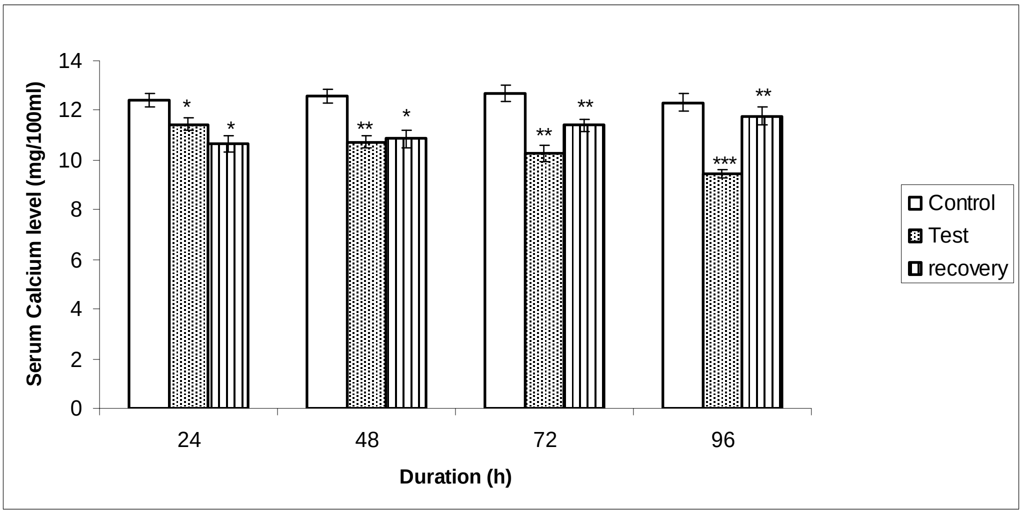
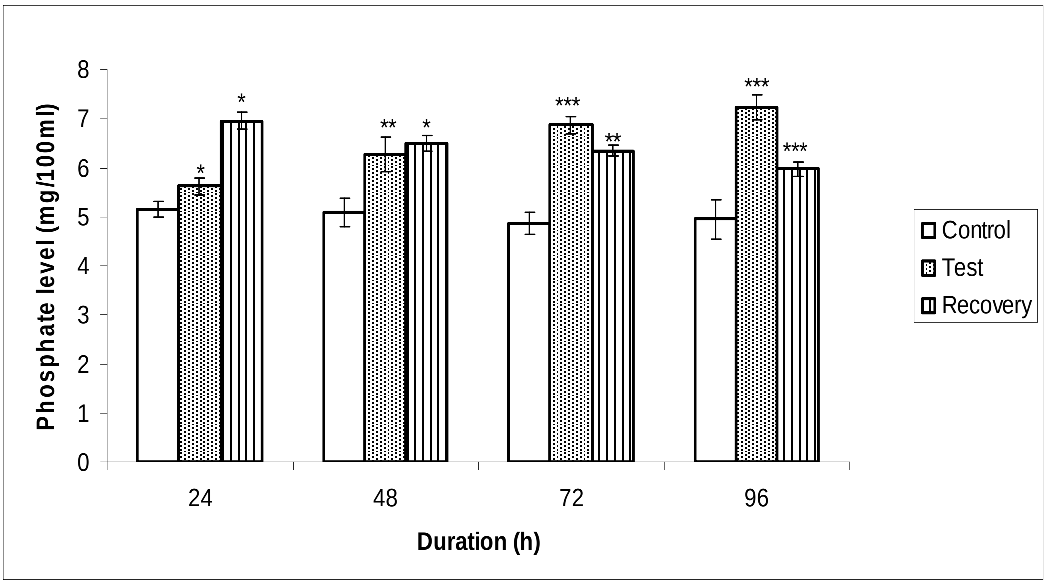


Figure 1: Opercular movement in *Heteropneustes fossilis* after cypermethrin exposure at different concentrations and time intervals [A two-way ANOVA depicts significant ($P < 0.05$) decline in OCM with respect to cypermethrin concentration and exposure time].



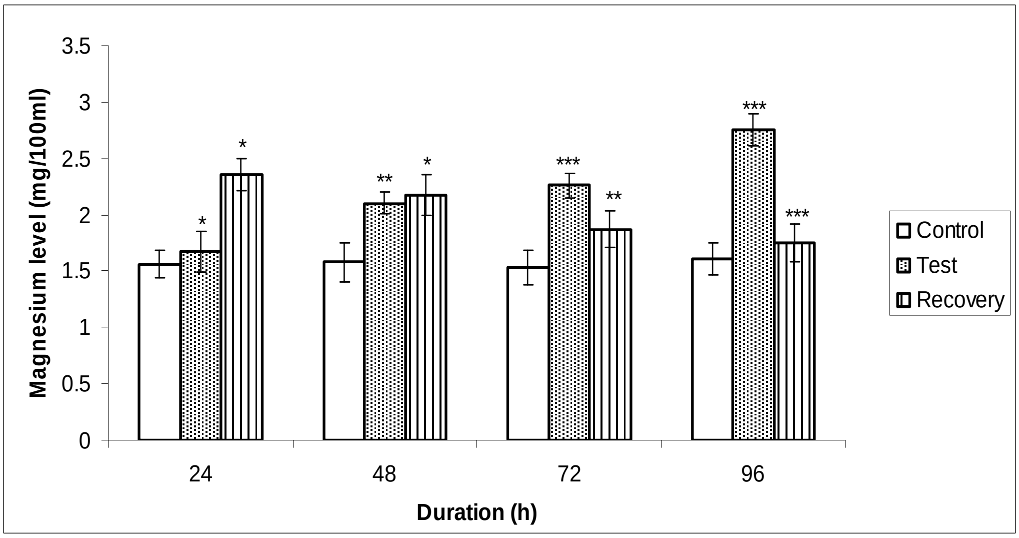
Values are Mean ±SD of six fish; * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ (Student's't' test)

Figure 2: Serum calcium level (mg/100ml) of *Heteropneustes fossilis* after cypermethrin (75% LC_{50} = 3.42 µg/l) exposure and recovery pattern.



Values are Mean ±SD of six fish.
 * P<0.05, ** P<0.01 and *** P<0.001 (Student's't' test)

Figure 3: Serum Inorganic Phosphate level of *Heteropneustes fossilis* after cypermethrin (75% LC_{50} = 3.42 µg/l) exposure and recovery pattern.



Values are Mean ±SD of six fish.
 * P<0.05, ** P<0.01 and *** P<0.001 (Student's't' test)

Figure 4: Serum magnesium level of *Heteropneustes fossilis* after cypermethrin (75% LC_{50} = 3.42 µg/l) exposure and recovery pattern.

with toxicant concentration and exposure time. Under stressful toxic condition, fish avoids uptake of water by reducing the opercular movement, simultaneously increasing the gulping frequency (Pandey et al., 2009). Similar observations were recorded by Saha and Kaviraj (2003) in cypermethrin treated *Heteropneustes fossilis*. Alteration in swimming behavior is marked by sudden short and swift movement followed by a brief period of calm settlement of fishes at the bottom of trough during early hours (within 2 h) of toxicant exposure. While swimming, fish vigorously shake their head, probably in an attempt to get rid of heavy mucus deposition on the gills. The loss of balance associated with body tremor in fish, with increased cypermethrin concentration and exposure time, may be attributed to the effect of toxicant on Na^+ channels in nerve membranes affecting the transmission of nerve impulse (Roberts and Hudson, 1998; Soderlund, 2002).

Cypermethrin exposure causes hypocalcaemia, hyperphosphataemia an hypermagnesaemia after 24, 48, 72 and 96 h whereas, the serum Mg^{+2} and serum inorganic phosphate content increased significantly during 48, 72 and 96 h exposure period when compared with control values. This is in agreement with the earlier results showing hypocalcaemia, hypermagnesaemia and hyperphosphataemia in teleosts exposed to sublethal concentration of aldrin (Singh et al., 1996), propoxur and formothion (Singh et al., 1997a). Hypocalcaemia and hypophosphataemia have also been observed in teleosts exposed to cypermethrin, deltamethrin, Metacid-50 and chlorpyrifos (Mishra et al., 2001, 2004, 2005; Atamanalp et al., 2002; Logaswamy et al., 2007). Thangavel et al. (2005) have reported hypocalcaemia and hypophosphataemia in *Oreochromis mossambicus* when exposed to dimecron and ziram. Velisek et al. (2006), however, reported no change in Ca^{+2} and phosphate levels in common carp exposed to deltamethrin. Earlier workers have reported that hypocalcaemic effect is apparently caused by inhibition of Ca^{+2} uptake by the gills. Pesticide exposed fishes generally show damage of gill epithelium (Yildirim et al., 2006; Velmurugan et al., 2007; Peebua et al., 2008). The damage in branchial epithelium probably inhibits the intake of Ca^{+2} from the ambient water causing hypocalcaemia, resulting in respiratory distress, hyper-excitability and tremor in *H. fossilis*. Increase in inorganic phosphate in blood and skeletal muscle have been observed in fish after endosulfan exposure (Gill et al., 1990, 1991). Singh et al. (1997a) also reported consistent hyperphosphatemia with hypocalcaemia and skeletal deformity in *H. fossilis* exposed to insecticides.

Hypermagnesaemia has been reported in a number of fishes exposed to variety of insecticides (Singh et al., 1996; Singh et al., 1997a, 1997b, 2002). However, Rangaswami and Padmanabha Naidu (1989), observed hypomagnesemia in tilapia exposed to endosulfan. Dabrowska et al. (1991) suggested an inverse correlation between Mg^{+2} and Ca^{+2} levels in common carps, while Thangavel et al. (2005) reported no change in serum Mg level. However, more evidence point to the fact that fishes having hypocalcaemia during insecticide exposure exhibit increased Mg level, which could be due to kidney damage. Gill et al. (1988, 1989) observed glomerular shrinkage and tubular atrophy in the kidney of Carbaryl and Cd exposed *Puntius conchonius*, which fail to excrete Mg ions. Earlier, Giles (1984) also noted hypermagnesemia with decrease in serum calcium and urinary Mg level in cadmium exposed rainbow trout. These reports support the present findings in *Heteropneustes fossilis* showing hypocalcaemia and hypermagnesemia in cypermethrin exposed fish.

The electrolyte levels (Ca^{+2}, Mg^{+2} and Pi) in *H. fossilis* exhibit a remarkable gradual pattern of recovery towards normal levels. Begum (2008) has reported a similar recovery response in gill and kidney tissues for protein, amino acid and certain enzymes in *Clarias batrachus* exposed to carbofuran.

It may be concluded from the present study that cypermethrin is highly toxic to fish *Heteropneustes fossilis*, though it is non-persistent in the environment. Recovery from the effects of exposure was fast and hence, controlled release of the toxicants can lead to restoration of affected aquatic life, particularly fish. Even the short term exposure from agricultural runoff may cause considerable damage to the fish population. Therefore, indiscriminate use of cypermethrin must be avoided and certain other insect pest control measures should be adopted.

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