Journal of Applied and Natural Science 2 (2): 258-262 (2010)

JANS

Behavioural shift of estuarine mudcrab as biomarker of arsenic exposure in Sundarbans estuary of West Bengal

Sanjib Saha, Mitali Ray and Sajal Ray*

Aquatic Toxicology Laboratory,Department of Zoology, University of Calcutta, Kolkata - 700019 (West Bengal), INDIA
***Corresponding author. E-mail: raysnailmail@rediffmail.com

Abstract: Mudcrab *Scylla serrata* (Crustacea: Decapoda) in an ecologically and economically important species of Sundarbans Biosphere Reserve was studied for its behaviour under the exposure of toxic arsenic - a common xenobiotic of this area. The behavioural profile of aquatic animals exposed to diverse toxicants are considered as an index to estimate the degree and nature of stress experienced by the animals both in nature and in experimental conditions. Present investigation involved study of selected behavioural shift of *S. serrata* under the sublethal concentrations of 1, 2 and 3 ppm of sodium arsenite for 1, 2, 3 and 4 days in controlled laboratory condition. Exposure to arsenic resulted an appearance of selected abnormal behavioural manifestation including tendency of avoidance, hypersecretion of mucoid element and release of excess excretory products. Toxin induced alteration of studied behaviour is indicative to possible shift in the overall physiological functions and biological activities of this important species in its natural habitat. Chronic exposure to 3 ppm of sodium arsenite for 30 days may lead to decline this economically important species in Sundarbans Biosphere Reserve.

Kew words: *Scylla serrata*, Sodium arsenite, Behaviour, Biomarker

INTRODUCTION

Sundarbans Biosphere Reserve of West Bengal is one of the large mangrove ecosystems in the world having hundred and two isle with a network of rivers, rivulets and creeks (Chaudhuri and Choudhury, 1994). Government of India announced the status of Sundarbans as one of the 15 Biosphere Reserves of India. Freshwater of the rivers mingles with the sea water and the ecosystem is characterized by fluctuations of salinity and water height. Dynamism of water height and salinity render the ecosystem an uniqueness. The entire area provides a suitable ground of reproduction and nutritional bed of diverse animal species (Naskar and Ghosh, 1989 and; Chaudhuri and Choudhury, 1994). As a result, Sundarbans Biosphere Reserve exhibits an array of biodiversity including both plants and animals. Estuarine mudcrab is an edible form of species and considered as an important member of Sundarban ecosystem (Ali *et al.*, 2004 and Saha and Ray, 2006). This intertidal species is under constant exposure of diverse xenobiotics including toxic metalloid arsenic (Sarkar *et al.*, 2002 and 2004 and Ghosh *et al.*, 2008). Arsenic, a major environment threat of the district of South 24-Parganas of West Bengal has dual sources of origin i.e. natural and anthropogenic (Gomez- Caminero *et al.*, 2001 and; Das and Roy Chowdhury, 2006). *Scylla serrata* mainly inhabits the soft muddy bottom of brackish water along the shoreline, mangrove areas, river mouths and deltas. They are scavengers, residing in the deep burrows around mangrove roots and in the banks of river and tidal creeks. Each burrow is oblique in shape and of 1-2 m deep and 8-16 cm in diameter at the opening. Natural and cultural habitat of *S. serrata* bears the environmental threat of hazardous xenobiotics, including arsenic from natural and anthropological sources (Krishnaja *et al.*, 1987; Saha *et al.*, 2007; 2008a; 2008b; 2009a; 2009b; 2009c and; 2010). The behavioural profiles of aquatic animals exposed to diverse toxicants are used as index to estimate the degree and nature of stress of animals in natural and experimental conditions (Victor, 1993 and; Vardhanan and Radhakrishnan, 2002). In this study, specific behavioural manifestations of *S. serrata* were studied under the exposure of arsenic. Present information would provide an important database concerning toxicity of arsenic in *S. serrata* in relation to its ethological profile. Toxicological information of arsenic on *S. serrata* in relation to behaviour is limited. It would also help to generate a sustainable strategy to restrict a degree of contamination and conservation of this economically important species in its natural habitat.

MATERIALS AND METHODS

Collection, transportation, acclimatization and maintenance of *S. serrata* : Live intermolt adult specimens of *S. serrata* weighing 65-75 gm were collected throughout the year manually from selected habitats of the district of south 24 Parganas of West Bengal, India which are free from arsenic contamination. Length and breadth of the carapace of experimental animals were 6 ± 0.5 cm and 9 ± 0.5 cm respectively. The crabs were carried alive in jute bags and transported immediately to the aquatic toxicology laboratory of department of Zoology of University of Calcutta. In the laboratory, live crabs were kept in large rectangular glass aquaria containing simulated marine salt water in controlled laboratory condition under constant aeration (Ali *et al.*, 2004 and; Saha and Ray, 2006). Live animals were maintained in laboratory aquarium and fed with fresh flesh of prawn and molluscs once a day. The water of glass aquaria was routinely replenished in every 24 hours to avoid residual toxicity. Animals were acclimatized in laboratory condition for 6-8 days prior to experimentation. Water quality parameters (Table 1) were kept constant. Temperature and acidity were monitored routinely and dissolved oxygen, hardness and salinity were estimated by standard protocols (Heasman and Fielder, 1983). A photoperiod of 12 h light-12 h darkness was provided. Tidal conditions were simulated in laboratory by increasing and decreasing the water level in every 12 hours of duration.

Sodium arsenite treatment

Determination of lethality: After acclimation to the laboratory conditions, acute toxicity study was carried out to determine the lethal (LC_{100}), median lethal (LC_{50}) and safe sublethal (LC_0) concentrations of sodium arsenite in *S. serrata*. Determination of LC_{50} was carried out by 'Behrens-Karber method' after Klassen (1991) whereas LC_0 and LC_{100} were determined after Hart *et al.* (1948); Vardhannan and Radhakrishnan (2002) respectively. The crabs were divided into many groups and each group contained 10 acclimatized crabs of same age and sex in triplicate which were introduced in the glass aquaria containing 10 liters of water. During acclimatization and treatment crabs were routinely checked for morbidity, mortality and dead crabs were instantly discarded to avoid additional toxicity due organic putrefication.

Rationale of selecting the experimental concentrations: In static water environment median lethal concentration (LC_{50}) of sodium arsenite is determined to be 15 ppm and 10 ppm in adult and juvenile crabs respectively for 96 hours of span. In this present study, all the experimental concentrations of sodium arsenite were selected to be less than 50% of LC_{50} value. According to Das and Roy Chowdhury (2006), concentration of arsenic in aquatic ecosystem of West Bengal ranges from 0.5 – 3.2 ppm in different seasons. Dynamics of concentration of dissolved arsenic is due to several environmental factors i.e. desiccation, dilution and natural availability. All the experimental concentrations of sodium arsenite were less than the highest reported natural concentration of arsenic.

Table 1. Water quality parameters.

Parameter	Value
Temperature	28 – 32 °C
pH	7.5 – 8.5
Dissolved oxygen	5 – 7 ppm
Hardness	170 ± 10 mg/l
Salinity	20-25 ppt

Treatment concentrations: Crabs were exposed to experimental concentrations i.e. 1, 2 and 3 ppm of sodium arsenite (E. Merck, Germany; 99% pure; CAS number 7784-46-5) dissolved in double distilled marine salt water taken in borosilicate glass containers. During treatment, mouths of test containers were partially covered by glass lids to prevent atmospheric desiccation.

RESULTS

Median lethal concentration of sodium arsenite in adult crab was estimated against different span of exposure i.e. 24, 48, 72 and 96 hours in static water environment (Figs. 1 and 2). In adult, the LC_{50} values of sodium arsenite 24 and 96 hours of span were determined as 100 ppm and 15 ppm respectively (Table 2). Safe concentration and safe application rate of sodium arsenite in adult *S. serrata* were 0.8 and 12.18 ppm respectively in static water environment (Table 2). Avoidance response: immediately after 4-5 minutes of exposure, the crabs in the treated set expressed physical restlessness in the static water environment of borosilicate glass jar (Figs. 2 and 3). All the crabs started moving along with periphery of the glass jar. After 10 – 15 minutes, all the crabs elevated the entire cephalothorax above the surface of water as typical toxin avoidance response under all concentrations of sodium arsenite (i.e.1, 2 and 3 ppm) (Fig. 2). The lowest concentration of sodium arsenite resulted low degree of avoidance behaviour against control and arsenic free water where as the exposure to higher concentrations of toxin resulted a steady avoidance pattern of crabs (Fig. 3). Hypersecretion of mucoid element: oral release of mucoid element associated with purgation and frothing began after 10 hours of exposure. Above symptoms continued for the entire period of exposure i.e. 96 hours under all experimental concentrations of arsenic (Table 3). Above mentioned symptom were absent in all the specimens of the control set (Fig. 1). Discharge of excess fecal matter: discharge of yellow coloured fecal elements initiated after 10 hours of exposure of sodium arsenite. The frequency of rectal release was significantly higher than that of the control set. This symptom continued throughout the entire span of exposure i.e. 96 hours under

Table 2. Median lethal concentration, safe concentration and safe application rate of sodium arsenite in *S. serrata*.

Span of exposure (h)	LC_{50} (ppm)	Safe concentration (ppm)	Safe application rate (ppm)
	Adult (6X9 ± 0.5 c.m)	Adult (6X9 ± 0.5 c.m)	Adult (6x9 ± 0.5 c.m)
24	100		
48	30		
72	20	0.8	12.18
96	15		

all experimental concentrations of sodium arsenite (Table 3). Nearly hundred percent animals of exposed sets exhibited this behaviour and these behavioural symptoms were completely absent in the members of control sets (Fig. 1). All the behavioural abnormalities mentioned above disappeared following maintenance of pre-exposed animals in arsenic free water for 48 hours.

DISCUSSION

Sundarbans Biosphere Reserve is a unique ecosystem and characteristic of diverse types of habitats ranging from mudflat to sandy beaches. Estuaries play a very important role in maintenance of the aquatic life by providing the necessary supplies for their growth and survival. Tidal submergence and periodic inflow of nutrients make this ecosystem dynamic in relation to physiological status of the animals. Generally, Sundarbans estuary plays as links between estuarine and marine ecosystems and the ecosystem of estuary receives diverse environmental toxins of known and unknown chemistry. These toxins are carried through water and silt and increase ecological crisis (Sarkar *et al.*, 2002 and 2004). Arsenic, a toxic metalloid of the environment, bears the potentiality to affect the normal physiology of crustaceans (Vijayavel *et al.*, 2006). Contaminated mudflats are the natural habitat of mud crab including *S. serrata*, an important inhabitant of this ecosystem (Naskar and Ghosh, 1989; Chaudhuri and Choudhury, 1994 and; Ali *et al.*, 2004). Arsenic is a naturally occurring metalloid toxin distributed in surface and ground water and gets concentrated in rivers and lakes upto a concentration of 5 ppm due to summer desiccation (Galloway and Depledge, 2001 and; Gomez - Caminero *et al.*, 2001). Trivalent arsenite is more mobile and stable than pentavalent arsenate especially at pH greater than 7 and the toxicity of arsenate is linked with reduction of arsenate to arsenite (Panthi *et al.*, 2006). During summer, the arsenate is chemically reduced to a highly toxic arsenite due to arsenites ability to react with sulfhydryl groups in the mud-water interface (Chakrabarti *et al.*, 2008). It was found that a large amount of these arsenic residues accumulated in the aquatic organisms namely fish and marine crustacean upto a concentration of 100 µg / g dry weight (Gomez-Caminero *et al.*, 2001). The arsenate dominates in oxidized sediments and is associated primarily with iron Oxyhydroxide and in reducing marine sediments arsenate is reduced to arsenite and associated with sulfide minerals. Marine algae are capable of accumulating arsenate from sea water and reduce arsenate to arsenite and then oxidize the arsenite to a large number of organoarsenic compounds and these compounds are bioaccumulated by human consumers of seafood products i.e. crabs, shellfish, prawn etc (Neff, 1997). No suitable markers of arsenic toxicity are available at present. Report on toxicity of arsenic in relation to behavioural patterns is absent in current literature. Median lethal concentration for 96 hours in adult *S. serrata* was determined as 15 ppm in static water environment. In this present study, the highest experimental dose (i.e. 3ppm) was $1/5^{th}$ of the 96 hours LC_{50} of adult (Table 2). The highest experimental dose was lower than the reported safe application rate for adult. Toxin induced shift in behavioural profile affects the life process of animal adversely. Exposure to arsenic resulted an appearance of abnormal behavioural manifestation including tendency of avoidance, hypersecretion of mucoid element and release of excess excretory products and these behaviours abruptly disappeared upon withdrawal of arsenic exposure in controlled experimental condition (Victor, 1993). Altered profile of ethology is indicative to physiological stress which may endanger the existence of the species in its natural environment. Vardhannan and Radhakrishnan (2002) reported the spectrum of abnormal behaviour of paddy field crab, *Paratelphusa hydrodromus* under the exposure of arsenic. Arsenic induced shift in the ethological profile

Table 3. Hypersecretion of mucoid element and discharge of excess fecal matter following an exposure of 3 ppm arsenic for 30 days.

S. serrata	Secretion of mucoid element by Mouth			Discharge of fecal matter		
Control		+			+	
	1 ppm/96 h	2 ppm/96 h	3 ppm/96 h	1 ppm/96 h	2 ppm/96 h	3 ppm/96
Specimen 1	++	++++	++++++	++	+++	++++
Specimen 2	++	+++	++++++	++	+++	+++++
Specimen 3	++	++++	++++++	++	+++	++++

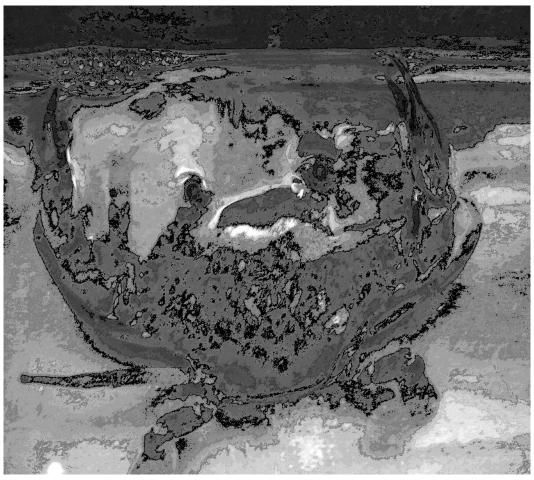

Fig. 1. *S. serrata in arsenic free water.*

Fig. 2. *Typical carapace elevation response of S. serrata exposed to sodium arsenite.*

of *S. serrata* is indicative to an alteration of related biological profile of the species in its natural habitat. Altered behavioural profile may affect the salient biological parameters like feeding, reproduction, predator ship and periodic activity. Such situation might reduce the ecological fitness of the species resulting dwindling of the population in the wild. They reported a profile of abnormal behaviour like wall climbing, mounting and mucous secretion in crab exposed to high concentration of toxin mentioned. In this present study behavioural profile of crab was studied in depth under the exposure of arsenic along with the control. *S. serrata* exposed to sodium arsenite expressed a typical avoidance response by attaining an elevated posture (Fig. 2). All the animals tended to climb the wall of aquaria and aggregated to form a pyramidal structure within 24 hours of exposure. The crab started releasing excess in yellow coloured fecal matter (Table 3). Simultaneously hypersecretion of mucoid element resulted frothing in the media (Fig. 2). Chronic exposure 3 ppm of sodium arsenite for 30 days may lead to alteration of physiological functions and behavioural profile of *S. serrata*. Moreover, the effect of arsenic on the behavioural physiology of crab is a less studied area. Current investigation would useful information of the toxicity of sodium arsenite on the behavioural physiology of Crustacea. Under this situation, *S. serrata* may become susceptible to microbial infection leading to mass mortality leading to possible decline of this economically important biological resource from Sundarbans Biosphere Reserve. However our present investigation suggests the studied behavioural profile of mudcrab as effective biomarker of arsenic exposure in the estuary. This experiment suggests minimizing the arsenic pollution in mud and estuary, so that, the estuarine mud crab of Sundarbans can properly be protected in their natural habitat. However, in depth study is awaited to understand the extent and magnitude of other heavy metals toxicity in *S. serrata* and allied species distributed in mangrove

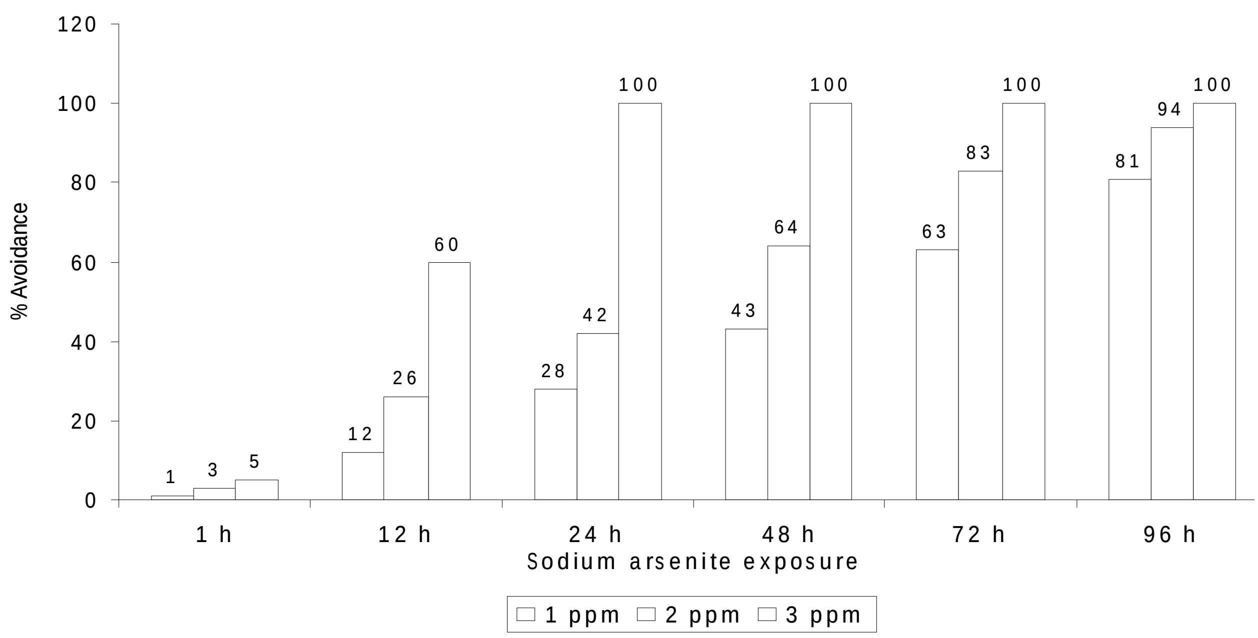

Fig. 3. *Avoidance response (climbing and pyramid formation) of crab exposed to 1, 2, and 3 ppm arsenic upto 96 h.*

ecosystem of Sundarbans.

ACKNOWLEDGEMENTS

Authors thankfully acknowledge the Department of Science and Technology and University Grants Commission of Government of India for providing financial support through FIST and SAP-DRS - 1 for grant support, instrumentation and other facilities.

REFERENCES

Ali, M. Y., Kamal, D., Hossain, S. M. M., Azam, M. A., Sabbir, W., Murshida, A., Ahmed, B. and Azam, K. (2004). Biological studies of the mud crab, *Scylla serrata* (Forskal) of the Sundarbans mangrove ecosystem in Khulna region of Bangladesh. *Pakistan J. Biol. Sci*., 7(11): 1981-1987.

Chakraborty, S., Ray, M. and Ray, S. (2008). Sodium arsenite induced alteration of hemocyte density of *Lamellidens marginalis* - an edible mollusk from India. *Clean*, 36 (2): 195-200.

Chaudhuri, A. B. and Choudhury, A. (1994). Mangrove of Sundarbans Vol. I: India (Zakir Hussain, M. and Acharya, G. Ed.) IUCN, Bangkok, Thailand. The World Conservation Union.

Das, S. and Roy Chowdhury, A. (2006). Arsenic: a global monster. *Sci. Cult.*, 72(7- 8): 230-237.

Galloway, T. S. and Depledge, M. H. (2001). Immunotoxicity in invertebrates: measurement and Ecotoxicological relevance. *Ecotoxicol.*, 10: 5- 23.

Ghosh, S., Ray, M. and Ray, S. (2008). Kinetics of nonself adhesion and viability of immunocytes of blood of economically important Mollusc, *Telescopium telescopium* of Sundarbans estuary. *Icfai J. Life Sci.*, 2(3): 7-14.

Gomez - Caminero, A., Howe, P., Hughes, M., Kenyon, E., Lewis, D. R., Moor, M., Nag. J., Aitio, A. and Becking, G. (2001). Summary in environmental health criteria 224, Arsenic and Arsenic compounds (Ed: 2nd), pp. 1-8: World Health Organization, Geneva.

Hart, W. B., Weston, R. F. and Demann, J. G. (1948). An apparatus for oxygenating test solutions in which fish are used as test animals for evaluating toxicity. *Tran. Am. Fish Soc*., 75: 228-239.

Heasman, M. P. and Fielder, D. R. (1983). Laboratory spawning and mass rearing of the mangrove crab (*Scylla serrata* Forskal), from first zoea to first crab stage. *Aquacult.*, 34: 303-316.

Klassen, C. D. (1991). Principles of toxicology. In: Gilman, A. G., Tall, T. W., Nies, A. S., Taylor, P. (Eds.), Pharmacological Basis of Therapeutics, 8th ed. McGraw Hill, pp. 49 – 61.

Krishnaja, A. P., Rege, M. S. and Joshi, A. G. (1987). Toxic effects of certain heavy metals (Hg, Cd, As, Se) on the intertidal crab *Scylla serrata*. *Mar. Environ. Res*., 21(2): 109-119.

Naskar, K. and Ghosh, A. (1989). Mangrove forest of the Sundarbans: Its impact on estuarine fisheries. Proceeding in Coast Zone Management of West Bengal, Sea Explores Institute, Calcutta, pp A 47-59.

Neff, J. M. (1997). Ecotoxicology of arsenic in the marine environment-review. *Environ. Toxicol. Chem*., 16(5): 917- 927.

Panthi, S. R., Sharma, S. and Mishra, A. K. (2006). Recent status of arsenic contamination in groungwater of Nepal – a review. *Kathmandu Uni. J. Sci. Engin. Tech*., 2(1): 1-11.

Saha, S. and Ray, S. (2006). Hemocyte profile of the estuarine mud crab, *Scylla serrata*. *Environ. Ecol*., 24S (3A): 818- 819.

Saha, S., Ray, M. and Ray, S. (2007). Analyses of total count of hemocytes of estuarine crab, under acute arsenic exposure. *Icfai J. Life Sci.*, 1(3): 75-78.

Saha, S., Ray, M. and Ray, S. (2008a). Kinetics of nonself surface adhesion and phagocytic response of hemocytes of *Scylla serrata* exposed to sodium arsenite. *Toxicol. Int.*, 15(1): 15-19.

Saha, S., Ray, M. and Ray, S. (2008b). Nitric oxide generation by immunocytes of mud crab exposed to sodium arsenite. In *Zoological Research in Human Welfare,* Zoological Survey of India, Kolkata Publication, paper 44: 425 – 428.

Saha, S., Ray, M. and Ray, S. (2009a). Recognition of antilymphocyte and antihemocyte sera by crab (*Scylla serrata*) hemocytes exposed to arsenic. *Research in Environment and Life Science,* 2(1): 1-6.

Saha, S., Ray, M. and Ray, S. (2009b). Activity of Phosphatase in the Hemocytes of Estuarine edible mud crab, *Scylla serrata* Exposed to Arsenic. *Journal of Environmental Biology*, 30(5): 655-658.

Saha, S., Ray, M. and Ray, S. (2009c). Effect of sublethal concentration of arsenic on hemocyte density in edible crab, *Scylla serrata*. *Animal Biology Journal,* 1(1): 17-26.

Saha, S., Ray, M. and Ray, S. (2010). Screening of phagocytosis and intrahemocytotoxicity in arsenic exposed crab as innate immune response. *Asian Journal of Experimental Biological Sciences*, 1(1): 47-54.

Sarkar, S. K., Bhattacharya, B., Debnath, S. Bandopadhaya, G. and Giri, S. (2002). Heavy metals in biota from Sundarbans wetland ecosystem, India: Implications to monitoring and environmental assessment. *Aquatic Ecosystem Health and Management*, 5(4): 467-472.

Sarkar, S. K., Franciskovic-Bilinski, S., Bhattacharya, M., Saha, M. and Bilinski, H. (2004). Level of elements in the surficial estuarine sediments of the Hugli River, northeast India and their environmental implications. *Environ. Int.*, 30 (8): 1089-1098.

Vardhannan, S. Y. and Radhakrishnan, T. (2002). Acute toxicity of copper, arsenic and HCH to paddy field crab, *Paratelphusa hydrodromus* (Herb.). *J. Environ. Biol*., 23(4): 387-392.

Victor, B. (1993). Responses of hemocytes and gill tissues to sublethal cadmium chloride poisoning in the crab, *Paratelphusa hydrodromous* (Herbst.). *Arch. Environ. Contam. Toxicol*., 24: 432-439.

Vijayavel, K., Anbuselvam, C., Balasubramanian, M. P., Samuel, V. D. and Gopalakrishnan, S. (2006). Assessment of biochemical components and enzyme activities in the estuarine crab, *Scylla traquebarica* from naphthalene contaminated habitants. *Ecotoxicol*., 15(5): 469-476.