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ORIGINAL ARTICLE

Influence of Chlorpyrifos Stress on Protein Metabolism of Edible Crab *Barytelphusa guerini*, and its Recovery

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The sublethal stress of chlorpyrifos on important metabolites and enzymes of protein metabolism was investigated in most important tissues (gills, muscle, hepatopancreas and nervous tissue) of freshwater edible crab, *Barytelphusa guerini*. The crabs were exposed to 0.07 mg/l (1/3 of LC_{50}) for 7, 14, 21 and 28 days. Subsequently, crabs were released into fresh water and kept in the same for 18 days in order to recovery study (3 days interval). Total protein content decreased whereas amino acids and ammonia increased in CPF exposed and maximum recovery was shown in cessation of intoxication. After cessation of intoxication recovery was observed. Urea content was decrease in all tissues and glutamine exhibited mixed response and recovery was highest in muscle. The activities of protease and aminotransferases were elevated in tissues for 28 days. Recovery of these enzymes activities was noticed during depuration. Acid phosphatase activity was inhibited in hepatopancreas and nervous ganglion and induced in gills and muscle. Alkaline phosphatase activity was enhanced in gills and hepatopancreas and inhibited in muscle and nervous ganglion. All these enzymes showed recovery after released of crabs into fresh water.

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The use of pest control chemicals has increased several folds in India and is likely to increase in the forthcoming years. It is a well known fact that indiscriminate use of pest controls in agriculture has resulted in widespread distribution in the environment and also has a direct and indirect impact on nontargeted organisms. In many cases less than 0.1% of an applied pesticide reaches the target pest, leaving 99.9% as an inadvertent pollutant in the environment, including in air, soil and water or on nearby flora (Pimentel 1995). Pesticides can move from the site of application via drift, volatilization, leaching, and runoff, which have various characteristics that determine how they act once in soil._Some commonly used pest chemicals (pesticides) persistent measurable residues in soil from three to five years (Torstensson *et al.,* 1989; Williams and Eagle 1979). More than 90% of water

and fish samples from all streams contained one, or more often, several pesticides. Besides that fish, other marine or freshwater animals are endangered by pesticide contamination. The pest controllers and weed-killers are moderately to highly toxic to aquatic invertebrates such as crab, shellfish and fish species (Caldwell *et al.,* 1979; Cheney *et al.,* 1997; Folmar *et al.,* 1979; Narra *et al.,* 2012).

Organophosphate (OP) insecticides are among the most heavily used classes of pesticides and both are considered highly toxic to aquatic animals. Members of these two pesticide classes overlap in crop and geographical usage. Their application often occurs during the dormant season, coinciding with spring rain events, which results in an increased likelihood of run-off into receiving waters (Epstein *et al.,* 2000), where they have been shown to co-occur in waters at concentrations toxic to aquatic organisms (Bacey *et al.,* 2005). It has been reported that as much as 70% of the pesticides used for pest control program in agriculture affect non target organisms inhabiting rivers, estuaries and adjacent aquaculture ponds that are fed by the rivers/estuaries (Ozha 1998; Selvakumar*et al.,* 2005). However, there have been several reports regarding the sensitivity of aquatic invertebrates and some fish species to pesticides (Werner *et al.,* 2002; Denton *et al.,* 2003). There is subsequently concern that the ecological consequences of increased pesticides application on aquatic ecosystems could be far-reaching. Crab fishery is fast developing and there is a vast scope for the crab meat due to its delicacy and nutritional richness. Some species of crabs are edible and a number of others are commercially important for fishmeal industry. Among the various resources, crab is a valuable and easily accessible of food. As a crab contains lipid of superior nutritional properties i.e., protein, carbohydrate and lipids, it is place in important category of seafood. These components 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; Narra *et al.,* 2012).

The organophosphate pesticides are known to affect the crustaceans viz; monocrotphos effected the neuroendocrine regulation in freshwater crab, *Barytelphusa guerini* (Patil *et al.,* 2008), chlorpyrifos effects survival and growth of *Palaemonetes argentines* (Montagna and Collins 2007) and also oxygen consumption and ammonia excretion of the freshwater crab *Trichodactylus borellianus* (Montagna and Collins 2008). The responses of non-target invertebrates (*Callinectes sapidus*) to sublethal concentrations of insecticide to reduced or impaired feeding ability, altered reproductive behavior, disruption of the molting cycle and inhibitory effect on chitin synthesis (Steve and Donald 1996). In these circumstances, it is noteworthy to mention that information on recovery of crab after cessation of toxicity is lacking. Therefore, the present study was undertaken to investigate the effect of sublethal concentration of chlorpyrifos on metabolites (total proteins, amino acids, ammonia, urea and glutamine) and enzymes (transaminases and phosphatases) of protein metabolism in metabolically active tissues (gills, muscle, hepatopancreas and nervous ganglion) of freshwater crab, *Barytelphusa guerini.* This crab is cultured as paddy cum crab and has high edible importance for rural population.

Chlorpyrifos (CPF), O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate is a broad spectrum insecticide commonly known as Dursban™. Chlorpyrifos is used extensively for the

management of domestic and agricultural pests and is effective in controlling of coleoptera, diptera, homoptera and lepidoptera in soil or on foliage in over 100 crops (Tomlin 2000). The study was conducted for 28 days at an interval of 7, 14, 21, and 28 day. Four weeks chlorpyrifos exposed crabs were released in freshwater in order to understand how metabolic alterations respond once the stress is withdrawn. The recovery study was carried out at an interval of 3 days up to 18 days.

MATERIALS AND METHODS

The freshwater crab, *Barytelphusa guerini* were collected from the local supplier and were brought to the laboratory in aerated large plastic tubs. They were acclimatized for 30 days in a huge cement tank with submerged water and fed with commercially available dry prawn. Crabs weighing 40±05 grams were transferred to plastic tubs for a further period of four weeks for conditioning. The natural photoperiod of 12:12 hrs was maintained. The average mean values of water quality during investigation are 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 $CaCO_3$, and chlorides 245.57±1.44 mg/l.

The insecticide chlorpyrifos (20% EC) was purchased from local market (NOCIL Mumbai) The 96h LC_{50} value of chlorpyrifos was determined in the laboratory using the semi-static method of (Finney 1971). The stock solution of CPF was prepared by dissolving in acetone. During sub-acute studies, a group of 60 crabs were exposed to sub-lethal concentrations of CPF 0.07 mg/l (1/3rd of 96 hrs LC_{50} 0.21 mg/l) for a period of 28 days. After four weeks remaining crabs were released in freshwater and kept in the same for 18 days in order to study the recovery pattern. Water was renewed daily and the required concentration was maintained by adding the toxicant directly in water. Crabs were starved 24 h prior to sampling. At an interval of 7 days sampling of control and exposed crab was done (up to 28 days), recovery sampling was carried out at an interval of 3 days for a period of 18 days. Crabs were sacrificed and gills, muscle, hepatopancreas and nervous ganglion was dissected carefully and used for biochemical estimations following various methods.

1% homogenate (w/v) of the tissues were prepared in trichloroacetic acid using a Poter-Elvehjam homogenizer for the estimation of total proteins (Lowry *et al.,* 1951), bovine albumen serum was used standard. Free amino acids (FAA) were estimated by the method of Moore *et al.,* (1954), tyrosine was used as standard. Levels of ammonia were determined using ammonium chloride as standard, urea by diacetyl monoxime method of Natelson (1971) and glutamine by acid hydrolysis method of Colowick and Kaplon (1967). For the estimation of aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT) enzyme activities 5% homogenate (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 µ L of phosphate buffer (pH 7.0) and 12 mg of denatured protein. The ALP activity was estimated by the method of Moss *et al.,* (1986) and ACP by the method of Jabeen (1984).

The experiments were repeated for three times and data was analyzed by student “t” test. There is no significant results observed within the controls, so summarized control values were taken. Graphs were plotted with percent variation over control.

RESULTS AND DISCUSSION

The freshwater crustacean, *Barytelphusa guerini* is one of the sources of nutritious food components for human beings. The nutritive values depend on biochemical constituents. The pesticides have their own target site of action; most of them are metabolic depressors. They generally affect the activity of biologically active molecules such as nitrogenous organic compounds (proteins), macromolecular substances (carbohydrates) and oily organic compound (lipids) (Singh *et al.,* 1996). Under extreme stress conditions, protein supply energy in metabolic pathways and biochemical reactions. Therefore an assessment of protein content in different tissues can be used as a diagnostic tool for determining the physiological status of an organism (Prasath and Arivoli 2008). Total protein content decreased in gill, muscle, hepatopancreas and nervous ganglion on all weeks of exposure (Fig. 1). The maximum decrease was noticed in nervous ganglion. The results of the present investigation demonstrated clear susceptibility of tissues to CPF intoxication. The decrease in protein content in gill, muscle, hepatopancreas and nervous ganglion of freshwater crab, *Barytelphusa* exposed to sublethale concentration of CPF for 28 days suggests active proteolysis. Pesticides are known to induce depletion in the protein content of crabs as reported in the tissues of *B. guerini* exposed to endosulfan (Reddy *et al.,* 1991). Sreenivasan *et al.,* (2009) also reported reduction in protein content of crab, *Spiralothelphusa hydrodroma* exposed to cypermethrin. Protein depletion in tissues constitutes a physiological mechanism with an important role in providing energy to cope with the stress of CPF intoxication. The quantity of protein is dependent on protein synthesis or on rate of degradation (Ogueji 2007). Decrease in DNA and RNA content in tissues of *S. serrata* exposed to napththalene is reported by Vijayavel and Balasubramanian (2006). The depletion in the levels of tissue protein observed in the present study might be due to the inhibition of RNA synthesis at the transcriptional level (Singh and Sharma 1998). The decrease in protein content could also be due to the production of heat shock proteins or destructive free radicals which could be due to the pesticide induced apoptosis as suggested by Epstein *et al.,* (2000). There is no reported literature on the recovery of crustaceans from the impact of toxicants. In the present study, when 4 week CPF exposed crabs were released into freshwater the recovery in protein content was observed. The hepatopancreas showed maximum recovery followed by nervous ganglion, gills and muscle.

The alterations in amino acids indicate the condition of the tissue, and their increase or decrease might be considered as the operation of the stress phenomenon at the tissue level (Shakoori *et al.,* 1976). An increase in free amino acids content was observed in all tissues (Fig. 2). The increase was gradual and the maximum was observed at the end of 28 days. Increase levels of free amino acid observed in the present study might be due to the decreased utilization and is also suggestive of catabolism of proteins. When *Barytelphusa guerini* were transferred to clean water the return in free amino acid content, indicates that these tissues may have recovered from CPF toxicity. At the end of 18 days of recovery period slight decrease (4%) in amino acids were observed in gill and muscle tissues.

Ammonia, amino acids and urea are the three principal end products of protein metabolism that are released to the environment through gills in

decapods (Regnault 1987). The ammonia level in the gill, muscle, hepatopancreas and nervous ganglion of CPF exposed crab exceeded those in controls on all sampling weeks. An elevation level of ammonia content was observed in all tissues (Fig. 3), highest increase in ammonia content was shown by gills and nervous ganglion respectively. Urea content decreased in all tissues, maximum decrease was observed in hepatopancreas followed by muscle, gill and nervous ganglion (Fig. 4). The elevated ammonia concentration might be due to increased ammoniogenesis in CPF intoxicated crab. Ammonia is a toxic metabolite and excess ammonia is known to trigger the operation of detoxification or utilization systems, chiefly by way of formation of less toxic nitrogenous substances namely urea and glutamine (Begum 2004). The decrease in urea content suggests its elimination from the body. After cessation of toxicity recovery in ammonia and urea content was observed.

Gill and hepatopancreas glutamine content increased throughout the exposure period, where as muscle and nervous ganglion showed decrease in glutamine content (Fig. 5). Mobilization of ammonia towards the formation of less toxic substance, glutamine is also evident from this study. According to (Philip and Rajasree 1996), glutamine production will be switched on when the tissue suspects the accumulation of ammonia. The mechanism of synthesis of glutamine from toxic ammonia might have helped the crab to overcome the stress of chlorpyrifos intoxication. Decrease in glutamine level in muscle and nervous tissue exhibited the tissue specific response. At the end of 18 days of recovery period glutamine level in gill tissue were near to control value. Protease activity was induced in all tissues and all days of exposure, maximum induction was shown by hepatopancreas followed by gill, nervous ganglion and muscle (Fig. 6). Increased protease activity caused proteolysis in order to get extra energy to face the toxicity of chlorpyrifos, a fact seen in the present study. After 18 days of permanence in clean water, recovery in protease activity was observed.

Aspartate aminotransaminase activity was induced in gill, muscle hepatopancreas and nervous during 4 weeks of CPF intoxication and there was recovery when the crabs were released into freshwater (Fig. 7). ALAT exhibited a pattern similar to that of AAT, maximum induction was shown by gill followed by hepatopancreas muscle and nervous ganglion (Fig. 8). Enhance activities of both the transaminases indicate stepped up transamination where feeding of amino acids into TCA cycle occurs in order to cope up with the enhance energy requirement under toxic stress of CPF. Similar increase in AAT and ALAT activity was reported in tissues of crab exposed to organophosphate pesticides (Reddy *et al.,* 1987) is drawn in support of present study. The elevation in transaminases might be due to the results of decrease protein content observed in gill, muscle, hepatoprancreas and nervous tissue. When CPF exposed crab were released into freshwater, recovery in AAT and ALAT was maximum in hepatopancreas and muscle. The difference between control and recovered muscle is 1.5 % only. The recovery might be due to increased enzyme molecules and / or by increased rates of the enzyme synthesis in order to compensate for the activity of lost enzyme as suggested (Ay *et al.,* 1999).

The analysis of marker enzymes such as phosphatases and transaminases serves as specific indications of water pollution induced changes in the enzyme activity of crustaceans (Vijayavel and

Balasubramanian 2006). Phosphatases hydrolyze various phosphate esters and liberate phosphate from the stored substrates of hepatopancrease during various physiological requirements (Zhou *et al.,* 2000). The inhibition in ACP enzyme activity was gradual in hepatopancreas and nervous ganglion and maximum inhibition occurred at the end of 4 weeks. Gill and muscle ACP activity was enhanced by CPF treatment, in contrast to hepatopancreas and nervous ganglion (Fig. 9). In the present investigation acid phosphatase was inhibited in hepatopancreas and nervous tissue, this could be due to the direct inhibitory action of CPF on ACP. Bhavan and Geraldine (2001) reported that comparable inhibition in acid phosphate in tissues of prawn exposed to endosulfan. Acid phosphatase was induced in gill and muscle tissue. The induction could be due to the destruction of lysosomal membrane which caused the release of enzyme. Acid phosphatase induction reflects proliferation of lysosomes an attempt to sequester the toxic xenobiotics (Gill *et al.,* 1992). Acid phosphatase in gill tissue recovered maximum after transfer of crab from CPF toxicant to freshwater.

ALP activity was enhanced in gill and hepatopancreas and inhibited in muscle and nervous ganglion up to 4 weeks of exposure (Fig. 10). In the present study CPF caused induction in the activity of alkaline phosphatase in gill and hepatopancrease which might be due to the accelerated membrane transport function related to anion hydroxide exchange across the lipid biomembranes mediated by organotin compound as suggested by Jaroli and Sharma (2005). Khan and Pandya (1986) reported that other possibility for the increase could be due to the destruction of smooth endoplasmic reticulum membrane. Inhibition in alkaline phosphatase activity in nervous tissue and muscle can be taken as an index of parenchymal damage, necrosis and uncoupling of oxidative phosphorylation in these tissues. When the crabs were transferred into CPF free water ALP activity recovered maximum in gill tissue.

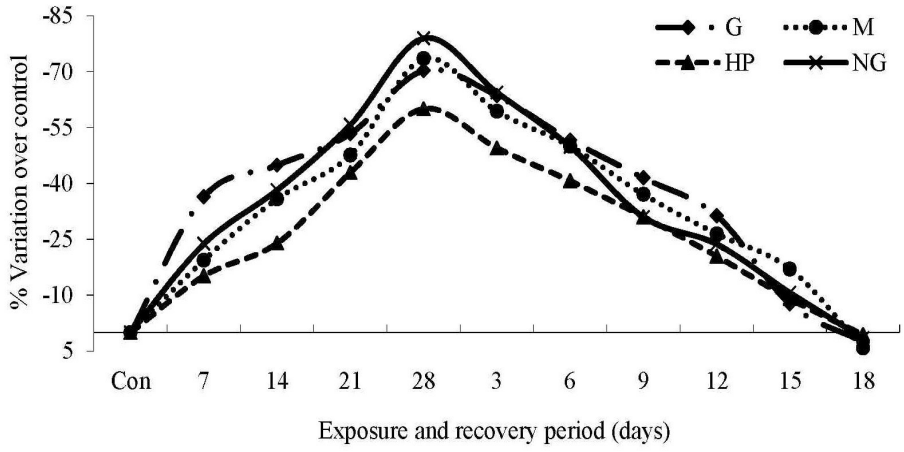

Figure 1 Total proteins content in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

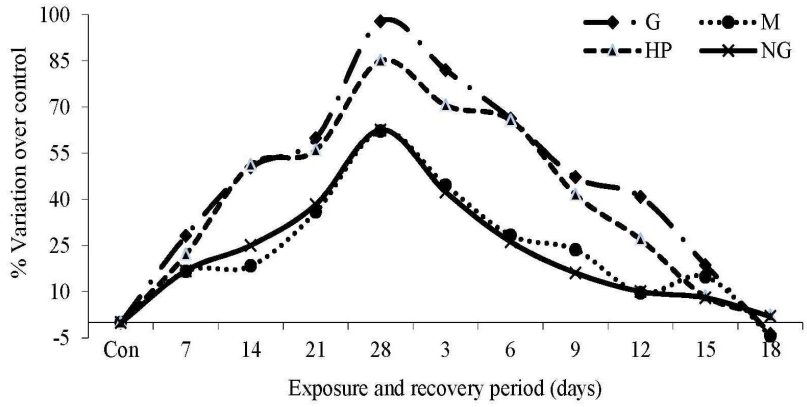

Figure 2. Free amino acids content in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

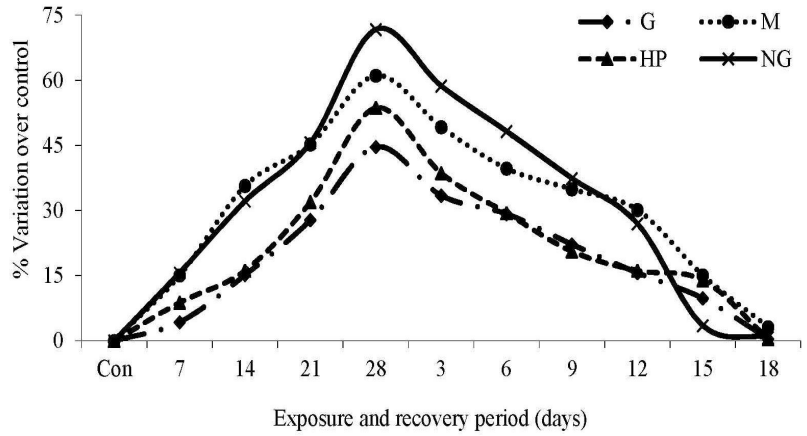

Figure 3. Ammonia levels in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

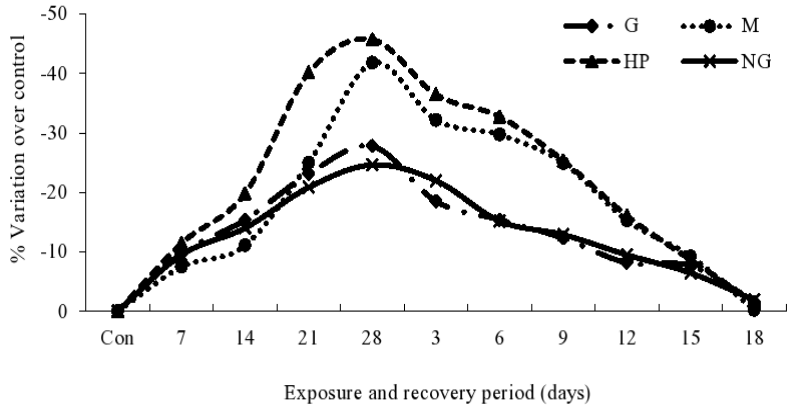

Figure 4. Urea levels in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

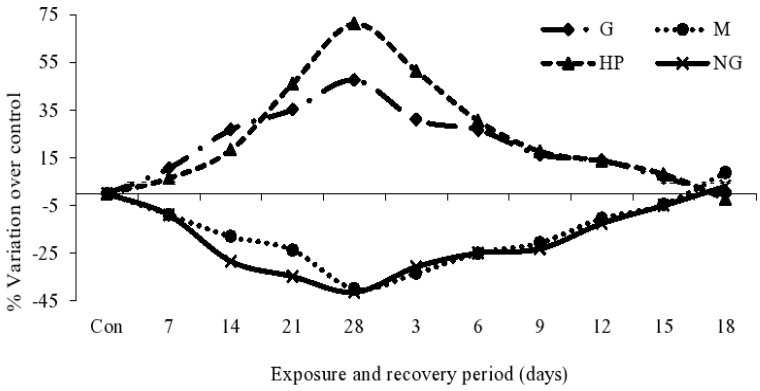

Figure 5. Glutamine content in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

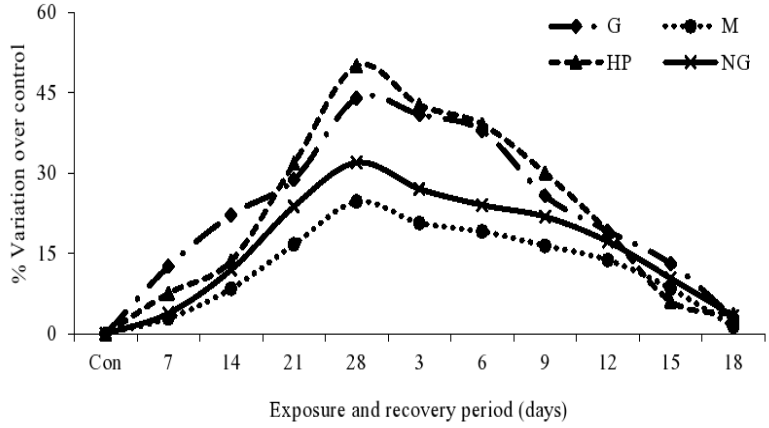

Figure 6. Protease activity in different tissues of *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

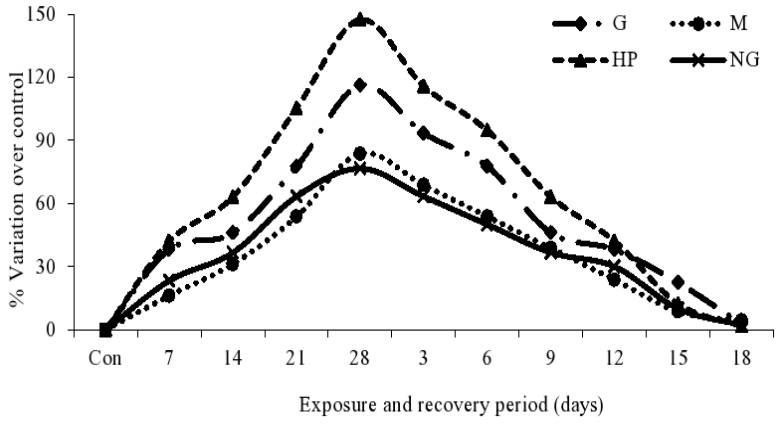

Figure 7. Aspartate aminotransferase activity *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

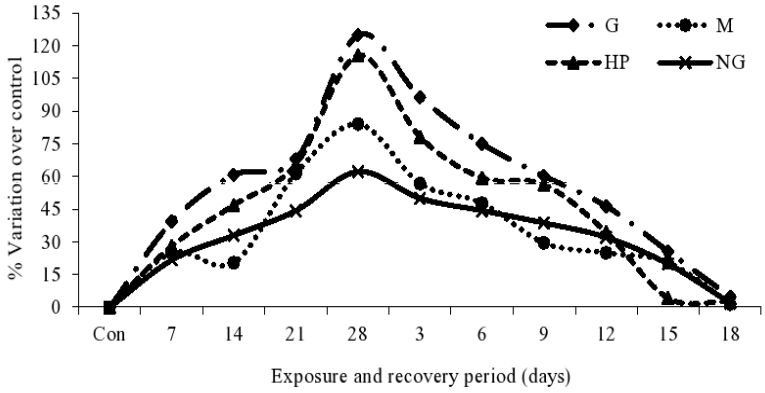

Figure 8. Alanine aminotransferase activity *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

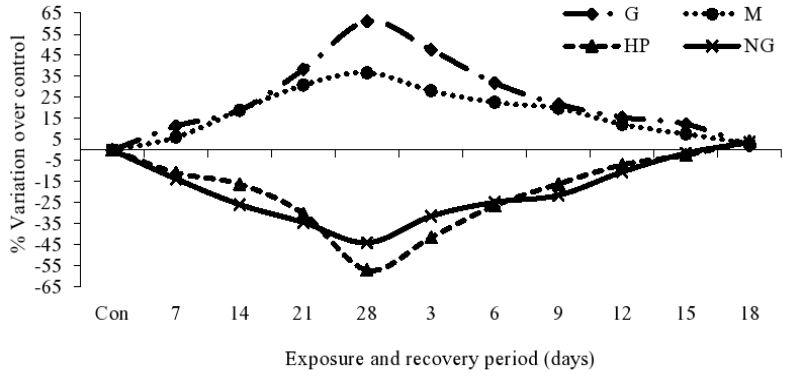

Figure 9. Acid phosphatase activity in different *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

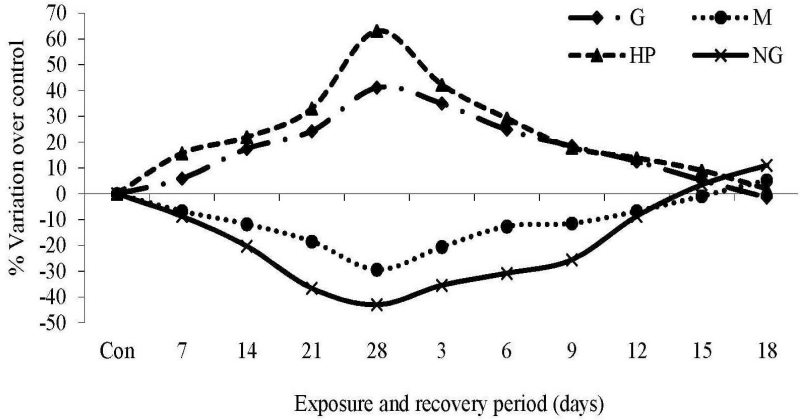

Figure 10. Alkaline phosphatase activity in different *Barytelphusa guerini* exposed to sub-acute concentration of CPF for 4 weeks followed by18 days of recovery. The values represent percent variation over control and n=6.

The present study demonstrates the sub-acute effect of CPF on protein metabolism caused alterations on crab physiology which affect not only the metabolites but also the enzymes. Some tissue specific response is also exhibited by crab under the impact of CPF. Transfer of crabs into freshwater improves the biochemical constituents of protein and enzymes metabolism. From the results it is clear that 21 days of recovery period is not sufficient, longer recovery period would be necessary in order to bring back the crab to normal metabolism. However, more investigations are

needed to know the exact compensatory role of protein metabolism and also to bring back the crabs to complete recovery state.

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REFERENCES

Almeida, J.A., Diniz, Y.S,, Marques, S.F.G., Faine, I.A., Ribas, B.O., Burneiko, R.C., Novelli, E.I.B. (2002) The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. *Environ. Toxicol*., **16**, 673-679.

Ay, O., Kalay, M., Tamer, L., Canli, M. (1999) Copper and lead accumulation in tissues of freshwater fish *Tilapia zilla* and its effects on the branchial Na^+, K^+ ATPase activity. *Bull. Environ. Contam. Toxicol.,* **5**, 160-168.

Bacey, J., Spurlock, F., Starner, K., Feng, H., Hsu, J., White, J., Tran, D.M. (2005) Residues and toxicity of esfenvalerate and permethrin in water and sediment, in tributaries of the Sacramento and San Joaquin rivers, California, USA. *Bull. Environ. Contam. Toxicol.,* **74,** 864-871.

Begum, G. (2004) Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clarias batrachus (linn) and recovery response. *Aquatic Toxicology*., **55**, 83-92.

Bhavan, P.S., Geraldine, P. (2001) Biochemical stress responses in tissues of the prawn Macrobrachium malcolmsonii on exposure to endosulfan. *Pesticide Biochemistry and Physiology,* **70,** 27-41

Caldwell, R.S., *et* . (1979) Toxicity of the herbicides 2,4-D, DEF, propanil and trifluralin to the Dungess crab Cancer magister. *Arch. Environ. Contamin Toxicol*., **8**, 383-396.

Cheney, M.A., Fiorillo, R., Criddle, R.S. (1997) Herbicide and estrogen effects on the metabolic activity of *Elliptio complanata* measured by calorespirometry. *Comp. Biochem. Physiol. C.,* **118**, 159-164.

Colowick, S.P., Kaplon, N.O. (1967) Methods in enzymology. Academic Press, New York.

Denton, D.L., Wheelock, C.E., Murray, S., Deanovic, L.A., Hammock, B.D., Hinton, D.E. (2003) Joint acute toxicity of esfenvalerate and diazinon to fathead minnow (*Pimephales promelas*) Larvae. *Environ. Toxicol. Chem*., **22**, 336-341.

Epstein, L., Bassein, S., Zalom, F.G. (2000) Almond and stone fruit growers reduce OP, increase pyrethroid use in dormant sprays. *California Agric*., **54**, 14-19.

Finney, D.J. (1971) Probit analysis. 2nd edition. Cambridge University Press. London and New York.

Folmar, L.C., Sanders, H.O., Julin, A.M. (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contamin Toxicol*., **8**, 269-278.

Gill, T., Tewari, H., Pande, J. (1992) Short and long-term effects of copper on the rosy carb (Puntius conchonius). *Ecotox. Environ. Safe*., **23,** 294-297.

Jabeen, K. (1984) Toxicological evaluation of some pesticides against chicken (*Gallus gallus domesticus*) and rat (*Rattus rattus norvegicus*) with special reference to haematology, blood and urine biochemistry. Ph.D thesis., Osmania

University.

Jaroli, D.P., Sharma, B.L. (2005) Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus*. *Asian Jour. Experiment Sci.,* **19**,121-129.

Khan, S., Pandya, K.P. (1986) Hepatoxiciy in albino rats exposed to n-octane and n-nonane. *Jour. Applied Toxicol*., **5**, 64-68.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with folin phenol reagent. *Jour. Biol. Chemistry.,* **193**, 265-275.

Montagna, M.C., Collins, P.A. (2007) Survival and growth of Palaemonetes argentinus (Decapods; Caridae) exposed to insecticides with chlorpyrifos and endosulfan as active element. *Arch. Environ. Contamin. Toxicol*., **53**, 371-378.

Montagna, M.C., Collins, P.A. (2008) Oxygen consumption and ammonia excretion of the freshwater crab *Trichodactylus borellianus* exposed to chlorpyrifos and endosulfan insecticides. *Pest. Biochem. Physiol.,* **92**, 150-155.

Moore, S., Stein, W.H.J., Farr, A.L., Randall, R.J. (1954) A Modified ninhydrin reagent for the photometric determination of amino acids and related compound. *Jour. Biol. Chem.*, **221**, 907-913.

Moss, D.W., Henderson, A.R., Kochmar, J.F. (1986) Enzymes: principles of diagnostic enzymology and the aminotransferases. In: *Text book of Clinical Chemistry*. (Tietz, N.W. ed) pp. 663-768. Saunders, Philadelphia.

Narra Madhusudan Reddy, Begum, G., Rajender, K., Rao, J.V. (2012) Toxic impact of two organophosphate insecticides on biochemical parameters of a food fish and assessment of recovery response. *Toxicol. Industrial Health*., **28(4)** 343-352

Natelson, S. (1971) Techniques of Clinical Chemistry. (Charles, G. ed) pp. 146. Thomas Publisher Springfield Illinois, USA.

Ogueji, E.O. (2007) Investigations of biochemical effects of acute concentration of lambda-cyhalothrin on African catfish *Clarias gariepinus*-Teugels. *Jour. Fish. Int*., **2**, 86- 90.

Ozha, D.D. (1998) Water and its formidable pollution: need immediate attention. *Research Jour. Chem. Environ*., **2**, 63-65.

Patil, C., Paul, R., Malkanna. (2008) Neuroendocrine reglulation and pesticidal impact on freshwater crab, *Barytelphusa guerini*. *Jour.Environ. Biol*., **29**, 887-892.

Phillip, G.H., Rajasree, B.H. (1996) Action of cypermethrin on tissue transamination during nitrogen metabolism in *Cyprinus carpio*. *Ecotox. Environ. Safe*., **34**, 174-179.

Pimentel, D. (1995) Amounts of pesticides reaching target pests: environmental impacts and Ethics. *Jour. Agric. Environ. Ethics*., **8**, 17-29

Prasath, P.M.D., Arivoli, S. (2008) Biochemical study of freshwater fish *Catla catla* with reference to mercury chloride. *Iranian Jour. Environ. Health Sci. Eng*., **3,** 109-116.

Reddy, M.S., Gouselazam, S., Babu, T.R., Rao, K.V.R. (1987) Changes in respiratory potentials of the penaeid prawn, *Metapenaeus monoceros* exposed to phosphomidon, DDT and fenvalerate. *Environ. Ecology*., **4**, 643-646.

Reddy, A.N., Venugopal, N., Reddy, S.L.N. (1991) Effects of endosulfan 35 EC on certain aspects of protein metabolism in various tissues of a fresh water field crab, *Barytelphusa guerini*.

Pest. Biochem. Physiol., **39**, 121-129.

Rao, J.V. (2006) Biochemcial alterations in euryhaline fish *Oreochromis mossambicus* exposed to sub lethal concentration of an organophosphorus insecticide monocrotophos. *Chemosphere.*, **65**, 1814-1820.

Regnault, M. (1987) Nitrogen excretion in marine and fresh water crustacean. *Biological Revs.*, **62**, 1-24.

Reitman, S., Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminases. *Ame. Jour.Clinic. Pathol*., **286**, 56-63.

Selvakumar, S., Geraldine, P., Shanji, S., Jayakumar, T. (2005) Stressor-specific induction of heat shock protein 70 in the freshwater prawn *Macrobranchium malcolmsonii* (H, Milne Edwards) exposed to the pesticides endosulfan and carbaryl. *Pest. Biochem. Physiol*., **82**, 125-132.

Shakoori, A.K., Zaheer, S.A., Ahmed, M.S. (1976) Effect of malathion, dieldrin, and endrin on blood serum protein and free amino acid pool of *Channa punctatus* (bloch). *Pak. Jour. Zoology*., **8**, 125-134.

Singh, R.K., Sharma, B. (1998) Carbofuran induced biochemical changes in *Clarias batrachus*. *Pest. Science*., 53, 285-290.

Singh, N.N., Das, V.K., Singh, S. (1996) Effect of aldrin on carbohydrate, protein, and ionic metabolism of a freshwater catfish, *Heteropneustes fossilis*. *Bull.Environ.Contamin Toxicol.,* **57,** 204-210.

Sreenivasan, R.S., Arivalagan, M., Vijayananth, S., Praveen Kumar, P.K., Moorthy, P.K., Deecaraman, M. (2009) Effect of cypermethrin, a pyrethroid compound on the nutritive value in a freshwater field crab, *Spiralothelphusa hydrodroma* (Herbst). *Res. Jour. Agric. Biological Science.,* **5**, 572-577.

Steve, R., Donald French, P. (1996) Effects of Dimilin on the Blue Crab, *Callinectes sapidus,* in Shallow-Water Habitats. *Estuaries*., **19(**2A) 279-267.

Tomlin, C.D.S. (2000) The pesticide manual. 12th ed. London: The British Crop Protection Council.

Torstensson, L., Lundgren, L.N., Stenstrom J. (1989) Influence of climate and edaphic factors on persistence of glyphosate and 2,4-D in forest soils. *Ecotox. Environ. Safe*.,**18**, 230-239.

Vijayavel, K., Balasubramanian, M.P. (2006) Fluctuations of biochemical constituents and marker enzymes as a consequence of naphthalene toxicity in the edible estuarine crab *Scylla serrata*. *Ecotox. Environ. Safe*., **63**, 141-147.

Werner, I., Deanovic, L.A., Hinton, D.E., Henderson, J.D., de Oliveira, G.H., Wilson, B.W., Krueger. W/, Wallender. W/W., Oliver, M.N., Zalom, F.G. (2002) Toxicity of storm water runoff after dormant spray application of diazinon and esfenvalerate (Asana) in a French prune orchard, Glenn county, California, USA. *Bull. Environ. Contamin Toxicol*., **68**, 29-36.

Williams, Eagle. (1979) Persistence of dichlobenil in a sandy soil and effects on plant Growth. *Weed Research.,* **19**, 315- 319.

Zhou, X.W., Chen, Q-X., Chen, Z., He, Z-Q., Zhou, H.M. (2000) Effects of oxodiperoxovanadate (V) complexes on the activity of green crab (*Scylla serrata*) alkaline phosphatase. *Biochemistry (Moscow).,* **65**, 1424-1428.