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A study on biochemical changes in the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) following exposure to sublethal doses of organochlorine pesticide (endosulfan)

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ABSTRACT

Endosulfan, a broad-spectrum non-systemic organochlorine (OC) pesticide is extensively used to control a wide variety of pests in agriculture, horticulture and public health programmes. Biochemical changes occurring in the metabolically active tissues of gills (GL), hepatopancreas (HP) and muscle (MU) of the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan were studied for 23 days of exposure (DoE). Sublethal doses of endosulfan significantly ($P < 0.05$) altered the levels of the total protein (TP), the total carbohydrates (TC), the glycogen (GLY), the total free sugars (TFS) and the total lipids (TL) in test shrimps. Concentrations of biochemical components significantly varied with the DoE but were dose-independent ($P < 0.05$). Percent decrease in all biochemical components increased with the progress of the DoE irrespective of the exposure concentrations. The order of percent decrease in the concentrations of the TP, TC, GLY, TL and TFS in different tissues at the end of 23 DoE was found to be MU > GL > HP, HP > GL > MU, MU > HP > GL, HP > MU > GL and MU > GL > HP, respectively. The results of the study revealed that sublethal doses of endosulfan significantly alters the proximate composition of major tissues, particularly the TP levels in the MU tissues thereby reducing the nutritive value of this economically important penaeid shrimp. Since *M. monoceros* exhibits significant biochemical changes on exposure to endosulfan, this species could possibly be used as biosensor of coastal marine and estuarine pollution by OCs.

1. Introduction

Fish constitute an integral part of the diet of a coastal population and is one of the major sources of cheap nutrition for the lower and middle income groups. The penaeid shrimp, *Metapenaeus monoceros* (Fabricius) is one of the economically and nutritionally important shrimp species that inhabits the mangrove swamps, estuaries and reclaimed estuarine flood plains (locally called *khazan* lands) along the central west coast of India. This species is harvested in considerable quantities from estuaries, coastal waters and traditional aquaculture ponds for human consumption (Achuthankutty et al., 1993).

Chemical pesticides are well recognized as an economic approach for controlling pests in agriculture and horticulture. Organochlorine (OC) class of pesticides (aldrin, dieldrin, endosulfan, heptachlor, malathion) account for two-thirds of the total consumption of pesticides in the country due to low cost and versatility in action against various pests (Jayashree and Vasudevan, 2007). It has been reported that as much as 70% of the chemical formulations used for pest control programmes in agriculture affect non-target organisms inhabiting rivers, estuaries and adjacent aquacultural ponds that are fed by the rivers/estuaries (Bhatnagar et al., 1992; Ozha, 1998; Selvakumar et al., 2005). Amongst different pesticides, OCs have been reported to be persistent in the environment and tend to accumulate in biological and non-biological materials (Jayashree and Vasudevan, 2007) and the consumption of fish contaminated by these pesticides has been reported to affect the immune response and cause severe health problems in human beings (Svensson et al., 1994; Colombo et al., 1995). However, no specific analytical data on pesticide contamination of the rivers connecting the estuaries, mangrove swamps and coastal wetlands along the central west coast of India is available.

Endosulfan, an OC compound belonging to cyclodiene group is extensively used as a broad-spectrum pesticide to treat a wide variety of invertebrate pests and bird repellent on fruit crops in more than 70 countries (Abraham, 2004). India is one of the largest manufacturer and consumer of pesticides in south Asia and about 81 000 metric tonnes of endosulfan was manufactured in India during 1999–2000 (Anonymous, 2001). Commercial endosulfan is a mixture of two stereoisomers, namely α and β, endosulfan at

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70:30 ratio and the latter is highly soluble in soil and other media. (Goebel et al., 1982). The principal metabolite, endosulfan sulphate has proven to be toxic or more toxic and persistent than parent compounds to fish and other aquatic organisms due to its chemical nature and solubility (Schimmel et al., 1977; Jayashree and Vasudevan, 2007) and predictably responsible for causing large fish kills in a tidal creek following massive agricultural run-off events (Scott et al., 1992).

The nutritional value of different species of fish and shellfish depend on their biochemical components such as protein, carbohydrate and lipids. These proximate components could serve as sensitive indicators for detecting potential adverse effects, particularly the early events of pollutant damage because their alterations appear before the clinical symptoms produced by the toxicant (Almeida et al., 2002; Rao, 2006). It is therefore important that potential effects of acute and chronic concentrations of pollutant on proximate composition are determined and interpreted to delineate mechanisms of pollutant action and possibly ways to mitigate adverse effects (Matos et al., 2007). Histopathological, biochemical, and physiological changes in different species of crustaceans after exposure to endosulfan have been widely reported (Omkar et al., 1984; Shukla and Omkar, 1984; Selvakumar et al., 1996). Behavioural abnormalities and severe impairment of metabolism and growth potential in freshwater prawn, *Macrobrachium malcolmsonii* following exposure to endosulfan has been reported (Bhavan and Geraldine, 1997, 2001). The suppression of growth and reproduction in zebrafish on exposure to mild doses of endosulfan has been recently reported by Balasubramani and Pandian (2008).

The lack of information on the effects of sublethal doses of endosulfan on the proximate composition of metabolically active tissues of coastal marine and estuarine shrimp species prompted us to undertake this study. Selection of *M. monoceros* as the test species in the present study was based on two criteria: this is one of the abundant and larger-sized shrimp species used in traditional aquacultural ponds in India (Achuthankutty et al., 1993) and this species is considered to be a sensitive indicator species of marine and estuarine pollution (Butler, 1966). The present communication describes changes in the concentrations of major biochemical components in the tissues of metabolically active organs (gills, hepatopancreas and muscle) of coastal marine penaeid shrimp species, *M. monoceros* on exposure to two sublethal doses of endosulfan over 23 days of exposure (DoE).

2. Materials and methods

2.1. Experimental animals and acclimation

Actively moving juveniles of penaeid shrimp, *M. monoceros* (total length, 62.2 ± 6.4 mm; weight, 1.8 ± 0.6 g; $n = 30$) with no visible signs of disease or morbidity were collected from a traditional aquaculture pond located in Goa (Chorao Island; Lat. 15°30′N; Long. 75°50′E). Immediately after the collection, juvenile shrimps were transferred to polythene bags containing seawater, filled with oxygen gas prior to packing and transported to the laboratory within few hours after collection. The juveniles were acclimatised to the laboratory conditions in two large FRP tanks (cap. 200 L) for more than 2 weeks before the initiation of experiments. The seawater used in acclimation tanks was treated by rapid sand filtration, bio-filtration and passed through ultraviolet radiation. Adequate aeration was provided using air blowers, and optimum water quality parameters were maintained during the acclimation period: temperature (29.5 ± 0.5 °C), salinity (32 ± 1.5 ppt), dissolved oxygen (6.1 ± 0.6 mg L^{-1}), pH (7.8 ± 0.3), NO_2–N (<0.02 mg L^{-1}) and NH_3/NH_4 (0 mg L^{-1}). The seawater used for acclimation and exposure experiments was free from residues of endosulfan. A photoperiod of 12 L (0700 h–1900 h):12 D (1900 h–0700 h) was maintained. Shrimps were fed *ad libitum* twice a day (0800 h and 1600 h) with commercial shrimp pellets (CP-Aquaculture, India; proximate composition: 38–40% protein, 5% lipids and 3% fibre). Faeces and uneaten feed was siphoned out twice a day (1000 h and 1730 h) and 50% of water exchanged daily (0730 h). In order to reduce the amount of excreted products in the test tanks, feeding was stopped 48 h prior to the commencement of acute bioassay tests. During the period of acclimation, the juveniles did not show any symptoms of stress or unusual behaviour. Care was taken to keep the mortality rate within 5% in the last 4 d before sublethal tests began. Dead shrimps, if any were removed immediately from the acclimation tanks.

2.2. Test chemical

The commercial-grade endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) marketed under the brand name 'EndoMinn' by M/s. Multimin Agro chemicals, Bangalore (India) was used in this study. The commercial-grade endosulfan which is in liquid form (an active ingredient of 35%), was diluted with deionised water to prepare solutions of required concentrations.

2.3. Acute bioassay tests

Acute toxicity experiments were performed in triplicate and the average mortality values were calculated using formula as described by Abbott (1925). The median lethal concentration (96-h LC_{50}) and 95% confidence intervals were determined with a computer-based program described by Finney (1971). The acute 96-h LC_{50} of *M. monoceros* juveniles to endosulfan was found to be 199.3 ng L^{-1} (95% confidence limits, upper = 221 ng L^{-1} and lower = 182 ng L^{-1}; $P < 0.05$).

2.4. Test solutions and sub-acute tests

It has been hypothesised that sublethal concentrations of pesticides offer an excellent scope for observing the behavioural and physiological changes in animals (Edwards, 1973). Two sublethal doses corresponding to 20% and 30% of 96-h LC_{50} were selected for sub-acute toxicity experiments. Concentrations of active ingredient of endosulfan present in two sublethal concentrations computed from commercial-grade composition were found to be 40 and 60 ng L^{-1}, respectively. The exact amount of active ingredient of endosulfan present in each sublethal solution however, was not quantified.

For evaluation of effects of sublethal concentrations, 450 randomly sampled shrimps of similar size (total length, 70 ± 7.2 mm; weight, 2.0 ± 0.4 g; $n = 30$) were divided into three groups, each group comprising of 150 intermoult juveniles of *M. monoceros*. One group served as control, while two other groups were exposed to two sublethal doses of endosulfan (one group to one sublethal dose). A total of three replicate aquaria (50 L capacity) were maintained for each dose and the control group (50 shrimps per concentration per replicate). During the exposure period, a mild aeration was provided using air pumps (BOYU, Japan) in order to maintain DO levels not less than 5 mg L^{-1}. Juveniles were fed with commercial shrimp pellets *ad libitum* and were deprived of feed 24 h before exposure experiments.

In toxicological studies, chronic tests of shorter duration (~21 d) have been recommended as an alternative to longer chronic tests (Maki, 1979). The experiment was run for a period of 23 DoE as the estimated intermoult period of *M. monoceros* un-

der the laboratory conditions was estimated to be 21 ± 1 d (personal observation). In order to maintain constant concentration of endosulfan in test solutions, the entire toxic medium in each aquarium was gently siphoned out daily (0900 h) and renewed with freshly prepared solution of respective sublethal concentrations of endosulfan. Aeration was suspended temporarily during water exchange and feeding, and care was taken that the disturbance caused to the shrimps was minimal. Neither mortality nor any visible signs of disease were observed in the shrimps exposed to sublethal concentrations of endosulfan. During the exposure period, the percentage mortality in both control and exposure tanks did not exceed 5%; dead shrimps, if any, were removed immediately and replaced with shrimps of similar size separately reared in the same medium.

2.5. Tissue samples and biochemical analysis

Sampling was done on 1, 7, 15 and 23 DoE and on each sampling occasion, 30 shrimps from each group (two experimental and one control) were sacrificed. From each shrimp, sample was extracted from the tissues of gills (GL), hepatopancreas (HP) and muscle (MU). Extracted tissue samples from respective organs of 10 animals were pooled to constitute a single sample; thus three such samples (GL, HP and MU) were made for each group. Shrimps from control group were similarly sampled at the same time as test shrimps.

Concentrations of biochemical constituents (proteins, carbohydrates, glycogen, free sugar and lipids) in different tissues were estimated by following standard procedures. The total protein (TP) and the total carbohydrate (TC) concentrations in different tissues were determined according to the methods of Lowry et al. (1951) and Roe (1955), respectively. Concentration of glycogen (GLY) in different tissues was estimated by the method of Carrol et al. (1956). Levels of the total free sugar (TFS) were calculated by subtracting the GLY content from the TC values. The total lipid (TL) content was estimated by the method of Barnes and Blackstock (1973). The accuracy of the analytical methods was tested against prepared standards and deviations from real standard values are expressed as coefficient of variation (c.v.%). For duplicate analysis, these were 5.0–17.5% for the TP, 4.0–16.5% for the TC, and 6–17.6% for the TL. For the purpose of comparison, concentrations of biochemical constituents are expressed in terms of mg g^{-1} of wet tissue. Variation in concentrations of biochemical constituents in test shrimps are expressed as the percent change from control (100%) and concentrations >100% and <100% indicate increase and decrease over control, respectively.

2.6. Statistical analysis

Fluctuations in concentrations of biochemical components in different treatment groups and organs were assessed by analysis of variance (ANOVA) (Underwood, 1997) with DoE and concentration as sources of variation. Because the variances were not homogenous and/or the residual departed from normality as indicated by Cochran's test (Winer et al., 1991), all the data were subjected to appropriate transformations. If ANOVA results were found significant, multiple comparisons between different means of biochemical components of control and test shrimps were then made by Tukey–Kramer test high significant differences (HSD) (Zar, 1996). Significance in all statistical tests was judged at a 'P' < 0.05 level. All statistical analyses were performed using Graph Pad Prism Software (Graph Pad, San Diego, CA, USA). Unless otherwise indicated, the results are presented as mean ± 1S.E. of replicate tanks.

3. Results

3.1. Physico-chemical parameters and biochemical analyses

Neither mortality nor visible disease signals were observed in the shrimps exposed to sublethal concentrations of endosulfan. Physico-chemical characteristics of seawater did not vary significantly both during the acclimatization and exposure periods ($P > 0.05$). Measured water quality parameters fell within the levels recommended for optimal growth and survival of penaeid shrimps (Chien, 1992). No statistical difference in the biochemical analyses was found between the replicate tissue samples of different organs ($P > 0.05$).

No shrimps died during exposure to sublethal concentrations of endosulfan during 23 DoE. The first reaction following exposure to endosulfan appeared after 7 DoE. At that time, it was noted that test shrimps failed to consume the normal ration of food. After 15 DoE, test shrimps completely rejected food, while the control shrimps continued to feed normally throughout 23 DoE.

3.2. Endosulfan induced changes in proximate composition

3.2.1. Changes in the TP concentrations

Levels of the TP in different tissues of control and exposed shrimps during the exposure period are depicted in Fig. 1. The TP concentrations were significantly lower in test shrimps than those of controls on all DoE ($P < 0.05$). The rate of depletion was found to be highly time and tissue dependent. The order of percent decrease of the TP concentrations in different tissues at the end of 23 DoE was observed to be MU > GL > HP. A progressive depletion in the TP levels of test shrimps was recorded in the tissues of GL and MU during the exposure period. No significant variation in the TP content between exposure concentrations of 40 and 60 ng L^{-1} was noticed ($P > 0.05$). The levels of hepatic protein of test shrimps were found to be almost similar to that of control shrimps on 1- and 7 DoE but depletion was more prominent on 15- and 23 DoE (Fig. 1). The magnitude of depletion in the hepatic protein was directly proportional to the concentration of endosulfan. Higher percent depletion in the hepatic protein was observed in test shrimps exposed to 60 ng L^{-1} compared to those exposed to 40 ng L^{-1} of endosulfan ($P < 0.05$).

3.2.2. Changes in the TC levels

Concentrations of the TC in different tissues of test shrimps and controls during the exposure period are shown in Fig. 2. The levels of the TC in the GL tissues of test shrimps were slightly higher (~3%) than the control shrimps on 1 DoE but thereafter concentrations reduced significantly with the progress of the exposure period (7, 15 and 23 DoE). No significant variation in the TC levels in the test shrimps between two exposure concentrations in the studied tissues was observed ($P > 0.05$). The depletion in the TC levels in the HP of test shrimps was significant with the progress in the period of exposure. Concentrations of hepatic carbohydrate in the test shrimps ranged from 117 ± 2% (1 DoE) to 45 ± 2% (23 DoE) over control shrimps (100%). The levels of the TC in the MU of test shrimps exhibited a biphasic pattern: higher concentrations on 1 DoE and 7 DoE and lower on 15 DoE and 23 DoE. The order of percent decrease in the TC levels in the studied tissues on the last day of exposure (23 DoE) was found to be HP > GL > MU (Fig 2).

3.2.3. Variations in the GLY content

Sublethal doses of endosulfan resulted in elevated levels of GLY on 1 DoE and lower levels beyond 1 DoE in all the tissues of test shrimps (Fig. 3). The decrement was significant in all the tissues of test shrimps ($P < 0.05$) irrespective of the sublethal concentration to which they were exposed to. The levels of GLY in the tissues

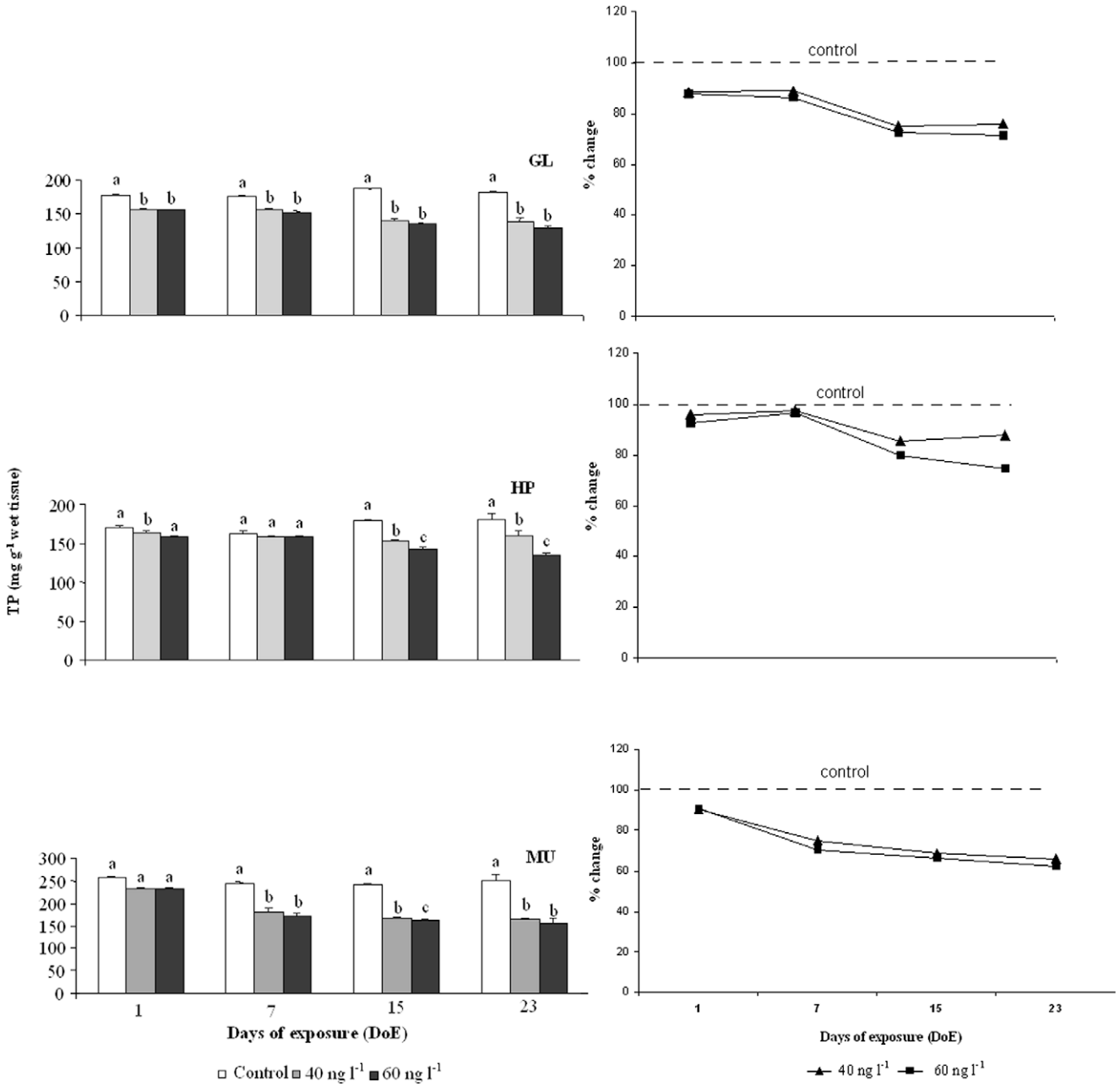

Fig. 1. Changes in the concentrations of the total protein (TP) in different tissues (mg g^{-1} of wet tissue) of the penaeid shrimp, *Metapenaeus monoceros* on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan. Values with different superscript are statistically significant ($P < 0.05$). Inset-percent change from control (100%). Vertical bars represent standard deviation ($n = 3$). GL-gills; HP-hepatopancreas; MU-muscle.

of MU and HP of test shrimps were 45% and 38% over control shrimps (100%) on 23 DoE (Fig. 3).

3.2.4. Changes in TFS levels

The levels of the TFS in the different tissues of test shrimps decreased progressively ($P < 0.05$) with the increase in the exposure period irrespective of the exposure concentrations (Fig. 4). The TFS level in all the tissues of test shrimps were relatively higher on 1 DoE and decreased significantly thereafter. The percent decrease in the TFS levels was more pronounced in the tissues of MU compared to GL and HP on 23 DoE (Fig. 4).

3.2.5. Variations in TL concentrations

Levels of the TL in different tissues of the test shrimps and controls during the exposure period are depicted in Fig. 5. In general, the TL concentrations in all the studied tissues of shrimps exposed to sublethal doses of endosulfan were significantly lower than those in controls ($P < 0.05$). The percent decrease in the hepatic lipid was higher in the HP than in the tissues of GL and MU and the order of percent decrease on 23 DoE was found to be HP > MU > GL (Fig. 5).

4. Discussion

The developing countries in south and southeast Asia continue to produce, sell and use endosulfan, for agricultural, horticultural and public health programmes, in spite of restrictions imposed on its use in most developed countries (Goebel et al., 1982; Salvo et al., 2008). In recent years, there is a growing concern worldwide on the environmental pollution due to indiscriminate use of pesti-

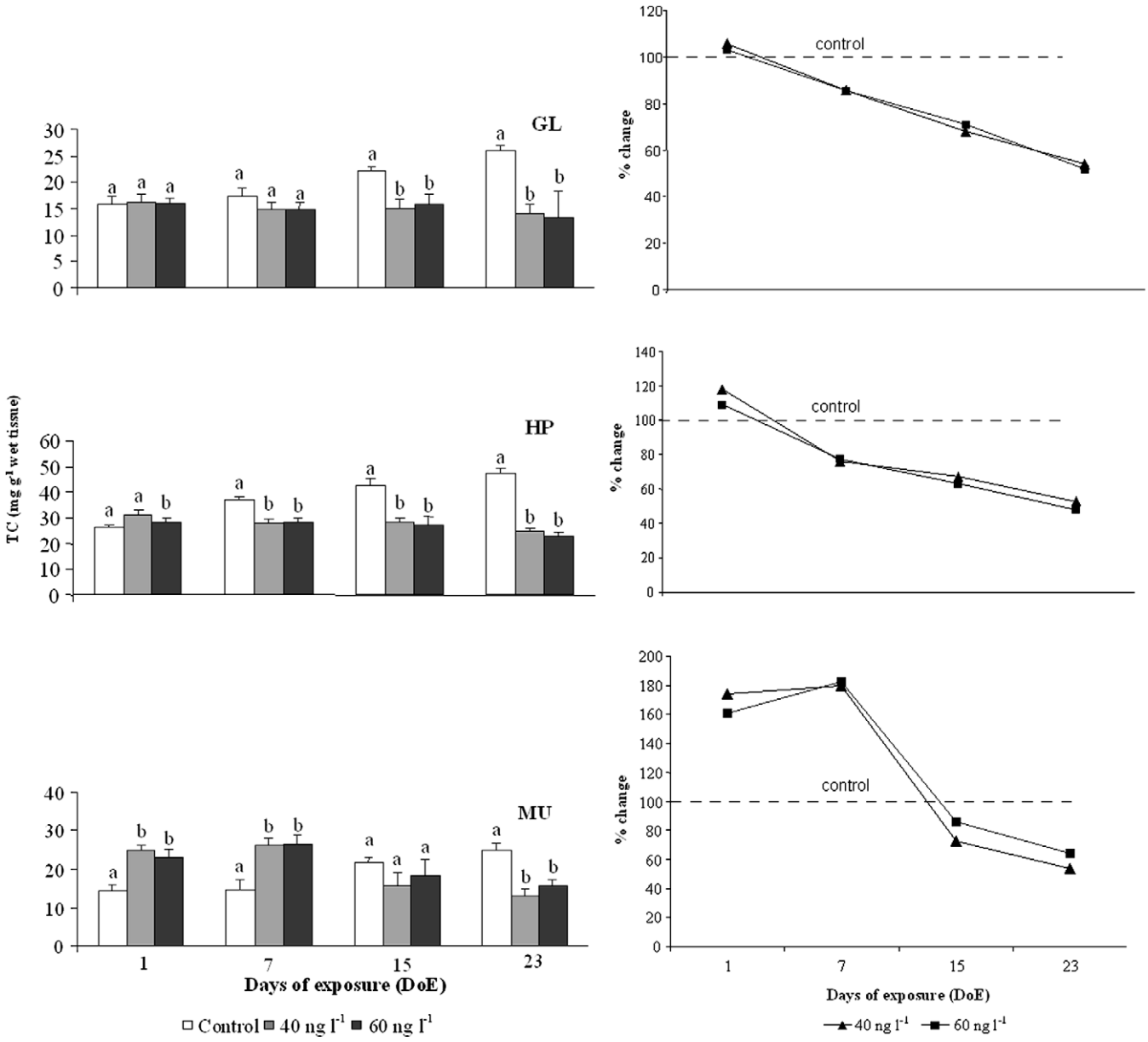

Fig. 2. Changes in the concentrations of the total carbohydrate (TC) in different tissues (mg g^{-1} of wet tissue) of the penaeid shrimp, *Metapenaeus monoceros* on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan. Values with different superscript are statistically significant ($P < 0.05$). Inset-percent change from control (100%). Vertical bars represent standard deviation ($n = 3$). GL-gills; HP-hepatopancreas; MU-muscle.

cides due to their persistence, toxicity at low concentrations and bioaccumulation by biota. The persistence and distribution behaviour of endosulfan in the rice fields has been recently studied by Jayashree and Vasudevan (2007). The detectable levels of endosulfan in Indian edible freshwater fishes have been reported (Amaraneni and Pillala, 2001; Zynudheen and Radhakrishnan, 2004). Biochemical changes induced by pesticidal stress is due to disturbed metabolism manifested by inhibition of enzymes, retardation of growth and reduction in the fecundity and longevity of the organism. Most of the pesticides act as metabolic depressors and generally affect the activity of biologically active molecules such as proteins, carbohydrates and lipids (Agrahari and Gopal, 2009). The exposure of aquatic organisms to even very low levels of pesticides causing alterations in the nutritional value of finfish and shellfish as well as their biochemical constituents, physiological and histological functions has been widely documented (Bhavan and Geraldine, 1997, 2001, 2002; Reddy and Rao, 1988a,b,c; Reddy et al., 1991).

The penaeid shrimp, *M. monoceros*, an economically important crustacean species is harvested in considerable quantities from estuarine mangrove environments and traditional aquaculture ponds of Goa, west coast of India which are connected by the rivers (Achuthankutty et al., 1993). This species in great demand due to its nutritional value and forms a cheap source of animal protein for lower and middle income groups. It is likely that these shrimps may get affected by the pesticides through the riverine runoff. Impairment of various biochemical and physiological mechanisms in different species of freshwater and marine prawns on exposure to most routinely used pesticides has been reported: in the freshwater prawn, *M. malcolmsonii* (Bhavan and Geraldine, 1997, 2001, 2002), in the white prawn, *Fenneropenaeus indicus* (also called *Penaeus indicus*) (Reddy and Rao, 1988a; Reddy et al., 1988) and

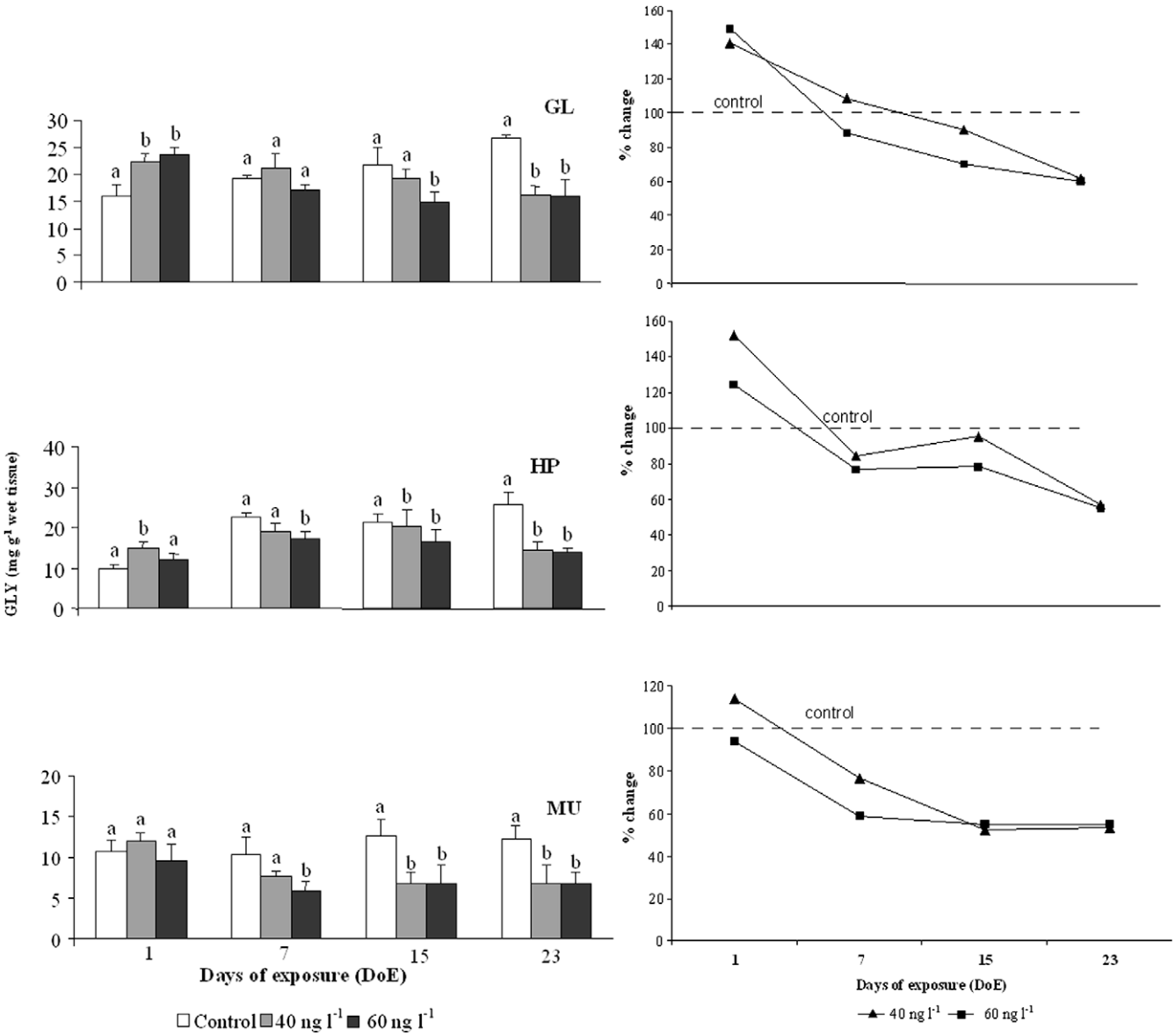

Fig. 3. Changes in the concentrations of the glycogen (GLY) in different tissues (mg g^{-1} of wet tissue) of the penaeid shrimp, *Metapenaeus monoceros* on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan. Values with different superscript are statistically significant ($P < 0.05$). Inset-percent change from control (100%). Vertical bars represent standard deviation ($n = 3$). GL-gills; HP-hepatopancreas; MU-muscle.

in the marine prawn, *M. monoceros* (Reddy and Rao, 1988b, 1988c, 1991). After 23 DoE, the tissues of GL, HP and MU exhibited significant depletion in the concentrations of major biochemical constituents perhaps due to stress induced by endosulfan. Juveniles of *M. monoceros* observed to suffer from certain physiological changes following exposure to sublethal concentrations of endosulfan as evidenced by the depletion in the levels of the TP, the TC, the GLY, the TFS and the TFL in GL, HP and MU tissues of test shrimps. All energy yielding metabolites such as the TP, the TC and the TL were found to be invariably altered following the exposure to sublethal concentrations of endosulfan in the present study.

4.1. Effect of endosulfan on the TP levels

Protein is one of the important biochemical components and plays an important role in metabolic pathways and biochemical reactions. Under extreme stress conditions, protein supply energy in metabolic pathways and biochemical reactions. Therefore, an assessment of the TP content in different tissues could be used as a diagnostic tool for determining the physiological status of an organism (Prasath and Arivoli, 2008). In the present study, concentrations of the TP in the tissues of GL and MU were found to be significantly lower than those in control shrimps on all sampled days ($P < 0.05$). The percent depletion progressively increased with DoE irrespective of exposure concentrations. A similar depletion in the TP content in different tissues of crustaceans on exposure to various pesticides has been documented: in the freshwater prawn, *Macrobrachium kistensis* on exposure to pesticides by Nagabhushanam et al. (1972); in the marine edible crab, *Scylla serrata* on exposure to dimecron, an OC pesticide, by Rao et al. (1987); in the white prawn, *F. indicus* on exposure to sublethal levels of phosphamidon and methylparathion by (Reddy et al., 1988); in the freshwater field crab, *Paratelphusa hydrodromous* following exposure to malathion by Singaraju et al. (1991). A marked decrement in the concentrations of the TP in the two freshwater field crab species, *Oziotelphusa senex senex* (Rajendra Prasad Naidu, 1985), *Barytel-*

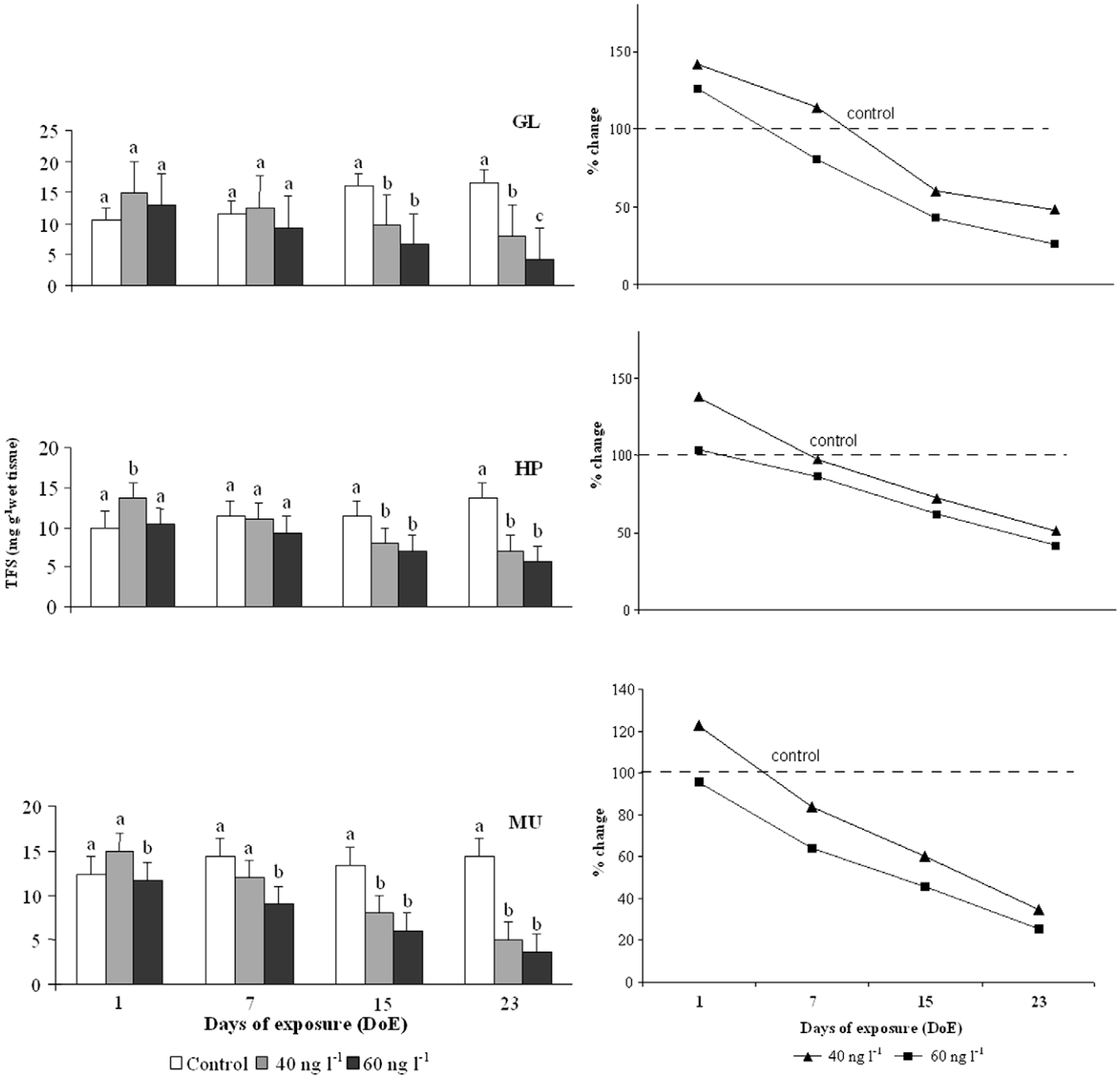

Fig. 4. Changes in the concentrations of the total free sugars (TFS) in different tissues (mg g^{-1} of wet tissue) of the penaeid shrimp, *Metapenaeus monoceros* on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan. Values with different superscript are statistically significant ($P < 0.05$). Inset-percent change from control (100%). Vertical bars represent standard deviation ($n = 3$). GL-gills; HP-hepatopancreas; MU-muscle.

phusa guerini (Reddy et al., 1991), and in the freshwater prawn, *M. malcolmsonii* (Bhavan and Geraldine, 1997) on exposure to endosulfan have been reported. A plausible explanation for such depletion of the TP levels in the tissues of GL and MU of test shrimps might be due to the enhanced proteolytic activity in these organs under pesticide stress. The depletion in the TP might be due to the diversification of energy to accomplish the impending energy demands when of animals are under toxic stress. The decreased protein concentrations might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery (Bradbury et al., 1987). Induction of proteolysis as a result of elevated protease activity reflecting in the decrease of the TP levels of different tissues during a short-term exposure study (96 h) of endosulfan on *B. Guerini* has been documented by Reddy et al., 1991). Depletion of the TP content in the tissues of may constitute a physiological mechanism under pesticidal stress, to provide intermediates to the Kreb's cycle or to enhance osmolality, by retaining free amino acid content in the haemolymph, to compensate osmoregulatory problems encountered due to the leakage of ions and other essential molecule, during the pesticide stress (Schmidt-Nielsen, 1974; Reddy et al., 1991; Yasmeen et al., 1991). Breakdown of various peptides in tissues and protein denaturation in freshwater prawn, *M. malcolmsonii* on exposure to endosulfan has been reported by Bhavan and Geraldine (2001).

The depletion in the TP levels of test shrimps was tissue-specific and time-dependent ($P < 0.05$). In the present study, the order of percent decrease in the concentrations of the TP in different tissues at the end of 23 DoE was found to be MU > GL > HP. This observa-

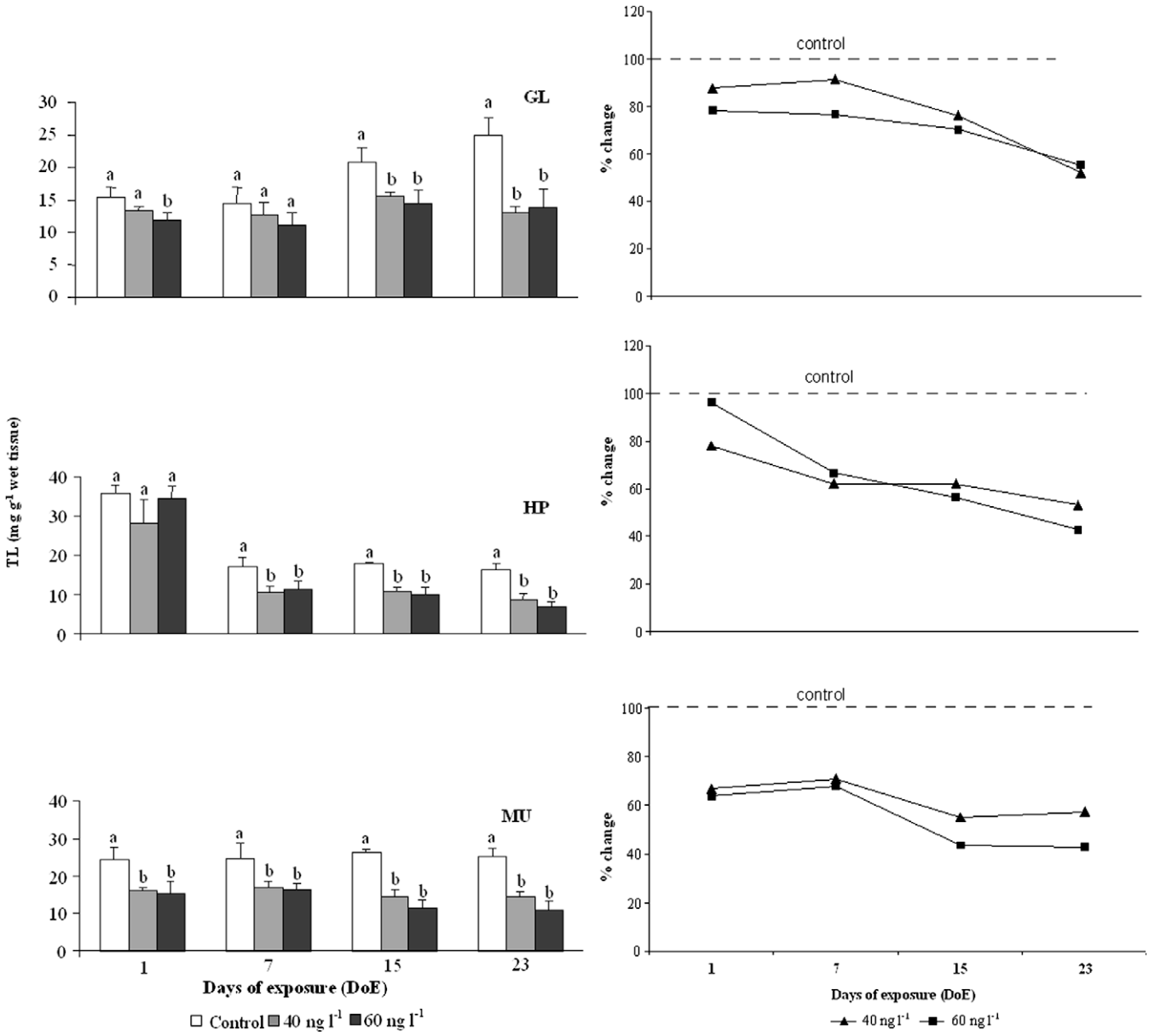

Fig. 5. Changes in the concentrations of the total lipids (TL) in different tissues (mg g^{-1} of wet tissue) of the penaeid shrimp, *Metapenaeus monoceros* on exposure to two sublethal doses (40 and 60 ng L^{-1}) of endosulfan. Values with different superscript are statistically significant ($P < 0.05$). Inset-percent change from control (100%). Vertical bars represent standard deviation ($n = 3$). GL-gills; HP-hepatopancreas; MU-muscle.

tion coincides with previous observation of where the muscle protein levels decreased, in most of the acute exposure of pesticides (Nagabhushanam et al., 1972). It is appropriate to mention here that the test animals were observed to be restless as indicated by their constant fast movements and frequent surfacing throughout the exposure period, more particularly during the initial stages of the exposure. Some energy is required to be spent for this kind of stress induced behaviour. Since, the extra energy required to offset the stress cannot be met with feeding materials alone, the extra energy demand should invariably fulfilled by the stored materials within the body only. It is known that the protein and carbohydrate are the primary sources for energy yielding process in aquatic organisms. Relatively higher depletion in the TP levels in MU tissues of test shrimps might be due to expenditure on additional energy constant movements aided by muscular action under pesticidal stress. Tissue-specific and time-dependent depletion of the TP has been reported in freshwater fish, *Channa punctatus* during BHC intoxication by Geetanjali (1988). Significant reduction in the TP levels in GL tissues of freshwater field crab, *O. senex senex* on short-term exposure to endosulfan has been documented by Rajendra Prasad Naidu, (1985). A decrement up to 33% in the HP and 42% in the MU in *M. monoceros* following 24 h exposure to sublethal concentration of phosphamidon has been reported by Vijayalakshmi and Rao (1985). The depletion in the TP levels up to 31%, 33% and 42%, respectively, in the tissues of GL, HP and MU of *M. malcolmsonii* on exposure to sublethal dose of endosulfan (32 ng L^{-1}) has been reported by Bhavan and Geraldine (1997).

In the present study, concentrations of the TP in GL tissues of test shrimps exhibited significant decrease during the exposure period. The levels of the TP in MU and GL tissues of test shrimps decreased significantly with the progress in the exposure period. On the other hand, concentrations in the hepatic protein showed a biphasic pattern: slight decrease (<5%) at the end of 1- and 7 DoE followed by a significant depletion at the end of 15- and 23

DoE. Higher concentrations of the hepatic protein on 1- and 7 DoE and lower on 15- and 23 DoE in *M. malcolmsonii* treated with sublethal doses of endosulfan has been observed by Bhavan and Geraldine (1997). Marginal depletion in the levels of hepatic protein during the initial stages of exposure might be due to the initial enhanced synthesis of protein, possibly to repair damaged cell organelles, to serve as a compensatory pool to restore enzymes lost due to tissue necrosis and to meet the increased demand to detoxify the pesticide, thus supporting the hypothesis of Gill et al. (1990). The subsequent decrement in the TP levels observed on 15- and 23 DoE might be due to the impairment of protein synthesis by endosulfan due to necrosis of hepatic cells which appears to be time- and dose-dependent. The quantity of protein is dependent on protein synthesis or on rate of its degradation (Ogueji and Auta, 2007). The decrease in the protein content in endosulfan treated shrimps may be either due to the inhibition of RNA synthesis at the transcriptional level (Singh et al., 1996) or due to impaired incorporation of amino acids into polypeptide chains (Ram et al., 2003). Further, the depletion in the protein content in the test shrimps might also be due to the production of heat shock proteins or destructive free radicals and could be a part of pesticide induced apoptosis (Selvakumar et al., 2005; Sobha et al., 2007).

4.2. Effect of endosulfan on carbohydrate metabolism

Carbohydrate is an important biochemical constituent of an animal tissue which acts as building blocks of the cells and is the primary and immediate source of energy. During exposure period, the TC levels decreased in all the studied tissues, the depletion was more significant in the HP followed by GL and MU tissues of test shrimps. This is consistent with the previous observations that have been reported in the marine prawn, *M. monoceros* on exposure to methylparathion (Reddy and Rao, 1991) and in the freshwater prawn, *M. malcolmsonii* following exposure to sublethal doses of endosulfan (Bhavan and Geraldine, 1997). The depletion of the TC may be due to its rapid utilisation to meet the reduced energetic efficiency under the impact of endosulfan.

Carbohydrate metabolism is broadly divided into the anaerobic segment or glycolysis in which the breakdown of glucose or glycogen through Embden–Meyerhaf pathway occurs and the aerobic segment that consists of oxidation of pyruvate to acetyl co-A to be utilized through citric acid cycle (Nelson and Cox, 2005). Insecticidal respiratory stress has been found to lead to a hypoxic/anoxic condition (Dezwaan and Zandee, 1972) and pesticides are also known to inhibit energy production by suppressing aerobic oxidation of carbohydrates leading to energy crisis in animals (Kohli et al., 1975). As a consequence of hypoxia, the metabolic pathway is shifted from aerobiosis to anaerobiosis and a strong suppression of the specific activities of enzymes involved in glycolysis and glycogen metabolism. These conditions might have depleted the TC levels in the shrimps exposed to endosulfan in order to meet the increased energy demands as carbohydrates form the major source of energy under stressful conditions (Hochachka and Somero, 1984). Carbohydrate metabolism is not considered to be a major energy source in fish (Walton and Cowey, 1982), but its importance increases during hypoxia because of activation of anaerobic glycolysis. This may explain the observed depletion of the TC levels in test shrimps during the later stages of exposure as a result of increased demand of these molecules to provide energy for the cellular biochemical processes under hypoxic conditions induced by endosulfan. At the end of 23 DoE, the percent decrease in the TC was maximum in the HP (50%) followed by GL (48%) and MU (42%). The crustacean hepatopancreas is the vital organ involved in such diverse metabolic activities as synthesis and secretion of enzymes and it is also the major organ of detoxification (Thaker and Haritos, 1989).

The levels of GLY in different tissues of test shrimps showed a decreasing trend as exposure progressed. Sublethal concentrations of endosulfan resulted in elevated levels of GLY in test shrimps on 1 DoE in both the exposure concentrations, after which a decrease in the concentrations of GLY was noticed. A steep fall in the glycogen level is an indication of its rapid utilisation to meet the enhanced energy demands in shrimps exposed to toxicant through glycolysis or hexose monophosphate pathway (Sobha et al., 2007). The depletion of GLY in the tissues is an indication of typical stress response when aquatic organisms are challenged with pesticides and glycogen depletion in different tissues after toxic stress has been reported in several studies with aquatic organisms (Omkar et al., 1984; Reddy and Rao, 1988a,b, 1991; Shobha et al., 1989; Bhavan and Geraldine, 1997). Elevated concentrations of GLY in test shrimps following immediate exposure to sublethal concentrations of endosulfan (1 DoE) may be attributed to the mobilisation of glycogen reserve in different tissues to overcome the stress. Further, the elevated levels in the GLY may also be a result of the enhanced synthesis of the sinus gland in order to meet the increased energy in the form of glucose (Omkar et al., 1984). The subsequent reduction in the GLY levels accompanied by the drastic depletion in the GLY levels in GL, HP and MU with the progress of the exposure period is possibly due to the increased glycogenolysis as a result of enhanced activity of glycogen phosphorylase enzyme.

The decrement in the GLY levels in the HP and MU tissues of endoulfan treated shrimps was relatively higher (~45%) than the levels in the GL tissues (39%) but the depletion rates between the tissues was, however insignificant ($P > 0.05$). Similar depletion of the glycogen reserves of HP and MU tissues in different crustacean species on exposure to different pesticides have been documented: in freshwater prawn species by Omkar et al. (1984) and Bhavan and Geraldine (1997); in marine prawn species by Reddy and Rao, 1988a,b) and in the field crab, *O. senex senex* by Reddy et al. (1986). Relatively higher depletion in the hepatic glycogen compared to muscular glycogen in the penaeid shrimps on exposure to methylparathion been reported by Reddy and Rao (1991). Such significant differences in the depletion of GLY levels in different studied tissues, was however not observed in the present study. This may indicate that the process of glycogenolysis initiated in all the studied tissues at same time and rate.

The concentrations of the TFS in the GL, HP and MU of *M. monoceros* exposed to endosulfan were significantly lower than the levels in the same organs of controls, post 1 DoE. A similar reduction in the TFS levels in different tissues in the prawn, *M. malcolmsonii* exposed to sublethal doses of endosulfan has been reported by Bhavan and Geraldine (1997). The reduction may be due to the rapid utilisation of free sugars generated by the breakdown of carbohydrate and glycogen in order to meet the increased energy requirements as result of toxic stress.

4.3. Effect of endosulfan on the TL levels

The TL concentrations in different tissues endosulfan treated shrimps in the present study were found to be significantly lower than the concentrations in the same organs of controls ($P < 0.05$). Similar observations have been made in the freshwater prawn, *M. kistensis* on exposure to pesticides (Nagabhushanam et al., 1972); in the marine prawn, *M. monoceros* exposed to phosphamidon, methylparathion and lindane (Reddy and Rao, 1988c, 1989) and in the freshwater prawn, *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 1997) and in the fishes, *Saratherodon (Oreochromis) mossambicus* and *Barbus conchonius* exposed to methylparathion and aldicarb, respectively (Rao and Rao, 1981; Pant et al., 1987). The decrement in the TL levels may be due to the increased activity levels of lipase, the enzyme responsible for the breakdown of lipid into free fatty acids and glycerol. Lipids con-

stitute the rich alternate energy reserves whose calorific value is twice that of an equivalent weight of carbohydrates and proteins and the mobilisation of lipid reserves may be due to the imposition of high energy demands to counter the toxic stress (Reddy and Rao, 1989).

The concentrations of the TL decreased in all the tissues significantly with the progress of exposure period irrespective of exposure concentrations. The order of percent decrease within the tissues was: HP > MU > GL. The considerable decrease in the TL in the HP and MU (~50%) on 1 DoE might be due to the drastic decrease in the GLY levels (~45%) in the same tissues. The HP of crustaceans is analogous to the liver of vertebrates and is the centre of lipid metabolism (Chang and O'Connor, 1983); higher levels of the TL could be expected in the HP compared to other tissues. The evidence of relatively higher lipid deposition in the hepatic tissues has been reported in climbing perch, *A. testudineus* exposed to pesticides (Bakthavathsalam and Reddy, 1981), in the fish, *B. conchonius* exposed to aldicarb (Pant et al., 1987), in the penaeid prawn, *M. monoceros* exposed to phophamidon, methylparathion and lindane (Reddy and Rao, 1989), in the freshwater prawn, *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 1997). In contrast, the concentrations of the TL decreased significantly in all the tissues of test shrimps with no apparent deposition of the TL in the HP in the present study. Absence of such deposition of the TL in the hepatic tissues of *M. monoceros* exposed to endosulfan might be attributed to the differential rates of lipid metabolism in the studied tissues of shrimps (Bakthavathsalam and Reddy, 1981).

In conclusion, the study revealed that the sublethal doses of endosulfan significantly altered concentrations of biochemical constituents in the metabolically active tissues of gills, hepatopancreas and muscles in *M. monoceros*. The reduction in nutritive value, particularly the protein content in the shrimps exposed to sublethal doses of endosulfan warrants the need to regulate the pollution caused by pesticides in general and OCs in particular. Further studies dealing with ratios of different biochemical components as well as the relationship between wet and dry weights could be useful in better understanding of the absorption of endosulfan by different tissues. Nevertheless, the results of the present investigation may be useful for assessing early warning signs of pesticide poisoning and support the possibility to use *M. monoceros* as biosensor of coastal marine and estuarine pollution by OCs.

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