In vivo Impact of Monocrotophos on Biochemical Parameters of a Freshwater Fish during Subacute Toxicity and Following Cessation of Exposure to the Insecticide

Madhusudan Reddy Narra[a], Ghousia Begum[b,*], Kodimyala Rajender[a], and J. Venkateswara Rao[b]

[a] Department of Zoology, College of Science, Osmania University, Hyderabad 500 007, A. P., India
[b] Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, A. P., India. Fax: +91 (40) 27193227. E-mail: ghousia@iict.res.in or ghousia786@yahoo.com

* Author for correspondence and reprint requests

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In vivo toxicity of monocrotophos on key metabolites and enzymes of the protein metabolism was investigated in important tissues of the freshwater fish *Clarias batrachus*. Fish were exposed to 1/10 and 1/20 of LC_{50} concentration for 28 days. After 28 days of exposure, some fish were transferred to monocrotophos-free water and kept in the same for 21 days (recovery period) in order to study the recovery response. Total protein, amino acid, and ammonia contents were decreased in gill, kidney, liver, and muscle tissues, and recovery was slight at the end of 21 days of transfer of fish into freshwater. Urea and glutamine levels were elevated, except in kidneys, and recovered at the end of the recovery period. The activities of protease, transaminase, and phosphatase enzymes were elevated in all tissues during 28 days of exposure and at both concentrations. Recovery of the activity of enzymes was more significant at the lower concentration as compared to the higher concentration.

Key words: Monocrotophos, Biochemical Alterations, Fish

Introduction

At present there is growing concern worldwide over the indiscriminate use of pesticides which results in environmental pollution and toxicity risk to non-target organisms. Organophosphorus (OP) compounds are widely used in household, agriculture, medicine, and industry. Overspray and/or runoff of pesticides from agricultural fields, industry operations, and household use may easily contaminate water bodies, resulting in serious damage to non-target species including fish. Responses to OP insecticides by aquatic organisms have a broad range depending on the exposure period, water quality, and species (Richmond and Dutta, 1992). Fish are particularly sensitive to environmental contamination of water and, therefore, pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fish (White *et al*., 1992; Storm *et al*., 2000).

Monocrotophos (MCP) is an OP insecticide with both a contact and systemic action. The extensive use of MCP poses a health hazard to animals and humans because of its persistence in soil and crops (WHO/IPCS, 1996). In humans, the main risk groups of higher-dose MCP exposure are its producers, workers in pesticide industries, and farmers. The majority of the population is exposed to lower doses of MCP via food, contaminated drinking water, or by application of household insecticides containing MCP (WHO/IPCS, 1996). Exposure to a low level of OP pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects reported in humans and experimental animals (Sultatos, 1994). As results of its widespread use, MCP has been detected in ground, surface, rain, urban, and rural water (White *et al*., 1992), and it may exert harmful effects in the organs of aquatic organisms (Nemcsok *et al*., 1987).

Fish are useful bioindicators and integrators of contaminants for various reasons, *viz*., their wide distribution in the freshwater environment, the fact of being free swimmers, their ability to

respond to environmental pollution, and their importance as a food source for human beings (Gupta *et al*., 2009). Hence, it is of interest to investigate the impact of MCP on some biochemical constituents and the activities of certain key enzymes in different tissues of the edible freshwater fish *Clarias batrachus*. This fish is a major source of animal protein for the rural population. It is cultivated in paddy fields in a "paddy cum fish" culture program in India, and is therefore often chronically exposed to insecticides (Begum, 2004).

The main objectives of the present study were to determine the toxicity of sublethal concentrations of MCP on metabolites and enzymes of the protein metabolism and to study the recovery in the freshwater fish *Clarias batrachus*, as the sublethal concentrations of pesticides offer an excellent scope to observe behavioural and biochemical changes in animals (Edwards, 1973). We have selected two sublethal concentrations of MCP based on LC_{50} studies and determined their effects on the levels of total proteins, free amino acids, end products of ammonia, urea and glutamine, and the activities of protease, acid and alkaline phosphatase (ACP, ALP), alanine and aspartate aminotransferases (ALAT, AAT) in gill, kidney, liver, and muscle tissues of *Clarias batrachus*.

Material and Methods

The freshwater fish *Clarias batrachus* (Linnaeus) were procured from local suppliers and brought to the laboratory in aerated drums. The procurement and acclimation of fish and the experimental conditions were according to the OECD (1992) guidelines. The fish were acclimatized for 15 d in a huge cement tank and fed with commercially available dry prawn and egg albumin. Fish weighing 20–25 g (length 15–20 cm) were transferred to aquaria of 40 L water capacity for a further period of 28 d for conditioning. The natural photoperiod of 12 h light:12 h dark was maintained. The average values of water quality during investigation were as follows: temperature, (25 ± 3) °C; pH 7.4 ± 0.4; dissolved oxygen, (8.24 ± 0.22) mg/L; total hardness, (415 ± 1.2) mg/L as $CaCO_3$; alkalinity, (348 ± 1.6) mg/L as $CaCO_3$; chlorides (245.57 ± 1.44) mg/L.

The test compound 36% EC monocrotophos was purchased from a local market [National Organic Chemical Industry Limited (NOCIL), Bombay, India]. The stock solution of MCP was prepared by dissolving it in 100% acetone. To determine the LC_{50} value, ten fish per 10 L of water were exposed to six serial concentrations of MCP. For each concentration the experiment was repeated three times with a parallel control, and mortality was noted after 96 h. The LC_{50} value was determined using the semi-static method of Finney (1971). A group of 42 fish exposed to two sublethal concentrations of MCP, *viz*. 1.072 and 2.114 mg/L (corresponding to 1/20th and 1/10th of the 96-h LC_{50} value of 21.44 mg/L) in 42 L of laboratory water for 28 d served as exposed group. After 28 d, 18 fish from the MCP-exposed group were released into fresh water and kept in the same for 21 d in order to study the depuration pattern. The depuration experiments were performed at the end of days 7, 14, and 21 only; whereas exposition studies were done at the end of days 7, 14, 21, and 28. The control experiment was performed by addition of the appropriate amount of the carrier solvent acetone. Water was renewed daily and the required concentration of MCP was added to the exposed group only. Fish were fed during the experiment but starved 24 h prior to sampling. Gill, kidney, liver, and muscle tissues were dissected, cooled to 4 °C, and used within an hour for the estimation of metabolites and enzymes. The experiments were repeated three times, and data was analysed by Student's *t*-test. There was no significant change in the values of control fish; hence summarized control values were taken. Graphs were plotted as percent variation relative to the control.

Homogenates (1% w/v) of the tissues were prepared in trichloroacetic acid (5% w/v) using a Potter-Elvehjem homogenizer (Heidolph, Berlin, Germany). Total protein was determined according to Lowry *et al*. (1951), with bovine serum albumin as standard. Free amino acids (FAA) were estimated by the method of Moore *et al*. (1954) with tyrosine as standard. The level of ammonia was determined using ammonium chloride as standard, of urea by the diacetyl monoxime method (Natelson, 1971), and of glutamine by the acid hydrolysis method of Colowick and Kaplon (1967). For estimation of the activities of aspartate aminotransferase (AAT; E.C. 2.6.1.1) and alanine aminotransferase (ALAT; E.C. 2.6.1.2), 5% homogenates (w/v) of tissues were prepared in 0.25 M ice-cold sucrose solution, and the assay was performed by the method of Reitman and

Frankel (1957). Protease activity was measured as described by Moore *et al.* (1954), the reaction mixture containing 100 μL of phosphate buffer (pH 7.0) and 12 mg of denatured protein. The alkaline phosphatase (ALP; E.C. 3.13.1) activity was determined by the method of Moss *et al.* (1986) and the acid phosphatase (ACP; E.C. 3.13.2) activity by the method of Jabeen (1984).

Results

The LC_{50} data are shown in Figs. 1A and B. The sublethal toxic concentrations of MCP did not cause any visible symptoms in the fish. No mortality was recorded during the exposure period of 28 days. The total protein content was decreased in all tissues during MCP exposure; the percent decrease was higher at the higher MCP concentration. At the higher concentration, the highest decrease was noted in gill (–73%), followed by liver, kidney, and muscle (Fig. 2A). Recovery in the protein content was observed after transfer of fish to clean water. The protein content reached near control values in kidney and muscle tissues at the lower concentration. A decrease in the free amino acid content was observed in gill, kidney, liver, and muscle tissues on all days of exposure and at both concentrations. The magnitude of depletion was directly related to the concentration of MCP. After transfer of *C. batrachus* into fresh water, kidney tissue showed maximum recovery at the lower concentration (Fig. 2B).

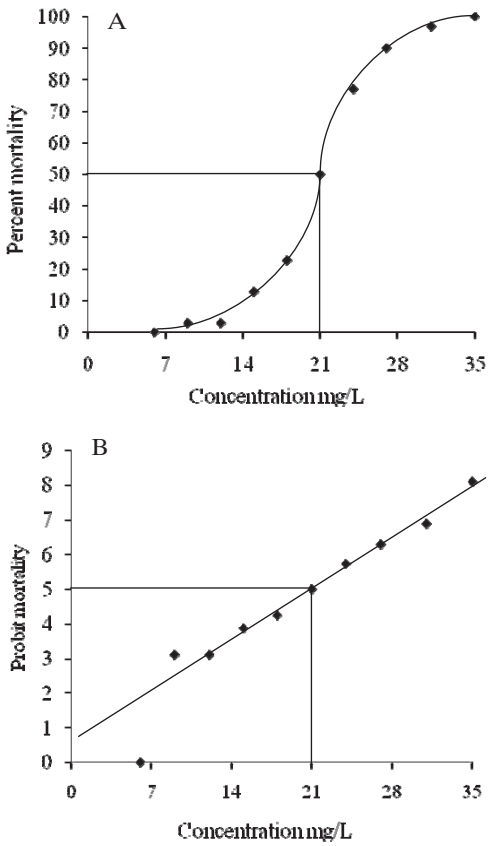

Fig. 1. (A) 96-h percent mortality versus MCP concentrations and (B) 96-h probit mortality versus MCP concentrations. Values are the mean of three replicates.

The ammonia content was decreased in all tissues on exposure to the two MCP concentrations. The highest decrease was observed in kidney (–81%) at the higher concentration. Recovery of the ammonia content was highest in liver, followed by gill, at the lower concentration (Fig. 3A). The urea content was increased in gill, liver, and muscle, whereas it decreased in kidneys at both concentrations (Fig. 3B). The glutamine levels exhibited a pattern similar to that of urea, *i.e.* decrease in kidney and increase in other tissues (Fig. 4A). Recovery in urea and glutamine contents, respectively, was noted in all tissues.

MCP intoxication in *Clarias batrachus* exerted adverse effects on protease, phosphatases and transaminases (Figs. 4B, 5, 6). Protease and aminotransferase activities were elevated in all tissues throughout the exposure period and at both concentrations. When the fish were transferred to clean water, protease and transaminase activities showed a recovery response. Muscle tissue at the lower concentration showed maximum recovery in protease as compared to other tissues, and the difference between recovered and control muscle was only 1.7%. Among transaminases, alanine aminotransferase recovery exceeded that of aspartate aminotransferase. Acid and alkaline phosphatase activities were reduced in liver throughout the exposure period, whereas in other tissues the activities were elevated. Maximum recovery in alkaline and acid phosphatases was seen in muscle and kidney tissues, respectively.

Discussion

The decrease in the protein content in tissues of *C. batrachus* was similar to that observed in the fish *Anguilla anguilla* exposed to an organophosphate insecticide (Sancho *et al.*, 1997). The

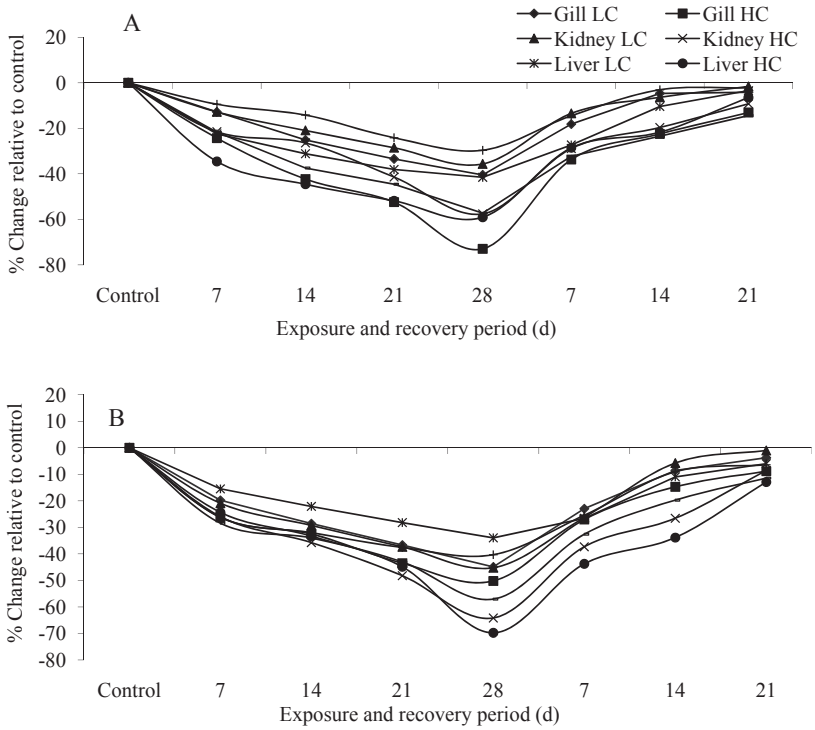

Fig. 2. (A) Total protein and (B) free amino acid contents in different tissues of *C. batrachus* exposed to two sublethal concentrations of monocrotophos for 28 days followed by 21 days of recovery. The experiment was repeated three times and the values represent percent changes from control. LC, lower concentration; HC, higher concentration.

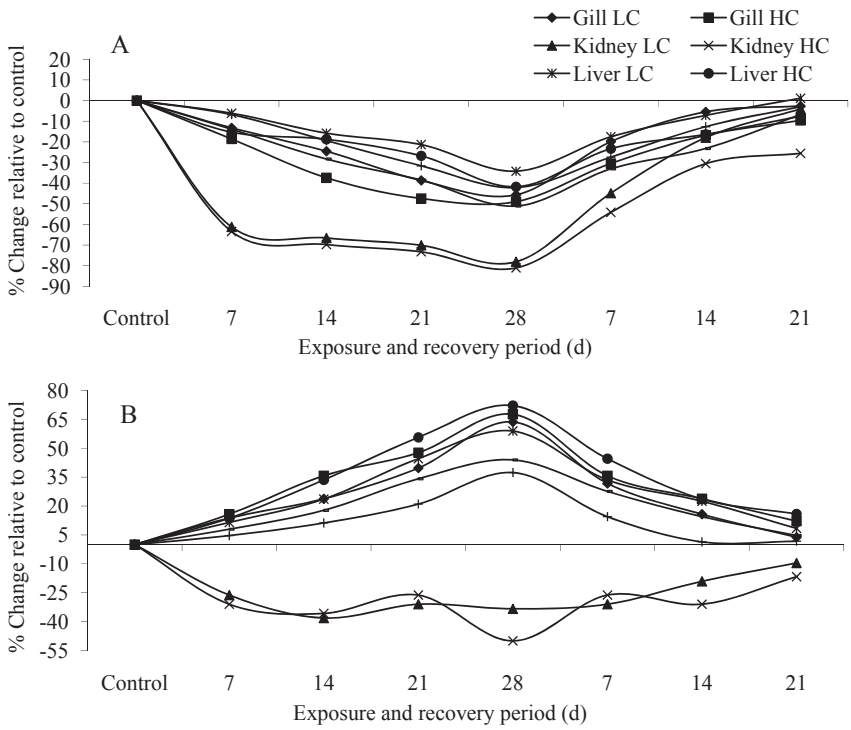

Fig. 3. (A) Ammonia and (B) urea levels in different tissues of *C. batrachus*. For statistics see Fig. 2.

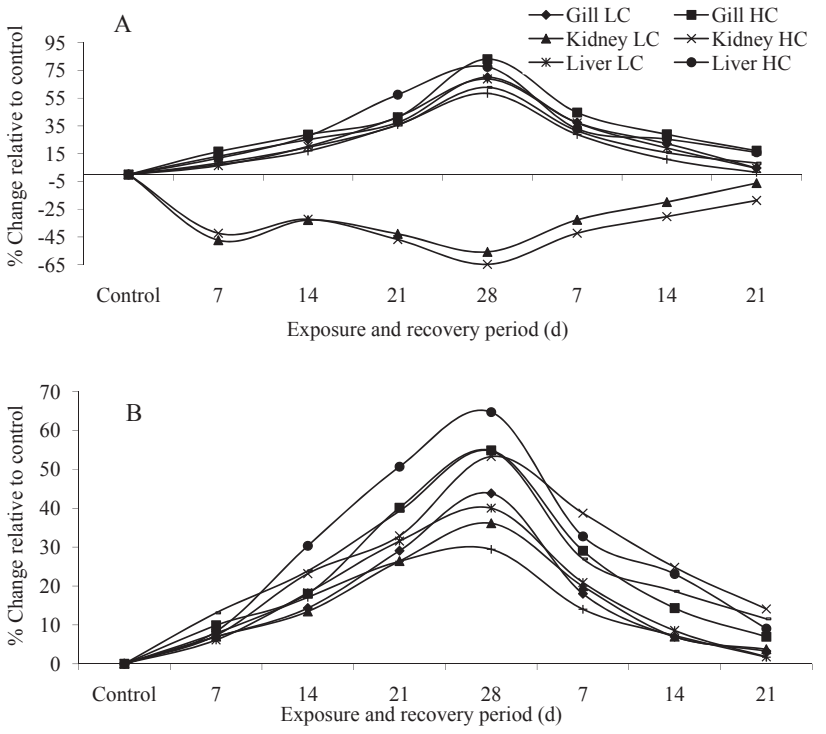

Fig. 4. (A) Glutamine content and (B) protease activity in different tissues of *C. batrachus.* For statistics see Fig. 2.

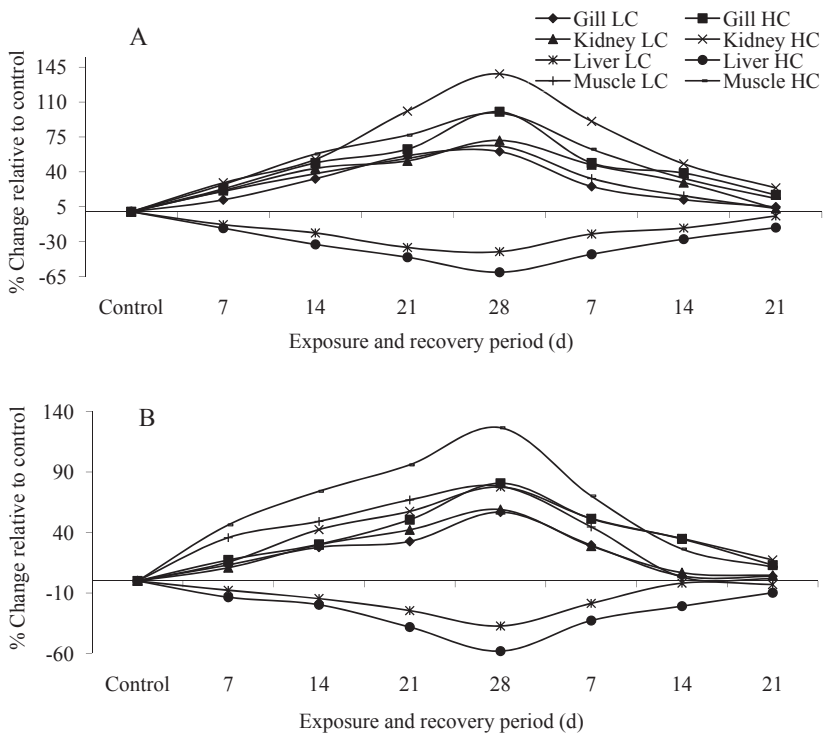

Fig. 5. (A) Acid phosphatase and (B) alkaline phosphatase activity in different tissues of *C. batrachus*. For statistics see Fig. 2.

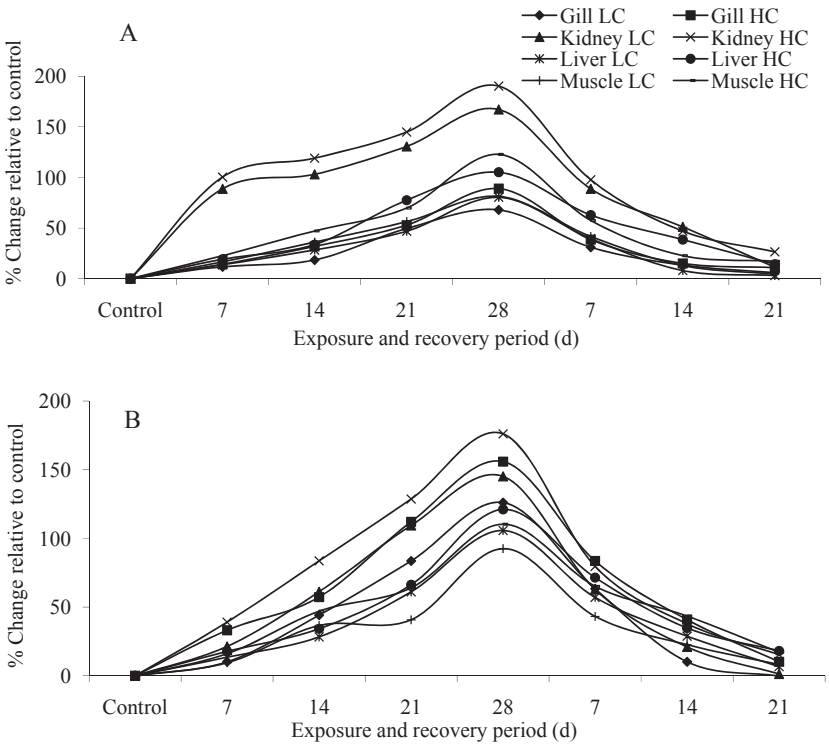

Fig. 6. (A) Aspartate aminotransferase and (B) alanine aminotransferase activity in different tissues of *C. batrachus*. For statistics see Fig. 2.

highest decrease in gills indicates that these are the primary route for the entry of pesticides. The decreased protein content could be due to blocking of the protein synthesis, or protein denaturation or inhibition of amino acid synthesis as suggested by Jha (1991). Another reason might be pesticide-induced apoptosis (Sobha *et al.*, 2007). When MCP-pre-exposed fish were released into fresh water, a reversal in the protein content was observed. The decrease in the free amino acid content could be due to the synthesis of nitrogenous compounds, through transamination and oxidative deamination as suggested by Philip and Rajasree (1996). The percent reduction was higher at the higher MCP concentration compared to the lower one. When MCP-exposed fish were released into fresh water, recovery was higher at the lower concentration.

MCP toxicity at both concentrations resulted in depletion of ammonia in all tissues, which suggests the removal and excretion of ammonia from the body of the fish. The kidneys of fish receive the largest portion of post-branchial blood for purification and are considered to be good indicators of environmental pollution (Ortiz *et al.*, 2003). The highest decrease in kidneys might be due to their purification capacity. The decrement of ammonia could be due to decreased fixation of ammonia through keto acids leading to glutamate formation by the action of glutamate dehydrogenase as suggested by David *et al.* (2004). The ammonia content recovered maximally in liver followed by gills after 21 days of recovery. The urea and glutamine levels in gill, liver, and muscle tissues of MCP-exposed fish exceeded those in controls, which may indicate that the fish adapted to the biosynthesis of glutamine and urea as a major pathway of detoxification of ammonia. The decrease in urea and glutamine levels in kidneys suggests that ammonia was directly excreted from this major excretory organ. Thus the kidneys exhibited a tissue-specific response to MCP intoxication. At the end of 21 days of recovery, urea and glutamine were near to controls in muscle tissue at the lower MCP concentration.

An increase in enzyme activities is a better indicator for tissue damage due to stress than hormones (Navrátil *et al.*, 1998). Increased protease activity promotes proteolysis to produce excess energy required to overcome the stress of MCP.

Slight to high recovery in the protease activity was noted at the end of 21 days of recovery. Exposure to pollutants is known to alter the phosphatase activity (Omkar, 1985). In the present investigation, acid and alkaline phosphatases were induced in all tissues except hepatic (Fig. 5). Similar results were observed in the fish *Oreochromis mossambicus* exposed to MCP (Rao, 2006). Acid phosphatase is used by lysosomes for the hydrolysis of foreign material; hence it plays a role in detoxification (Jaroli and Sharma, 2005). The induction of acid phosphatase activity in gill, kidney, and muscle tissue indicates the destruction of the lysosomal membrane and proliferation of lysosomes in an attempt to sequester the toxic xenobiotic (Gill *et al*., 1992). Alkaline phosphatase is a membrane-bound enzyme found at the bile pole of hepatocytes, in pinocytic vesicles, in the golgi complex, and more predominantly in the microvilli of bile canaliculi. It is often employed to assess the integrity of the plasma membrane (Akanji *et al*., 1993). Inhibition of the alkaline phosphatase activity in hepatic tissue could be taken as an index of hepatic parenchymal damage, hepatocytic necrosis, and uncoupling of oxidative phosphorylation. It reflects an organ-specific response related to the liver's function in determining the exposure route, distribution, and bioaccumulation of pollutants, as well as the defensive capacity (Ahmad *et al*., 2006). Elevation in the alkaline phosphatase activity of gill, kidney, and muscle tissues might be due to the accelerated membrane transport function related to hydroxy ion exchange across the lipid biomembranes (Jaroli and Sharma, 2005) and also due to the destruction of the smooth endoplasmic reticulum (Khan and Pandya, 1985). Transaminases play an important role in the utilization of amino acids for oxidation and/or gluconeogenesis (Samsonova *et al*., 2005). The induction in AAT and ALAT enzymes in gill, kidney, liver, and muscle tissues of *C. batrachus* is the response to MCP stress to generate keto acid derivatives, such as ketoglutarate and oxaloacetate, for utilization in gluconeogenesis and/or energy production to cope with the elevated energy demand created by the MCP toxicity. When the fish were released into fresh water, the ALAT and AAT activities recovered (Fig. 6). Recovery in the ALAT activity was highest in kidney and hardly differed from the control. The recovery in the AAT activity was highest in liver followed by muscle, kidney, and gill. The recovery may be due to increased rates of enzyme synthesis in order to compensate for the activity of lost enzyme as suggested by Ay *et al*. (1999).

Conclusion

The results show that the effects of MCP on *C. batrachus* were more severe at the higher concentration and that a uniform response pattern of the investigated organs, but also some organ-specific responses, was observed. It can be concluded that 21 days of recovery are not sufficient for complete reversal of the toxic effects. Further investigations are required to provide information on complete recovery of the fish and also on phenotypically not detectable effects which would have implications on aquaculture, including fishery management.

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