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Alterations in certain biochemical parameters of the intertidal clam, *Gafrarium divaricatum*, due to chronic exposure of xylene, benzene and gear oil-WSF

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Abstract: *Gafrarium divaricatum (Gmelin) was exposed to sublethal concentrations of xylene (4.25 and 8.50 mg/l), benzene (4.35 and 8.70 mg/l) and gear oil-WSF (1 and 2%) for 30 days. There was a decline in condition index of the treated clams while moisture content showed an increase on day 30. Glycogen content of hepatopancreas of the clams treated with the pollutants registered an initial increase on day 4 whereas the level declined in gill and adductor muscle during this period while this constituent in hepatopancreas, gill and adductor muscle of the treated clams recorded drastic decline by day 30. Lactic acid, pyruvic acid and protein content in the three tissues increased significantly on day 4 and 30 of the treatment. It appears that the clams resorts to anaerobic mode of respiration under the pollution stress induced due to prolonged chronic exposure of xylene, benzene and gear oil-WSF.*

Key words: Biochemical parameters, Xylene, Benzene, Gear oil-WSF, *Gafrarium divaricatum.*

Introduction

The intertidal organisms are adaptive to environmental variations like temperature, salinity and pH by physiological and biochemical mechanisms (Vernberg, 1979; Gosling, 1982; Sukhotin and Portner, 1999). Among the biochemical adjustments, tissue metabolites play an important role during such adaptations. The effects of salinity, temperature and pH variations on tissue metabolites like glycogen, lactic acid, puruvic acid, proteins, fats and water content in the intertidal organisms have been recorded (Mane, 1974; Schoffeniels, 1976; Nagabhushanam and Mane, 1978; Rodriguez, 1981; Akaberali and Truman, 1985). The measurement of a specific biochemical metabolite in the tissues of intertidal organisms exposed to environmental stressors provides a sensitive method for predicting the effects of such stress on metabolism of these organisms. In toxicological investigations also, the change in the tissue metabolites proved to be a sensitive indicator of stress caused by toxicants. Therefore, the tissue metabolites like glycogen, proteins, fats, lactic acid and pyruvic acid in fishes exposed to various pollutants under field and laboratory conditions have been monitored (Venkatrama and Radhakrishnaiah, 1987; Al-Akel *et al*., 1988; Ferrando and Andreu-Moliner, 1991; Durairaj and Selvarajan, 1992). These studies have proved the utility in monitoring of such metabolites to know the toxic mode of action of pollutants on fishes. Such methods have also been applied in assessment of the effects of various pollutants on metabolism of invertebrates like crustaceans and molluscs (Mane *et al.*, 1986; Biswas, 1986; Krishna *et al*., 1987; Shankara *et al*., 1989; Shreedevi *et al*., 1992; Reddy and Bhagyalakshmi, 1994; Roy, 1994).

Bayne *et al.* (1982) showed that xenobiotics induced stress in intertidal bivalve, *Mytilus edulis* could be detected by assessing a number of metabolic parameters such as blood sugar levels, disruption of energy balance represented by alterations in glycogen, proteins

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and lipid content of tissues. Similarl, effects of oil and related hydrocarbons on metabolic parameters of aquatic organisms have also been recorded (Sabo and Stegeman, 1977; Augenfeld *et al*, 1980; Dange and Masurekar, 1982; Kulkarni, 1983; Deshmukh, 1983; Axiak and George, 1987; Neff *et al.*, 1987; Moles *et al.*, 1987; Kostylev, 1988; Khan and Nag, 1993). In India, considerable importance has been given to the studies on ecophysiological aspects of marine bivalves (Deshmukh, 1972; Mane, 1975) and some intertidal bivalves have also been employed as test organisms in toxicological research (Mane and Nagabhushanam, 1975; Eapen, 1987; Kulkarni and Kulkarni, 1987; Eapen and Patel, 1989; Kulkarni, 1990; Roy, 1994). Since the observations pertaining to biochemical responses to tissue metabolites in intertidal clams exposed to petroleum products is very scanty, the present investigation was conducted to assess the effects of sub-lethal exposure of oil and petroleum hydrocarbons on certain biochemical parameters in the vital tissues of *Gafrarium divaricatum*.

Materials and Methods

Gafrarium divaricatum (Gmelin)were collected during low tide period from Nariman Point area of Bombay coast and after cleaning with sea water were brought to the laboratory as single stock. The clams were acclimated for 24 hours in a medium sized aquarium (60x30x30) containing sea water of salinity 30-32 ppt, temperature 27-29^{0}C and pH 7.7-8.0. The same conditions were maintained throughout the bioassay tests. No special food was given to the clams as these animals thrived well under laboratory conditions. The sea water used during the acclimation and experimentation was brought from Nariman Point coastal area. Only those sets of stock animals which did not show any mortality during the acclimation period were used for the acute toxicity experiments. The active clams of more or less uniform size (30-32 mm) were selected for the bioassay experiments. The clams with protruding siphon and foot were considered as active clams.

The clams were exposed to sub-lethal concentrations of xylene (4.25 and 8.5 mg/l), benzene (4.35 and 8.7 mg/l) and gear oil water-soluble faction (WSF) (1 and 2 %) for the test period of 4 (96 hours) and 30 days. At the end of each period, the control and exposed clams were sacrificed and hepatopancreas (HP), gills (G) and adductor muscles (AM) were removed for the analysis of glycogen, lactic acid, pyruvic acid, proteins and moisture content. A brief account of the methods followed to assess the various metabolites is given below. Optical density (OD) of various solutions and extracts were measured on Spectronic 20.

Condition index: At the end of the exposure period of 30 days, the control and exposed clams were removed from aquaria and their shell length was measured. Then they were sacrificed to remove the whole soft tissues inside their shell. They were dried in an oven at 60^{0}C and weighed accurately. The condition index was calculated from the following formula.

$$\text{Condition index} = \frac{\text{Dry weight}}{\text{Shell length}} \times 100$$

Moisture content: Moisture content was determined according to the method described by AOAC (1975). The whole of the soft tissue from the clam was removed and dried in an oven at 60^{0}C. The dried tissue was weighed to a constant weight. The percentage moisture content was then calcuklated with the help of following formula.

$$\%\text{ moisture content} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight of tissue}} \times 100$$

Glycogen: The tissue glycogen content was estimated by anthrone method of Seifter *et al.* (1950). Freshly excised tissues (hepatopancreas, gills and adductor muscles) were taken in test tubes and digested in 3 ml 30% KOH kept in water bath at 70^{0}C. Each KOH digest was diluted to 100 ml with distilled water. To 5 ml aliquot of this solution, 10 ml of freshly prepared anthrone reagent (0.2% in 95%

concentrated H_2SO_4) was added gradually. Similarly, blank and standard tubes were also prepared using distilled water and glucose solution, respectively. The mixture was then boiled for 10 minutes in boiling water bath. After cooling, the optical density of the mixture was read at 625 nm. Standard calibration curve was also prepared for calculating glucose values. The glucose values thus obtained were converted into glycogen using the Morrisons factor 1.11.

Lactic acid: Lactic acid levels were determined according to the methods of Barker and Summerson (1941). A 2 ml aliquot of the deproteinized supernatant was treated with 1 ml of 20% $CuSO_4$ solution and then diluted to 10 ml with distilled water. 1 gm of $Ca(OH)_2$ powder was added and the mixture was shaken vigorously and allowed to stand for 30 minutes. The mixture was then centrifuged at 5,000 rpm for 5 minutes. To 1 ml of supernatant, 0.05 ml 4% $CuSO_4$ solution was added followed by 6 ml concentrated H_2SO_4. The solution was heated in a boiling water bath for 5 minutes and cooled at room temperature. After cooling, 0.1 ml p-hydroxydiphenyl reagent (1.5 gm p-hydroxydiphenyl dissolved in 10 ml of 5% NaOH and diluted to 100 ml with distilled water) was added and the solution was incubated at 30^0C for 30 minutes. The test tubes were then placed in a vigorously boiling water bath for 90 seconds and then allowed to cool down to room temperature. The optical density was read at 560 nm and the amount of lactic acid was calculated using a standard graph.

Pyruvic acid: Pyruvic acid content of the tissues from the control as well as experimental clams were determined by the method of Friedmann and Hawgen (1943) as modified by Chaykin (1966). Tissue of known weight was homogenized in 3 ml of 30% trichloroacetic acid. The mixture was centrifuged at 5,000 rpm for 5 minutes and clear protein-free supernatant was used for pyruvic acid assay. An appropriate aliquot of the deproteinized supernatant was diluted to 2 ml with distilled water and then treated with 0.5 ml 2,4-dinitrophenyl hydrazine reagent (0.1% in 2 N HCl) followed by 3 ml 2.5 N NaOH. The reaction mixture was allowed to stand for 10 minutes and OD was read at 540 nm. The amount of pyruvic acid was calculated using standard graph.

Soluble proteins: Soluble proteins were assayed by the method of Lowry *et al*. (1951). Freshly removed hepatopancreas, gills and adductor muscles were weighed and homogenized with chilled glass distilled water. The homogenates were centrifuged at 10,000 rpm for 10-15 minutes and the clear supernatants were used for estimation of soluble proteins. 0.2 ml supernatant was diluted to 1.0 ml by glass distilled water. 5 ml alkaline copper solution (prepared freshly by mixing 50 ml 2% $NaHCO_3$ in 0.1N NaOH and 1 ml of 0.5% $CuSO_4$ in 1% Na-K tartrate) was added. After incubation for 10 minutes at room temperature, 0.5 ml folin-phenol reagent was added rapidly and the solution was thoroughly mixed. After 30 minutes, the optical density was read at 500 nm. A standard graph was also prepared using bovine serum albumen and protein content from different tissues was calculated from this graph.

Results and Discussion

Condition index: The condition index of clam showed significant reduction after their exposure to all the concentrations of xylene, benzene and gear oil WSF after 30 days testing period (Table 1).

Moisture content: A significant elevation in total moisture content of *G. divarcatum* was noticed at all the test concentrations after 30 days exposure (Table 2).

Glycogen: Except a slight elevation in hepatopancreas, glycogen content of clam tissues exposed to all test pollutants for 96 hours was depleted. After 30 days of test period, glycogen content in all test clams was reduced significantly. The reduction in gills and adductor muscles was more pronounced after 30 days exposure (Tables 3-5).

Lactic acid: The lactic acid levels in all tissues

of exposed clams was found to be elevated after short (96 hours) and long-term (30 days) exposure to xylene, benzene and WSF of gear oil (Tables 6-8).

Pyruvic acid: The pyruvic acid content in clam tissues exposed to all test concentrations was found elevated after 96 hours of exposure whereas an extended exposure of clams to xylene, benzene and gear oil WSF resulted in marked depletion in pyruvic acid content (Table 9-11).

Proteins: Results indicate that there was an elevation in protein content in all tissues of clams exposed to both the concentrations of all the three pollutants for 96 hours whereas in case of cl;ams exposed for 30 days to these pollutants the protein content in all the tissues was found depleted significantly (Tables 12-14).

Table 1. Condition index in *G. divaricatum* exposed to xylene, benzene and gear oil.

Days	Control	Xylene concentration (mg/l)		Benzene concentration (mg/l)		Gear oil concentration (mg/l)	
		4.25	8.50	4.35	8.70	1	2
30	8.03±0.32	5.89±0.51 **	2.86 ±0.36 **	5.99 ±0.34 **	3.91 ±0.44 **	6.73 ±0.4 6 **	4.68 ±0.24 **

Values are mean ± S D of 5 determinations. Significant response: ** P <0.01.

Table 2. Moisture content (gm%) in *G. divaricatum* exposed to xylene, benzene and gear oil WSF.

Days	Control	Xylene concentration (mg/l)		Benzene concentration (mg/l)		Gear oil concentration (mg/l)	
		4.25	8.50	4.35	8.70	1	2
30	76.58±2 .38	80.33±0.27 **	83.13±1.30 **	80.85±1.04 **	81.35±0.88 **	79.26±0.7 0*	79.85±0.7 3*

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, ** P <0.01.

Table 3. Glycogen content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to xylene.

Days	Tissues	Control	Xylene concentration (mg/ml)	
4			4.25	8.50
	HP	26.60±2.63	28.08±1.49	30.59±2.90
	G	29.45±2.40	18.48±2.37**	14.40±1.20**
	AM	29.49±1.66	18.73±1.87	13.41±0.93**
30	HP	24.63±2.84	13.78±0.51**	12.13±0.25**
	G	27.88±2.84	10.50±0.86**	9.15±0.44**
	AM	30.75±3.24	20.74±1.51**	18.17±2.55**

Values are mean ± S D of 5 determinations. Significant response: ** P <0.01.

Table 4. Glycogen content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to benzene.

Days	Tissues	Control	Benzene concentration (mg/ml)	
4			4.3	8.7
	HP	23.47 ±3.16	25.33 ±3.52	28.43 ±1.69*
	G	27.97 ±2.61	22.44 ±3.22 *	15.41 ±1.32**
	AM	30.49 ±2.98	20.51 ±2.15**	16.151±1.52 **
30	HP	24.57 ±3.97	14.38 ±0.48**	13.18 ±0.63**
	G	25.72 ±2.33	10.60±0.82**	9.81 ±0.36**
	AM	29.53 ±2.69	13.90 ±0.62**	17.23±2.51 **

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, ** P <0.01.

Table 5. Glycogen content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to gear oil WSF.

Days	Tissues	Control	Gear oil concentration (mg/ml)	
4			1	2
	HP	25.58±4.82	26.47±3.43	28.36±1.55
	G	28.44±2.39	24.26±3.58	17.48±2.50**
	AM	30.81±3.16	20.70±2.24**	18.541±2.46**
30	HP	23.38±1.44	12.94±2.16**	12.38±1.78**
	G	20.46±2.75	8.53±0.97**	7.44±0.72**
	AM	23.42±0.75	9.10±0.67**	15.26±2.36**

Values are mean ± S D of 5 determinations. Significant response: ** P <0.01.

Table 6. Lactic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to xylene.

Days	Tissues	Control	Xylene concentration (mg/ml)	
4			4.25	8.50
	HP	4.53±1.90	5.41±1.73	6.39±2.03
	G	7.40±2.05	8.47±1.75	11.62±3.88
	AM	8.65±1.47	10.53±3.46	11.46±2.46
30	HP	3.32±1.24	5.76±1.53*	17.28±2.06*
	G	6.25±1.88	7.34±2.17	9.15±0.44**
	AM	7.45±2.10	8.47±1.53	11.43±2.56*

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, ** P <0.01.

Table 7. Lactic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to benzene.

Days	Tissues	Control	Benzene concentration (mg/ml)	
4			4.35	8.7
	HP	5.34±1.70	6.58±2.12	6.36±1.89
	G	7.43±1.87	7.69±2.04	9.41±3.36
	AM	11.34±2.40	12.47±2.43	14.66±1.72*
30	HP	5.56±1.80	6.42±1.97	8.35±1.56*
	G	6.62±1.74	6.50±2.06	9.62±3.28
	AM	8.61±1.61	9.39±3.06	11.58±2.30*

Values are mean ± S D of 5 determinations. Significant response: *P<0.05.

Table 8. Lactic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to gear oil WSF.

Days	Tissues	Control	Gear oil concentration (mg/ml)	
4			1	2
	HP	4.42±2.12	4.43±2.17	5.45±1.65
	G	6.52±1.95	7.51±2.03	8.56±1.54
	AM	7.58±1.88	8.44±1.60	10.35±3.59
30	HP	3.31±1.23	4.42±2.07	6.60±1.92
	G	7.22±2.03	8.23±1.58	12.56±2.26**
	AM	5.37±1.55	6.27±1.79	7.47±2.12

Values are mean ± S D of 5 determinations. Significant response: ** P <0.01.

Table 9. Pyruvic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to xylene.

Days	Tissues	Control	Xylene concentration (mg/ml)	
4			4.25	8.50
	HP	8.41±2.46	11.47±1.37*	12.36±1.34*
	G	5.36±1.11	7.37±1.59*	9.35±2.54*
	AM	4.40±1.24	5.43±1.11	7.50±1.43**
30	HP	9.57±2.64	7.34±1.61	4.39±1.40**
	G	4.50±1.51	3.38±1.01	2.70±0.94
	AM	5.26±1.21	4.30±1.42	3.26±1.17*

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, ** P <0.01.

Table 10. Pyruvic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to benzene.

Days	Tissues	Control	Benzene concentration (mg/ml)	
			4.23	8.7
	HP	7.34±1.62	9.46±2.59	10.61±1.89*
	G	5.29±1.34	7.38±1.56	8.37±2.68
	AM	6.19±1.77	7.45±1.57	8.54±2.58
30	HP	8.37±2.69	7.41±1.75	4.39±1 .39*
	G	5.21±1.28	4.30±1.53	4.29±1.29
	AM	6.31±1.73	5.32±1.15	4.07±1.42

Values are mean ± S D of 5 determinations. Significant response: *P<0.05.

Table 11. Pyruvic acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to gear oil WSF.

Days	Tissues	Control	Gear oil concentration (mg/ml)	
4			1	2
	HP	8.26±2.61	10.24±3.22	11.52±2.48
	G	4.48±1.46	6.20±1.81	7.55±1.69*
	AM	5.21±1.18	6.17±1.86	6.34±1.75
30	HP	7.27±1.52	6.59±1.87	4.23±1.49*
	G	6.25±1.71	5.43±1.03	5.28±1.27
	AM	4.66±1.24	3.26±1.07	3.52±1.08

Values are mean ± S D of 5 determinations. Significant response: ** P <0.05.

Table 12. Protein acid content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to xylene.

Days	Tissues	Control	Xylene concentration (mg/ml)	
4			4.25	8.50
	HP	21.69±1.60	23.94±4.56	30.37±2.25**
	G	25.17±3.81	27.28±3.85	28.96±2.95
	AM	18.08±2.37	20.33±1.45	24.96±4.70*
30	HP	18.68±3.78	10.48±1.82**	10.35±1.42**
	G	21.51±5.08	12.21±4.87*	11.34±1.52**
	AM	17.85±3.42	9.60±1.14**	10.59±1.99**

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, ** P <0.01.

Table 13. Protein content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to benzene.

Days	Tissues	Control	Benzene concentration (mg/ml)	
			4.23	8.7
	HP	20.10±2.31	24.12 ±4.76	27.08±2.60*
	G	24.34±2.12	26.28 ±1.80	28.24±2.61*
	AM	14.34±2.42	15.33 ±2.64	18.30±3.09
30	HP	16.25±1.82	15.88±1.80	11.32 ±1.30**
	G	22.40±3.14	12.72±2.45*	12.63±1.09**
	AM	14.75±2.81	13.11 ±2.50	12.33±2.33

Values are mean ± S D of 5 determinations. Significant response: *P<0.05, *P<0.01.

Table 14. Protein content (mg/gm wet weight) in tissues of *G. divaricatum* exposed to gear oil WSF.

Days	Tissues	Control	Gear oil concentration (mg/ml)	
4			1	2
	HP	18.25±2.64	20.38 ±1.50	24.59±2.60**
	G	23.43±2.13	24.11 ±2.08	24.42±3.49
	AM	14.29±2.46	16.10 ±2.24	17.20±2.75
30	HP	16.05±6.87	15.30±2.02	12.51±1.47
	G	18.72±5.71	9.33±2.72*	11.95 ±3.87
	AM	13.39±1.72	9.00±1.62	9.30±3.32*

Values are mean ± S D of 5 determinations. Significant response: ** P <0.05, P<0.01.

The results on the biochemical metabolites in clam, *Gafrarium divaricatum*, subjected to sublethal exposure of oil and petroleum hydrocarbons are in agreement with the earlier reports (Bayne *et al*., 1982; Deshmukh, 1983; Axiak and George, 1987; Neff *et al.*, 1987; Moles *et al*., 1987; Kostylev, 1988; Patel and Eapen, 1989). The clams exposed to different concentrations of petroleum hydrocarbons and oils showed many signs of stress, probably as a result of negative energy balance (Table 3-14). The rate of growth is a fundamental measure of physiological fitness and therefore growth has often been used as measure of environmental quality and pollution effects. However, it is often difficult to quantify and interpret the growth in adult bivalves, especially in relation to pollution. Therefore, the condition index can be utilized as an indication of whole body response of bivalve to the environment. There have been laboratory studies in which condition index was used to assess the toxic effects of various pollutants (Augenfeld *et al.*, 1980; Axiak and George, 1987; Eapen, 1987). The observed decrease in condition index of the experimental clams may be due to the use of energy reserves demanded by stressful conditions resulting into the decrease in dry weight. Decrease in condition index was noticed in *Maconma balthica* (Stekoll *et al*., 1980), *Venus verrucosa* (Axiak and George, 1987) and *Anadara granosa* (Patel and Eapen, 1989) when exposed to higher concentrations of oil, petroleum hydrocarbons and naphthalene, respectively.

Lipophilic compounds such as oil and petroleum hydrocarbons are readily taken up into tissues of bivalves and concentrate to the levels greatly above those in surrounding water (Clement *et al*., 1980; Awad, 1987; Axiak *et al*., 1988; Lin-Qin *et al*., 1990; Lin-Qin and Xin-Xiaoping, 1991; Readman *et al*., 1992). Accumulation of petroleum hydrocarbons might be disturbing the general osmotic balance in the tissues of *Gafrarium divaricatum* which might be leading to water uptake thereby resulting in increased moisture content. Stekoll *et al.* (1980) and Patel and Eapen (1989) have recorded increased moisture content in the tissue of marine bivalves as a result of oil and petroleum hydrocarbons exposure.

There is ample evidence to show that the current view of mammalian bioenergetics also applies to marine bivalves. Moreover, most of the enzymes which are intermediate of glycolysis and Krebs cycle have also been reported in marine bivalves (Bennet and Nakada, 1968; Engel and Neat, 1970; de zwaan and Zamdee, 1971; Usuki and Okamura, 1986). Most of the intertidal bivalves are facultative anaerobics *i.e.* they can live either aerobically or anaerobically but prefer to use oxygen when available as it allows much more economical use of fuel molecules. However, the environmental conditions or organism level anaerobiasis is very common in intertidal bivalves (Sukhotin and Portner, 1999). The maintenance of high reserves of glycogen is one of the adaptations in intertidal bivalves against anoxic conditions and under anaerobic conditions, carbohydrates are the sole or main source of energy. The high power output mode during anaerobiasis in marine invertebrates including the intertidal bivalves is linked to classical glycolysis. Furthermore, the increase in anoxia tolerance with larval development appears to go along with shift from functional anaerobiasis to environmental anaerobiasis (Widdows *et al.*, 1989). Therefore, the observed depletion of glycogen in the tissues of oil and hydrocarbon exposed clams might be due to mobilization of glycogen reserves to meet the energy demand. The reduction of glycogen reserves in bivalves under anaerobic conditions has been reported by de Zwaan and Zandee (1971) and Badman and Chin (1973). Similarl, utilization of glycogen reserves in bivalves exposed to various pollutants is reported (Dunning and Major, 1974: Theede, 1985, Roy, 1994). Such depletion of glycogen reserves has also been observed in marine crustaceans under pollutant stress (Deshmukh, 1983; Kulkarni, 1983; Reddy and Bhagyalakshmi, 1994). The histo-pathological damage observed in the gill of *Gafrarium divaricatum* under such treatments is supportive to hypoxic condition of the clam (Agwuocha *et al*., 2009).

The reaction step of EMP pathway in which glucode-6-phosphate is converted to pyruvate has been recommended for *Mytilus edulis* both in terms of enzyme activities (Churchill and Livingstone, 1989) well as intermediates (Ebberink and de Zwaan, 1980). During the environmental anoxia, six end products of pyruvate metabolism have been identified in molluscs. The lactate is one of the six end products of anaerobic pyruvate metabolism in marine bivalves. Futhermore, marine bivalves accumulate a variety of organic acids in addition to small amounts of CO_2, opines and lactate during environmental anoxia (Sukhotin and Portner, 1999). The end product lactate is formed in the cytoplasm. The observed changes in lactic acid (Table 6-8) and pyruvic acid (Table 9-11) content in tissues of experimental *Gafrarium divaricatum* suggest the disturbed carbohydrate metabolism. The upward trend of lactic acid with respect to pyruvic acid in the tissues of experimental clams also suggested severe respiratory stress leading to anaerobic metabolism. Similar trend of lactic acid and pyruvic acid in tissues of organisms including clams exposed to different pollutants has been recorded (Ferrando and Andreau-Moliner, 1991; Roy, 1994; Reddy and Bhagyalakshmi, 1994).

Proteins form one of the major fuels in marine clams. The marked depletion in protein content

(Table 12-14) in tissues of *Gafrarium divaricatum* exposed to xylene, benzene and gear oil WSF is symptomatic to severe stress caused by these pollutants which resulted in increased proteolysis. Such depletion in protein content in the tissues of the clam, *Macoma balthica* exposed to oil has been reported (Stekoll *et al*., 1980). Furthermore, it is possible that proteins and amino acids from tissues are mobilized under stressful condition of pollutants and released into circulatory system. Eapen and Patel (1989) observed increased protein levels in serum of clam, *Anadara granosa* exposed to naphthalene. Similarly, Kulkarni (1983) has reported elevation of protein levels in the serum of the crab, *Scylla serrata* exposed to naphthalene. The observed increase in protein of the tissues of experimental *Gafrarium divaricatum* after 96 hours of exposure in the present investigation also suggested the mobilization of proteins which might have released into the blood of clam during chronic exposure period.

References

Agwuocha, S., Kulkarni, B.G. and Pandey, A.K. (2009) Histopathological changes in gill of intertidal clam, *Gafrarium divaricatum* (Gmelin) exposed to xylene, benzene and gear oil-WSF. *J. Ecophysiol. Occuop.Hlth*., **9**: 37-42.

Akberali, H.B. and Truman, R.R. (1985) Effect of environmental stress on marine bivalve molluscs. In: *Advances in Marine Biology* (J.H.S. Blaxter Y. Russell and M. Younge, eds.). **22**, 102-183.

Al-Akel, A.S., Shamsi, M.J.K., Al-Katim, H.F., Chowdhary, M.A. and Ahmed, Z. (1988) Effect of cadmium on cichlid fish, *Oreochromis niloticus*: behaviour and physiological responses. *J. Univ. Kuwait* (*Sci*.), **15**, 341-346.

A.O.A.C. (1975) Official Method of Analysis of the Association of Official Analytical Chemists. (William Hovwitz, ed). 12th Edition.

Augenfield, J.M., Anderson, J.W., Woodruff, D.L. and Webester, H.J.L. (1980) Effects of Prudhoe Bay crude oil contaminated sediments on *Protothaca staminea* (Mollusca: Pelecypoda). *Mar. Environ. Res*., **4**, 135-143.

Awad H. (1987) Aromatic hydrocarbons in some organisms of coastal Red Sea. *Indian J. Mar. Sci*., **16**, 114-116.

Axiak, V. and George, J.J. (1987) Bioenergetic responses of the marine bivalve, *Venus verrucosa* on long-term exposure to petroleum hydrocarbons. *Mar. Environ. Res*., **23**, 33-47.

Axiak, V., George, J.J. and Moore, M.N. (1988) Petroleum hydrocarbons in the marine bivalve, *Venus verrucosa:* accumulation and cellular responses. *Mar. Biol.,* **97**, 225-230.

Badman, B.D. and Chin, S.N. (1973) Metabolic responses of the freshwater bivalve, *Pleurobema coccineum* (Conard) to anaerobic conditions. *Comp. Biochem. Physiol*., **44B**, 27-32.

Barker, S.B. and Summerson, W.H. (1941) Colorimetric determination of lactic acid in biological materials. *J. Biol. Chem*., **138**, 535-554.

Bayne, B.L., Widdows, J., Moore, M.N., Salkeld, P. Worrall, C.M. and Donkin, P. (1982) Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. *Phil. Trans. R. Soc. (Lond.)*, **297B**, 219-239.

Bennet, R. and Nakada, H.I. (1968) Comparative carbohydrate metabolism of marine molluscs. *Bull. Comp. Biochem. Physiol*., **24**, 787-797.

Biswas, R. (1986) Toxic effects of an organochlorine insecticide, thiodon, on marine crab, *Scylla serrata (*Forskal). *Ph.D.* Thesis, University of Bombay, Bombay.

Chaykin, S. (1966) *Biochemistry: Laboratory Techniques*. Wiley Eastern Pvt. Ltd., New Delhi. 196 p.

Churchill and Livingstone (1989) As cited in Cellular Biochemistry and Endocrinology by DeZwaan, A. and Mathieu, M. (1992). In: *The Mussel, Mytillus: Ecology, Physiology, Genetics and Culture: Development in Aquaculture and Fisheries Science* (E. Gosling, ed). Elsevier Publishers, Netherlands.

Clement, L.E., Stekoll, M.S. and Shaw, D.G. (1980) Accumulation, fractionation and release of oil by the intertidal clam, *Macoma balthica. Mar. Biol*., **57**, 41-50.

Dange, A.D. and Masurekar, V.B. (1982) Naphthalene induced changes in carbohydrate metabolism in *Sarotherodon mossambicus* Peters (Pisces: Cichlidae). *Hydrobiologia*, **94**, 163-177.

Deshmukh, N. (1983) Some aspects of toxicity of petroleum hydrocarbons to the speckled prawn, *Metapeneus monoceros* (Fabricus). *Ph.D. Thesis*, University of Bombay, Bombay.

Deshmukh, R.S. (1972) Some aspects of biology of *Meretrix meritrix*. *Ph.D. Thesis*, Marathwada University, Aurangabad.

de Zwaan, A. and Zandee (1971) The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* (L.). *Comp. Biochem. Physiol*., **93B**, 47-54.

Dunning, A. and Major, C.N. (1974) The effect of cold seawater extracts of oil fractions upon the blue mussel, *Mytilus edulis*. In: *Pollution and Physiology of Marine Organisms* (F.J. Vernberg and W.B. Vernberg, eds.). Academic Press, New York. 349-366.

Durairaj, S. and Selvarajan, V.R. (1992) Influence of Quinolphos, an organophosphorus pesticide, on biochemical constituents of the tissues of fish, *Oreochromis mossambicus*. *J. Environ. Biol.*, **13**, 181-185.

Eapen, J.T. (1987) Physiological and biochemical responses of an arcid clam, *Anadara granosa* exposed to naphthalene. *Ph.D. Thesis.* University of Bombay, Bombay.

Eapen, J.T. and Patel, B (1989) Haematological evaluation of naphthalene intoxication in the tropical arcid blood clam, *Anadara granosa. Mar. Biol*.,**100**, 223-226.

Ebberink and de Zwaan (1980). As cited in Cellular Biochemistry

and Endocrinology by DeZwaan and Mathieu (1992). In: Development in Aquaculture and Fisheries Science (E. Gossling, ed). Elsevier Publishers, Netherlands. 25

Engel, H.R. and Neat, M.J.C. (1970) Glycolytic and gluconeogenic enzymes in the Quahog, *Mercenaria mercenaria*. *Bull. Comp. Biochem . Physiol*., **37**, 397-403.

Ferrando, M.D. and Andreu-Moliner, E. (1991) Changes in selected biochemical parameters in the brain of fish, *Anguilla anguilla* (L.) exposed to Lindane. *Bull. Environ. Cotam. Toxicol*., **47**, 459-464.

Friedmann, T.K. and Hawgen, G.E. (1943) Pyruvic acid. II. The determination of ketoacids in blood and urine. *J. Biol. Chem*., **47**, 415-442.

Gosling, E. (1992) *The Mussel, Mytilus: Ecology, Physiology, Genetic and Culture. Development in Aquaculture and Fisheries Science*. Elsevier Pub., Amstardam.

Khan, R.A. and Nag, K. (1993) Estimation of hemosiderosis in sea birds and fish exposed to petroleum. *Bull. Environ. Contam. Toxicol*., **50**, 125-131.

Kostylev, Eh.F. (1988) Changes in metabolic rates in bivalves under conditions of water pollution with detergents and oil products. In: *Aquatic Toxicology and Optimization of Bio-production Management Processes in Aquaculture* (S.A. Patin, ed.), Moscow. 83-93.

Krishna, P., Ventatarani, L., Ravi, C. and Indira, K. (1987) Metabolic consequences of methylparation exposure in the bivalve, *Lamellidens marginalis. Bull. Environ. Contam. Toxiciol*., **38**, 509-514 .

Kulkarni. B.G. (1983) Toxicity studies: Effects of some petroleum hydrocarbons on some marine animals. *Ph.D. Thesis*. University of Bombay, Bombay.

Kulkarni. B.G. (1990) Effect of copper exposure on tissue enzyme activities in the intertidal clam, *Gafrarium divaricatum* (Gmelin). *Nat. Acad. Sci. Letters*, **13**, 4-6.

Kulkarni, B.G. (1990) ATPase activity in the gills and hepatopancreas of the intertidal clam, *Meretrix casta* var. *ovum* (Hanley) expose to naphthalene. *J. Environ. Biol*.,**11**, 275-278 .

Kulkarni, B.G. and Kulkarni, R.G. (1987) The effect of naphthalene exposure on tissue aminotranferase activities in the clam, *Meretrix casta* var. *ovum* (Hanley). *Poll. Res*., **7**, 9-12.

Lin-Qin and Xin-Xiaoping (1991). Petroleum hydrocarbons in marine organisms from Honghai Bay, north-eastern South China. *Seq. Mar. Sci. Bull.* (Haiyang Tongbao), **10**, 33-38.

Lin-Qin, Xin-Xiaoping and Lu-Xiaoyu (1990). Petroleum hydrocarbons in marine animals from the Pearl river estuary. *Mar. Sci.* (Haiyang Kexue) No. **3**, 34-38.

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

Mane, U.H. (1974) Adaptations of the estuarine clam, *Katelysia opima* to the salinity fluctuations. *Rev. Biol*., **6**, 73-107.

Mane, U.H. (1975) Study on the rate of water transport of the clam, *Katelysia opima* in relation to environmental conditions. *Hydrobiologia*, **47**, 439-451.

Mane, U.H. and Nagabhushanam, R (1975) Body distribution and seasonal changes in the biochemical composition of the estuarine mussel, *Mytilus viridis* at Ratnagiri. *Riv. Hydrobiol*., **14**, 163-175.

Mane, U.H., Akarte, S.R. and Kulkarni, D.A. (1986) Acute toxicity of fenthion to freshwater lamellibranch mollusc, *Indonaia caeruleus* (Prasad, 1918) from Godavari river at Paithan. A biochemical approach. *Bull. Environ. Contam. Toxicol*., **37**, 622-628.

Moles, A., Babcock, M.M. and Rice, S.D. (1987) Effects of oil exposure on pink salmon, *Oncorhynchus gorbuscha* alevins in a simulated intertidal environment. *Mar. Environ. Res*., **21**, 49-58.

Nagabhushanam, R. and Mane, U.H. (1978) Seasonal variation in the biochemical composition of *Mytilus viridis* at Ratnagiri on the west coast of India. *Hydrociologia*, **571**, 69-72.

Neff, J.M., Hillman, R.E., Carr, R.S., Buhl, R.L. and Laney, J.I. (1987) Histopathological and biochemical responses in arctic marine bivalve molluscs exposed experimentally to spilled oil. *Arctic* (Suppl. **1**), 220-229.

Patel, B. and Eapen, J.T. (1989) Biochemical evaluation of naphthalene intoxication in tropical arcid clam, *Anadara granosa . Mar. Biol.,* **103**, 203-209.

Readman, J.W., Fowler, S.W., Villeneuve, J.P., Cattini, C., Oregioni, B. and Mee, L.D. (1992) Oil and combustion product contamination of the Golf marne environment following thw war. *Nature*, **358**, 662-665,

Reddy, P.S. and Bhagyalakshmi, A. (1994) Changes in oxidative metabolism in selected tissues of crab, *Scylla serrata* in response to cadmium toxicity. *Ecotoxicol. Environ. Safety*, **29**, 255-264.

Rodriguez, G.A. (1981) Osmoregulation and total serum protein in two species of Penaidean shrimps from Pacific coast of Mexico. *J. Crust. Biol*., **1**, 392-400.

Roy, S.S. (1994) Effects of copper on ecophysiological aspects of the clam, *Gafrarium divaricatum. M.Sc. Thesis*, University of Bombay, Bombay.

Sabo, D.J. and Stegeman, J.J. (1977) Some metabolic effects of petroleum hydrocarbons in marine fish. In: *Physiological Responses of Marine Biota to Pollutants* (F.J. Vernberg, Calabrese, A., Thurberg, F.P. and Vernberg, V.B., eds.). Academic Press, New York. 279-287.

Schoffeniels, E. (1976) Biochemical approaches to osmoregulatory processes in Crustacea. In: *Perspectives in Experimental Biology. Vol. 1.* (P.S. Davis, ed.). Pergaman Press, Oxford. 107-124.

Seifter, S., Dayton, S., Noovic, B. and Muntwyler, I. (1950) Estimation of glycogen with anthrone reagent. *Arch. Biochem*., **25**, 191-200.

Shankara, C.R., Babu, N.S.A., Indira, K. and Rajendra, W. (1989) Metabolic response to methyl parathion toxicity in bivalve, *Lamellidens marginalis*. *Curr. Sci*., **58**, 767.

Shreedevi, P., Sivaramakrishna, B., Suresh, A. and Ramakrishnaiah, K. (1992) Effect of nickel on some aspects of protein metabolism in the gill and kidney of the frseshwater fish, *Cyprinus carpio* L. *Environ. Pollut.,* **77,** 59-63.

Stekoll, M.S., Clement, L.S. and Shaw, D.G. (1980) Sublethal effects of chronic oil exposure on the intertidal clam, *Macoma bolthica*. *Mar. Biol*., **57**, 51-60.

Sukhotin, A.A. and Portner, H.-O. (1999) Habitat as a factor involved in the physiological response to environmental

anaerobiosis of White Sea *Mytilus edulis*. *Mar. Ecol. Progr. Ser.*, **184**, 149-160.

Theede, (1985) Some problems of pollution with heavy metals under the environmental conditions of the Baltic Sea. *Symposium Biol. Hung*., **29**, 223-236.

Usuki, I. and Okamura, N. (1986) Glycolytic in oyster gill. *Sci. Rep. Tohuku Univ*. Fourth Ser., **22**, 225-232.

Venkatrama, P. and Radhakrishnaiah, K. (1987) Lethal and sublethal effects of copper on the protein metabolism of freshwater teleost, *Labeo rohita. Trends in Life Sci*., **2** (2), 82-86.

Vernberg, W.B. (1979) Marine Pollution: Functional Responses. Academic Press, New York & London.

Widdows, J., Bayne, B.L., Livingstone, D .R., Newell, R.I.E. and Donkinn, P. (1979). Physiological and biochemical responses of bivalve molluscs to exposure to air. *Comp. Biochem. Physiol.*, **62A**, 301-308.