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OIL EFFLUENT EXPOSURE INDUCED BIOCHEMICAL MODULATIONS IN SUBCELLULAR / TISSUES (CYTOSOL,MICROSOMES) OF FOOT, GILL AND DIGESTIVE GLANDS OF *LAMELLIDENS MARGINALIS.*

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ABSTRACT

Sub-cellular fractions (cytosolic and microsomal) were prepared from the tissues (foot, gill and digestive gland) of freshwater mussel *Lamellidens marginalis* and were scrutinized to investigate the modulation of biochemical components (protein, carbohydrate and lipids) after exposure of sub-lethal concentrations of 1/4th (11.88 ppt) and 1/10th (8.55 ppt) of 96-hr LC 50 of oil effluent, and recovery period (depuration-without oil effluent) after seven days interval i.e., 1st, 8th, 15th 22nd and 30th days. At the end of 30th day, biochemical components in both exposure and depuration periods, were analysed from the tissues of mussels. The accumulation of oil effluent in the tissues gradually increased, when exposed to both sub-lethal concentrations of oil effluent and significantly and gradually decreased levels of protein, carbohydrate and lipid contents in the sub-cellular tissues of mussels were observed. During recovery period (without adding oil effluent), all depleted biochemical contents were recovered, increased, gradually, significantly from 30th day to Ist day ($P<0.05$) and one-way ANOVA showed progressive positive regression coefficient values ($P<0.05$) in all days. The enhanced and recovered levels of biochemical components in oil-exposed mussels demonstrate a well-established defense mechanism in mollusc, and this response offers the possibility of use as a biomarker for the early detection of oil pollution.

Keywords: Biochemical modulations, protein, carbohydrates, lipids, cytosol, microsomes *Lamellidens marginalis,* oil effluent exposure.

INTRODUCTION

Aquatic pollution by various industrial effluents and anthropogenic activities are considered as a serious problem to aquatic inhabitants and affect the normal life of animals. Polycyclic aromatic hydrocarbons (PAHs), and Polychlorinated biphenyl (PCBs) are recognized that to assess the impact of environmental, molecular, cellular disturbance in animals and as well as ecosystem level (Moore *et al.* 2000). Environmental pollution by polycyclic aromatic hydrocarbon is increasingly posing a grave threat to man's resources since oil-derived hydrocarbons may affect on aquatic organisms (Fernadez-Casalderrey *et al*., 1995). Bivalve mussel performs an indispensable role in the transformation of the energy of food chains coupled with their sessile mode of life, and which can be important as suspension-feeder, influencing water clarity, chemistry, and organization, the functioning of ecosystems. Accumulation and elimination of xenobiotics in the tissues of aquatic organisms occur from the sediment, contaminated water column, and food chain that cause deleterious effects (Balamurugan and Subramanian, 2003). Cellular reactions to chemical pollutants can provide early-warning distress signals of injurious change in animals (Moore *et al*., 1994, 1999; Balamurugan and Subramanian, 2003). Though numerous studies have been carried out on the effect of different industrial effluents on fishes (Haniffa and Sundravathanam 1983). Everarts *et al*. (1994) a load of anthropogenic contaminants induce detrimental environmental conditions, affecting the biological integrity of ecosystems as well as physiological functions of individual organisms. Through the understanding of the basic mechanisms of toxic action and biochemical effects of environmental pollutants are of crucial importance to determine their effects at a high level of biological hierarchy (Fent 1996). Laboratory studies hitherto performed have revealed that exposure to lethal or, as more frequently occurs, the sublethal concentration of toxicants results in adverse alterations in various biochemical and physiological functions in aquatic organisms (Kumar *et al*., 1998).

Biochemical indices are often very sensitive to stressors and the magnitude of the biochemical changes is often related to the severity of the toxicants (Livingston, 1985). The biochemical components like protein, carbohydrate, and lipid that play a significant role in body construction, energy production, and storage are also affected by aquatic pollution (Palanichamy *et al*., 1989). Marine bivalve mollusks, in particular, have been the subject of many investigations on the relations among body size, metabolic rate, protein

metabolism, fecundity, and resistance to environmental stress (Scott and Koehn, 1990). Fernandez-Reiriz *et al.* (1996) have been studying the environmental influence on the biochemical composition (protein, carbohydrate, glycogen, lipid classes and fatty acids of total lipids) in two zones in the Ria Sada (Spain) on *Mytilus galloprovincialis* (Lmk). Krishnamoorthy (1993) have studied the effect of sublethal doses of copper on the biochemistry (protein, carbohydrate, and lipid) of the prawn *Macrobrachium lamerrei lamarrei* during accumulation and depuration period.

Proteins play a vital role in biology, physiology of living organisms. Protein is one of the most important and complete groups of biological materials comprising the nitrogenous constituent of the body and performing different biological functions. If any alteration takes place in the protein turn over it may have an adverse influence on complex groups of biological materials, comprising the nitrogenous constituents of the body and the food intake, and thus performing different biological functions. Proteins are involved in major physiological events to maintain the homeostasis of the cell. Therefore, the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of the cell. Tissue proteins of aquatic animals under toxic stress are known to play a pivotal role in the activation of compensatory mechanisms (Venkataramana and Radhakrishnaiah, 1987). Proteins are highly sensitive to heavy metals and are one of the earliest indicators of heavy metals poisoning (Jacobs *et al*., 1977). Proteins that are responsible for the transport, accumulation and detoxification of cadmium have been studied in major cadmium accumulation organs such as the liver and kidney of different fish species (George and Olsson, 1994). Ramaswamy (1987) has emphasized that proteins play an important role in the maintenance of blood glucose and major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants, which enter the animal's body.

Carbohydrate is an important energy source of all vital activities of an organism and supplies a major portion of energy to the living system. Ramakrishnan *et al*. (1980) have stated that in addition to energy yield, glycogen may be converted into glucose, fat, and amino acid. Disturbance in carbohydrate metabolism is the most outstanding biochemical lesion arising from the action of toxic compounds. Glucose is one of the most essential fractions of carbohydrates and it serves as an immediate and major metabolic fuel. Alterations in biochemical components like protein and carbohydrates are responsible for environmental stress. The heavy metals are also known to elicit changes in the biochemical constituents of fish thereby altering the metabolic pathway (Sarkar and Medda, 1993). Baskaran *et al*. (1989) have observed a decrease in carbohydrate reserves in muscle and liver of *Oreochromis mossambicus* when exposed to textile mill effluent. Changes in carbohydrate metabolism and skeletal deformations may be related to exposure to heavy metals and organic pollutants (Andersson *et al*., 1988). Contaminant accumulation in bivalves is regulated by several physiological parameters such as age, size, and lipid content (Stegeman and Teal, 1973).

Lipid levels in bivalves vary throughout seasonally. Because lipid reserves are the primary site of non-polar hydrophobic xenobiotic storage and are an important factor for determining hydrophobic compound accumulation. Napolitund *et al*. (1992) studied the lipid composition of egg and adductor muscle in giant scallops *P. magellanicus*. Many researchers from India devoted to study on the effect of various pollutants on freshwater bivalves in relation to biochemical, histology, reproduction, physiology etc. Since the information on the tissues and subcellular distribution of biochemical modulations in mussels is sparse, to fill up the gap, a preliminary study has been carried out on the freshwater mussel, *Lamellidens marginalis*.

MATERIALS AND METHODS

Sample Collection

Almost uniform size of freshwater mussel *Lamellidens marginalis* (total length 6-7cm and weight 25-27 g) were collected from the River Cauvery Tiruchirappalli, India, and maintained in the laboratory.

Acute toxicity experimental design

The aqueous oil effluent originated from the coal conversion plant, turbine section of boiler units in the Boiler plants of Bharat Heavy Electricals Limited (BHEL) situated 14 km away from Tiruchirappalli, are collectively released into a drainage canal. It consisted mainly of hydrocarbons. Initial experiments were conducted to assess the minimum concentration of oil effluent to obtain maximum mortality, for freshwater mussel, *Lamellidens marginalis*, over a 96-hr exposure. After confirming the minimum concentration, 10 animals in 5L of tubs (each) and exposed to various concentrations of oil effluent, ranging from 4ppt to 16ppt for a period of 96-hr to ascertain LC_{50} concentration. In addition, a control was also maintained. The 96-hr LC_{50} values with 95% confidence limits were calculated using National Crop Production Centre Technical Bulletin (1986).

Exposure experiment

Based on the 96-hr LC_{50} value of oil effluent, sublethal concentrations of $1/4^{th}$ (11.88ppt) and 1/10th (8.55 ppt) of LC_{50} were prepared and used for biochemical study. In this study, two sets of 10^{-1} plastic tubs were used. In each tub, mussels were exposed to 11.88 ppt or 8.55 ppt of oil effluent. A control was also run simultaneously without the addition of oil effluent. At seven days interval, mussels were sacrificed for biochemical analysis. After 30 days, the treated mussels were released into freshwater 30-day depuration (recovery) study was conducted. Four mussels were randomly chosen and removed from each of the two tubs (n=8) for dissection.

Preparation of Sub-cellular fractions

Sub-cellular fractions (Cytosol and microsomes) were prepared from gill, foot, and digestive gland tissues of freshwater mussels by the procedure of (Livingstone and Farrar. 1984). All the preparation procedure was carried out at 4ºC. Pooled tissues were homogenized in 20 mM Tris–HCl (pH 7.6) (1 g/4 ml) containing 0.25 M sucrose; 0.15 M KCl; 1 mM EDTA and 1 mM DTT and 100 lM PMSF. All the preparation procedures were carried out at 4°C. The homogenization samples were centrifuged at first on 600g for 10 min to sediment nuclei and cell fragments. Then, without transferring these fragments, samples were re-centrifuged at 12,000g for 45 min. Consequently, the supernatant was collected and used as a mitochondrial fraction. The resultant pellet was re-suspended in the homogenizing buffer and re-centrifuged at 100,000g for 90 min, then, the supernatant was

collected and used as a cytosolic fraction. Resuspension buffer consisting of 20 mM Tris pH 7.6, 1 mM Dithiothreitol, 1 mM EDTA, and 20% v/v glycerol were added into the remaining pellet. This resuspended pellet was used as a microsomal fraction.

Biochemical Analysis

The carbohydrate, protein and lipid estimations were carried out by following the methods of Dubois *et al.* (1956), Lowry *et al.* (1951), and Folch *et al.* (1957) respectively

Statistical Analysis

Results concerning biochemical parameters (protein, carbohydrate, and lipid) are presented as mean ± SEM. Each value is the mean of three determinations significant differences between groups of values were established at the P=.05 level using one-way ANOVA followed by Dun can's test (Duncan Multiple Range Test-DMRT, Zar, 1984). Regression analysis was used and P<0.05 was accepted as significant.

RESULTS

Total Protein

The control mussels protein content level for 1/4th and 1/10th exposure concentrations and depuration study are presented in Table-1 A and B. Accumulation of oil effluent, in the cytosol, microsomes of foot, gill, and digestive gland of mussels exposed to 1/4th and 1/10th concentrations, protein level decreased gradually, and attained maximum value at 30 day (Table 1A) .Figure 1A,B,C, and D).Also protein content decreased on day 30 were many fold higher when compared to Day 1 (Figure 1A,B,C, and D). In both concentration of exposure period, when compare to subcellualr fractions, microsomal fractions are quickly decreased than the cytosol. During depuration phase, the protein content levels gradually increased from a Day 30 and attained a maximum value of 3.265±5.11 E-03 cytosol, 3.326±5.56 E-03 microsomes of foot; 3.235±4.24 E-03 cytosol, 3.311±5.74 E-03 microsomes of gill; 3.746±5.60 E-03 cytosol, 3.530±5.80 E-03 microsomes of 1/4th; 3.316±5.95 E-03 cytosol, 3.413±5.61 E-03 microsomes of foot, 3.339±4.01 E-03 cytosol, 3.413±6.06 E-03 micrsosomes of gill, 3.791±8.12 E-03 cytosol, 3.644±4.49 E-03 microsomes of digestive gland of 1/10th.

Table 1A: Protein content alterations in the sub-cellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10thexposure period of oil effluent.

Conc./ Tissues	Subcellular fractions	Accumulation Period Days					
		Control	1	8	15	22	30
1/4th Conc. oot	Cytosol	4.464±1.14E-02	3.093±9.28E-03	2.929±7.68E-03	2.347±6.18E-03	2.032±7.08E-03	1.667±7.66E-03
	Microsomes	4.372±1.82E-02	3.122±4.93E-03	2.900±3.84E-03	2.381±1.10E-02	2.034±7.31E-03	1.415±6.26E-03
Gill	Cytosol	4.479±1.44E-02	3.081±9.52E-03	2.923±6.04E-03	2.511±6.81E-03	2.073±4.69E-03	1.442±4.89E-03
	Microsomes	4.417±3.43E-02	3.125±6.71E-03	2.905±5.74E-03	2.460±5.16E-03	2.018±4.09E-03	1.126±5.77E-03
Digestive gland	Cytosol	4.419±2.04E-02	3.167±1.15E-02	2.947±3.23E-03	2.515±6.87E-03	2.511±6.61E-03	1.929±4.69E-03
	Microsomes	4.334±8.49E-03	3.158±1.26E-02	2.917±8.67E-03	2.464±4.78E-03	2.460±5.16E-03	1.236±4.69E-03
1/10th Conc. Foot	Cytosol	4.464±1.14E-02	3.475±4.89E-03	2.849±1.11E-02	2.460±5.16E-03	2.029±6.36E-03	1.946±6.16E-03
	Microsomes	4.372±1.82E-02	3.466±5.33E-03	2.835±5.11E-03	2.343±4.39E-03	2.034±7.31E-03	1.740±6.25E-03
Gill	Cytosol	4.479±1.44E-02	3.533±5.06E-03	2.934±4.24E-03	2.627±3.62E-03	2.073±4.69E-03	1.928±4.69E-03
	Microsomes	4.417±3.43E-02	3.500±4.49 E-03	2.901±5.53E-03	2.511±6.61E-03	2.018±4.09E-03	1.564±5.11E-03
Digestive gland	Cytosol	4.436±1.05E-02	3.592±6.80E-03	2.968±4.09E-03	2.650±1.03E-02	2.093±5.74E-03	1.964±1.03E-03
	Microsomes	4.334±8.49E-03	3.567±5.53E-03	2.931±4.69E-03	2.387±4.44E-02	2.081±7.26E-03	1.742±4.07E-03

* Protein values (mg/g) are mean ± SEM of five determinations

Table 1.B : Protein content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10th depuration period.

Conc./ Tissues	Subcellular fractions	Depuration Period Days					
		Control	1	8	15	22	30
1/4th Conc. Foot	Cytosol	4.464±1.14E-02	2.252±4.28E-03	2..332±574E-03	2.553±7.51E-02	2.623±1.25E-03	3.265±5.11E-03
	Microsomes	4.372±1.82E-02	1.172±6.60E-03	1.466±7.97E-03	1.485±4.69E-03	2.588±4.53E-03	3.326±5.56E-03
Gill	*Cytosol*	4.479±1.44E-02	1.563±6.32E-03	2.029±6.36E-03	2.034±9.27E-03	2.281±7.45E-03	3.235±4.24E-03
	Microsomes	4.417±3.43E-02	1.146±5.80E-03	1.410±6.65E-03	1.466±5.33E-03	2.608±4.55E-03	3.311±5.74E-03
Digestive gland	Cytosol	4.436±1.05E-02	2.274±4.24E-03	2.597±5.22E-03	2.650±1.03E-02	3.738±4.31E-03	3.746±5.60E-03
	Microsomes	4.334±8.49E-03	1.295±8.59E-03	1.761±8.80E-03	1.792±8.09E-03	3.501±4.69E-03	3.530±5.80E-03
1/10th Conc. *Foot*	Cytosol	4.464±1.14E-02	2.871±3.63E-02	2.932±5.74E-03	2.952±6.60E-03	3.052±4.49E-03	3.316±5.95E-03
	Microsomes	4.372±1.82E-02	1.858±5.32E-03	1.949±5.77E-03	2.269±5.58E-03	3.335±5.74E-03	3.413±5.61E-03
Gill	Cytosol	4.479±1.44E-02	2.332±5.74E-03	2.373±3.06E-03	2.573±7.90E-03	3.255±7.37E-03	3.339±4.01E-03
	Microsomes	4.417±3.43E-02	1.617±4.69E-03	1.822±8.91E-03	2.239±7.51E-03	3.329±5.16E-03	3.413±6.06E-03
Digestive gland	Cytosol	4.436±1.05E-02	2.841±7.06E-03	2.969±5.56E-03	3.006±4.82E-03	3.667±5.16E-03	3.791±8.12E-03
	Microsomes	4.334±8.49E-03	1.904±6.03E-03	2.073±4.69E-03	2.340±9.15E-03	3.551±5.95E-03	3.644±4.49E-03

* Protein values (mg/g) are mean ± SEM of five determinations

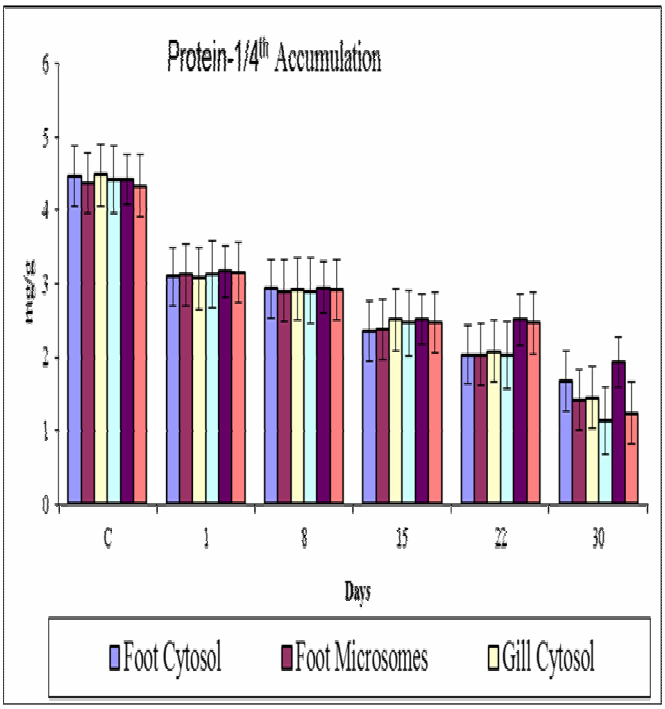

Fig. 1.A : Protein content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4thexposure period of oil effluent.

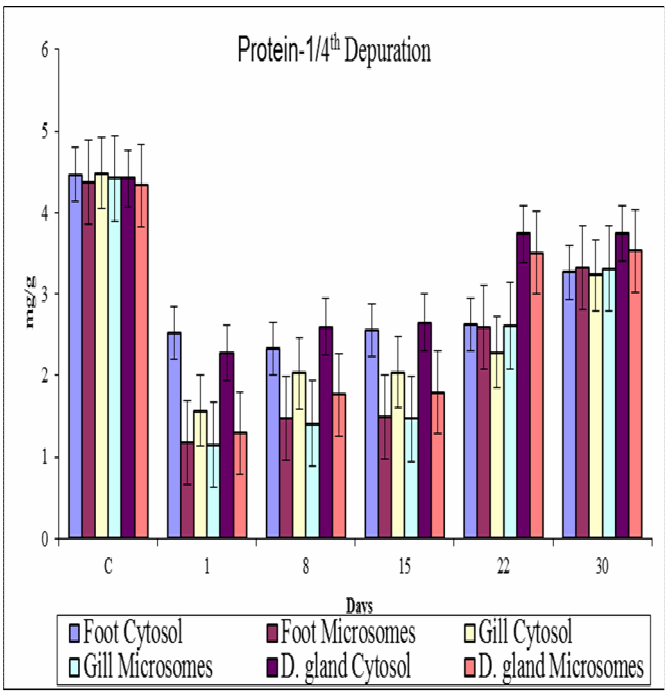

Fig. 1.C : Protein content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th depuration period.

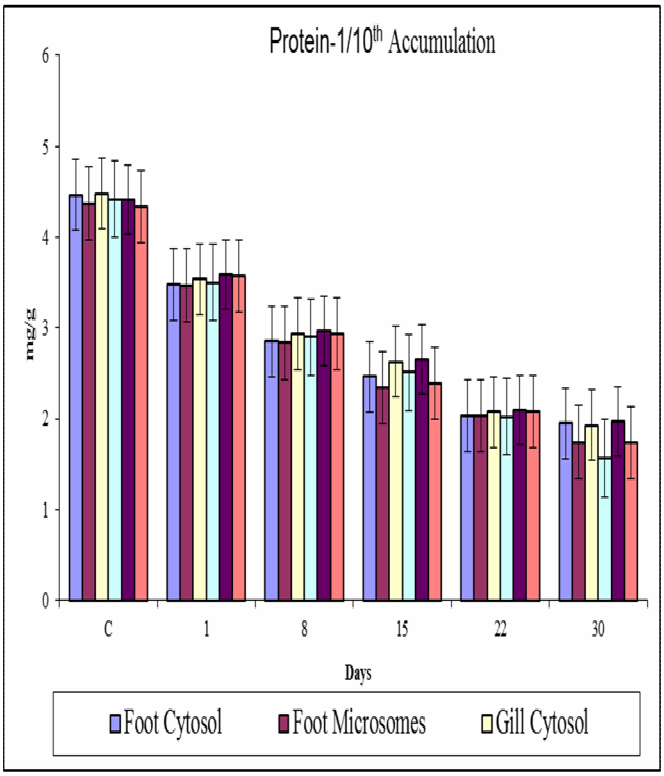

Fig. 1.B : Protein content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/10thexposure period of oil effluent.

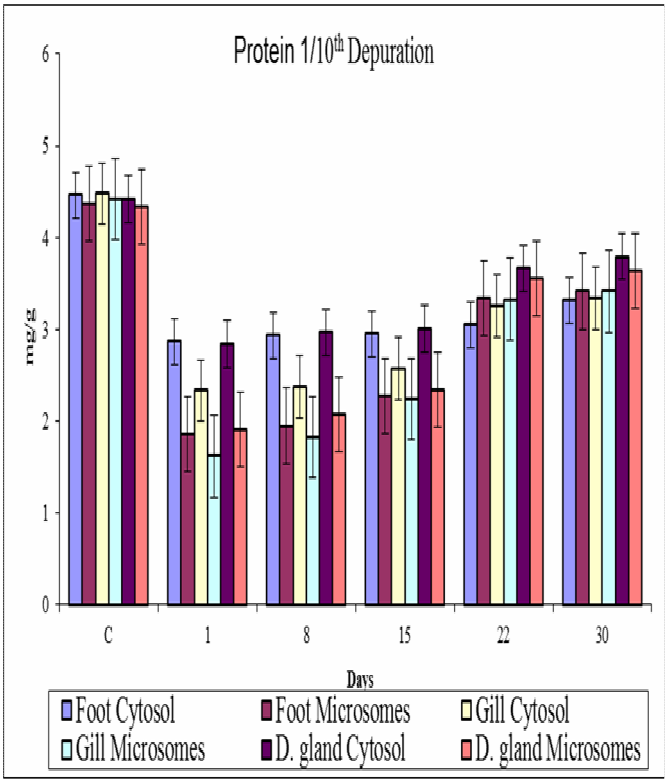

Fig. 1.D : Protein content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/10th depuration period.

Total Carbohydrates

The control mussels carbohydrate content level for 1/4th and 1/10th exposure concentrations and depuration study are presented in Table-2 A and B. Compared to control, the content of carbohydrate decreased by gradually from a Day 1 to a Day 30. Many fold higher decreasing level attained at a day 30, when compared to Day 1 in all exposure periods and tissues. During depuration period, the content of carbohydrates increased and reached 60 -80 % level on the Day 30 (Figure 2 A,B,C and D).

Table 2.A: Carbohydrate content alterations in the sub-cellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10thexposure period of oil effluent.

Conc./ Tissues	Subcellular fractions	Accumulation Period Days					
		Control	1	8	15	22	30
1/4th Conc. Foot	Cytosol	1.591±1.44E-03	1.020±1.36E-03	0.995±2.63E-03	0.880±1.56E-03	0.755±2.60E-03	0.666±2.15E-03
	Microsomes	1.669±1.36E-03	1.089±2.27E-03	1.020±1.36E-03	0.912±1.08E-03	0.772±1.36E-03	0.652±1.63E-03
Gill	Cytosol	1.550±2.48E-03	0.998±1.85E-03	1.190±0.20E-03	0.963±1.58E-03	0.772±1.56E-03	0.612±2.56E-03
	Microsomes	1.810±1.21E-03	1.007±1.92E-03	0.996±1.03E-03	0.906±2.25E-03	0.751±2.16E-03	0.565±1.92E-03
Digestive gland	Cytosol	1.974±1.41E-03	1.092±3.11E-03	1.079±1.44E-03	0.995±1.08E-03	0.963±1.58E-03	0.613±2.32E-03
	Microsomes	1.934±2.06E-03	1.101±1.57E-03	1.068±2.06E-03	0.993±1.98E-03	0.912±1.08E-03	0.659±2.20E-03
1/10th Conc. Foot	Cytosol	1.591±1.44E-03	1.040±2.20E-03	0.997±2.23E-03	0.883±3.20E-03	0.896±2.80E-03	0.771±1.77E-03
	Microsomes	1.669±1.36E-03	1.097±2.77E-03	1.015±2.96E-03	0.991±1.78E-03	0.869±1.28E-03	0.796±3.03E-03
Gill	*Cytosol*	1.550±2.48E-03	1.001±1.69E-03	0.993±1.36E-03	0.936±1.44E-03	0.835±2.20E-03	0.772±1.56E-03
	Microsomes	1.810±1.21E-03	1.022±9.61E-03	0.996±1.08E-03	0.959±1.70E-03	0.870±1.63E-03	0.753±1.76E-03
Digestive gland	Cytosol	1.974±1.41E-03	1.112±1.63E-03	1.079±1.44E-03	0.980±1.21E-03	0.931±1.85E-03	0.663±3.20E-03
	Microsomes	1.934±2.06E-03	1.118±2.14E-03	1.068±2.06E-03	0.997±2.23E-03	0.959±1.10E-03	0.659±2.20E-03

* Carbohydrate values (mg/g) are mean ± SEM of five determinations

Table 2.B: Carbohydrate content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10thdepuration period.

Conc./ Tissues	Subcellular fractions	Depuration Period Days					
		Control	1	8	15	22	30
1/4th Conc. Foot	Cytosol	1.591±1.44E-03	0.849±1.98E-03	0.948±2.50E-03	0.968±2.12E-03	1.104±1.98E-03	1.135±1.41E-03
	Microsomes	1.669±1.36E-03	1.045±2.42E-03	1.062±2.48E-03	1.080±2.06E-03	1.170±1.50E-03	1.214±1.43E-03
Gill	Cytosol	1.550±2.48E-03	0.898±1.78E-03	0.968±2.43E-03	0.977±2.34E-03	1.101±1.43E-03	1.124±1.21E-03
	Microsomes	1.810±1.21E-03	1.017±2.94E-03	1.034±2.63E-03	1.063±2.28E-03	1.165±1.0E-038	1.206±1.93E-03
Digestive gland	Cytosol	1.974±1.41E-03	0.862±2.98E-03	0.964±1.91E-03	0.975±2.14E-03	1.134±1.41E-03	1.175±1.73E-03
	Microsomes	1.934±2.06E-03	1.065±2.35E-03	1.072±2.34E-03	1.092±3.11E-03	1.208±2.22E-03	1.231±1.12E-02
1/10th Conc. Foot	Cytosol	1.591±1.44E-03	0.906±2.35E-03	0.974±1.77E-03	0.990±8.60E-04	1.125±1.77E-03	1.156±1.24E-03
	Microsomes	1.669±1.36E-03	0.993±1.98E-03	1.079±1.44E-03	1.103±1.21E-03	1.206±1.41E-03	1.252±2.06E-02
Gill	Cytosol	1.550±2.48E-03	0.970±1.28E-03	0.991±1.78E-03	1.001±1.63E-03	1.113±1.84E-03	1.139±2.06E-03
	Microsomes	1.810±1.21E-03	1.006±1.95E-03	1.055±2.15E-03	1.077±1.63E-03	1.192±2.91E-03	1.241±1.21E-03
Digestive gland	Cytosol	1.974±1.41E-03	0.974±2.87E-03	0.991±1.78E-03	0.990±1.36E-03	1.252±1.93E-03	1.260±2.06E-03
	Microsomes	1.934±2.06E-03	1.032±2.08E-03	1.085±2.08E-03	1.106±1.56E-03	1.273±2.04E-03	1.294±6.77E-03

*Carbohydrate values (mg/g) are mean ± SEM of five determinations

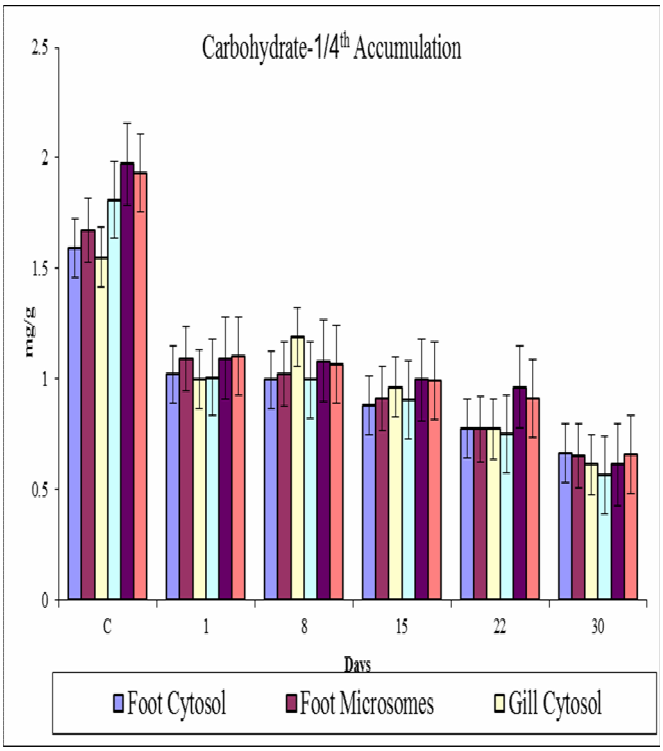

Fig. 2.A: Carbohydrate content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th exposure period of oil effluent.

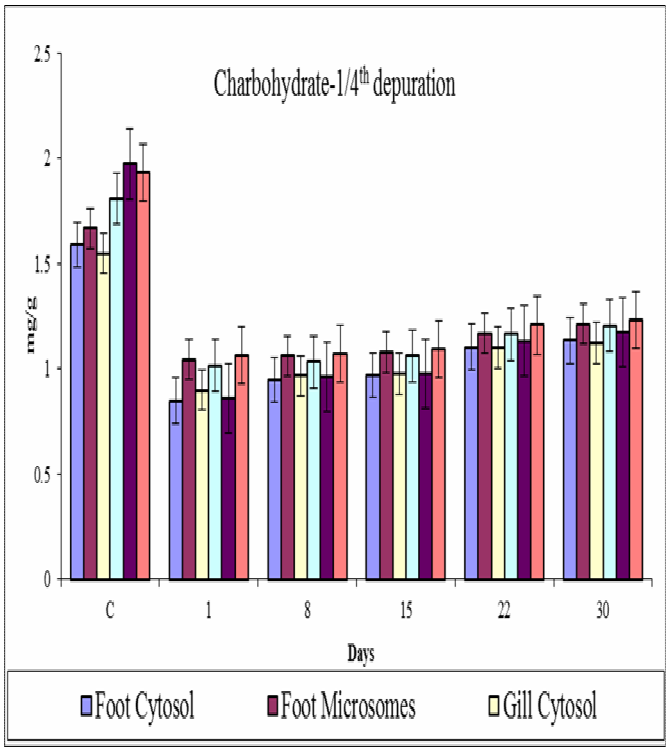

Fig. 2.C : Carbohydrate content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4thdepuration period.

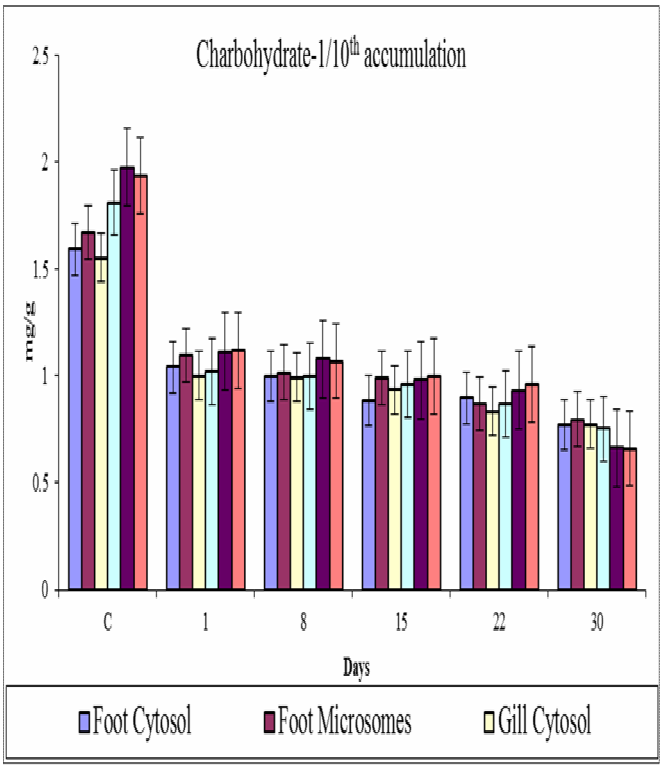

Fig. 2.B: Carbohydrate content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during1/10thexposure period of oil effluent.

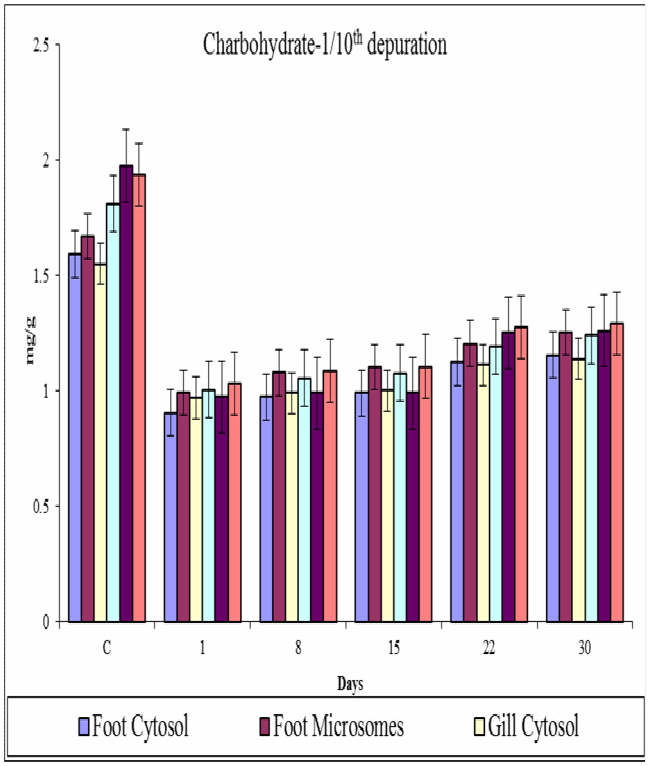

Fig. 2.D: Carbohydrate content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/10thdepuration period.

Total Lipids

The control mussels lipid content level for 1/4th and 1/10th exposure concentrations and depuration study are presented in Table-3 A and B. On the exposure to oil effluent, the lipid content level decreased gradually from a Day 1.Maximum level of decreased at a Day 30 on exposure to both sublethal concentration of oil effluent. During the depuration phase, the lipid contents increased gradually and reached the control value (1.075±1.91 E-03 cytosol, 1.007±1.96 E-03 microsomes of foot, 1.086±1.63 E-03 cytosol,1.020±1.96 E-03 microsomes of gill 1.074±1.41 E-03 cytosol, 1.053±1.71 E-03 microsomes of digestive gland of 1/4th phase and 1.090±1.56 E-03 cytosol, 1.016±1.21 E-03 microsomes of foot,1.044±1.44 E-03 cytosol, 1.073±1.64 E-03 microsomes of gill,1.060±1.21 E-03 cytosol, 1.091±1.72 E-03 microsomes of digestive gland of 1/10th phase on Day 30 (Figure 3 A,B,C and D).

Table 3.A: Lipid content variations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10thexposure period of oil effluent.

Conc. / Tissues	Subcellular fractions	Accumulation Period Days					
		Control	1	8	15	22	30
1/4th Conc. Foot	Cytosol	1.114±1.66E-03	1.037±1.21E-03	1.001±1.83E-03	0.986±2.55E-03	0.905±1.41E-03	0.662±1.93E-03
	Microsomes	1.022±1.89E-03	0.997±1.96E-03	0.962±2.20E-03	0.951±1.66E-03	0.884±2.34E-03	0.616±1.77E-03
Gill	Cytosol	1.098±2.28E-03	1.005±2.34E-03	0.998±1.56E-03	0.995±1.91E-03	0.852±1.93E-03	0.721±1.43E-03
	Microsomes	1.101±2.13E-03	1.038±2.27E-03	1.005±2.38E-03	0.993±2.21E-03	0.850±1.85E-03	0.626±1.28E-03
Digestive gland	Cytosol	1.159±1.36E-03	1.079±1.36E-03	1.007±1.98E-03	0.993±2.29E-03	0.932±1.72E-03	0.650±1.65E-03
	Microsomes	1.139±1.21E-03	1.091±1.78E-03	1.038±2.27E-03	0.980±1.70E-03	0.905±1.41E-03	0.677±1.96E-03
1/10th Conc. Foot	Cytosol	1.114±1.66E-03	1.073±1.84E-03	1.036±2.75E-03	0.983±2.21E-03	0.868±1.99E-03	0.623±2.39E-03
	Microsomes	1.022±1.89E-03	1.010±1.63E-03	0.884±2.34E-03	0.818±1.77E-03	0.787±2.27E-03	0.716±2.08E-03
Gill	Cytosol	1.098±2.28E-03	1.013±2.99E-03	0.807±0.17E-03	0.923±1.56E-03	0.883±2.20E-03	0.787±2.27E-03
	Microsomes	1.101±2.13E-03	1.048±1.63E-03	1.021±2.56E-03	0.986±2.55E-03	0.882±2.06E-03	0.721±1.43E-03
Digestive gland	*Cytosol*	1.159±1.36E-03	1.080±1.21E-03	1.006±1.56E-03	0.962±1.85E-03	0.907±1.21E-03	0.677±1.96E-03
	Microsomes	1.139±1.21E-03	1.099±2.14E-03	1.060±1.58E-03	1.002±2.08E-03	0.986±1.77E-03	0.696±1.91E-03

*Lipid values (mg/g) are mean ± SEM of five determinations

Table 3.B: Lipid content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th and 1/10th depuration period.

Conc./ Tissues	Subcellular fractions	Depuration Period Days					
		Control	1	8	15	22	30
1/4th Conc. Foot	Cytosol	1.114±1.66E-03	1.021±2.56E-03	1.023±2.65E-03	1.053±1.71E-03	1.064±1.56E-03	1.075±1.91E-03
	Microsomes	1.022±1.89E-03	0.948±1.92E-03	0.951±1.84E-03	0.986±2.55E-03	0.998±1.56E-03	1.007±1.96E-03
Gill	Cytosol	1.098±2.28E-03	0.734±1.63E-03	0.818±1.77E-03	0.932±1.72E-03	0.952±1.24E-03	1.086±1.63E-03
	Microsomes	1.101±2.13E-03	1.004±2.50E-03	1.013±2.99E-03	1.038±2.27E-03	1.034±1.96E-02	1.020±1.96E-03
Digestive gland	Cytosol	1.159±1.36E-03	1.012±2.27E-03	1.021±2.20E-03	1.041±2.71E-03	0.957±1.43E-03	1.074±1.41E-03
	Microsomes	1.139±1.21E-03	0.911±2.42E-03	0.921±2.49E-03	0.942±1.28E-03	1.022±1.91E-03	1.053±1.71E-03
1/10th Conc. Foot	Cytosol	1.114±1.66E-03	0.962±2.20E-03	1.006±1.56E-03	1.023±2.75E-03	1.036±2.75E-03	1.090±1.56E-03
	Microsomes	1.022±1.89E-03	0.873±2.92E-03	0.923±1.56E-03	0.974±1.98E-03	1.011±2.99E-03	1.016±1.21E-03
Gill	Cytosol	1.098±2.28E-03	0.926±1.58E-03	0.950±1.16E-03	0.986±1.77E-03	1.021±2.42E-03	1.044±1.44E-03
	Microsomes	1.101±2.13E-03	0.891±2.06E-03	0.907±1.21E-03	0.957±1.43E-03	1.041±2.71E-03	1.073±1.64E-03
Digestive gland	Cytosol	1.159±1.36E-03	0.923±1.56E-03	0.944±2.44E-03	0.976±2.50E-03	1.002±4.21E-03	1.060±1.21E-03
	Microsomes	1.139±1.21E-03	0.986±2.55E-03	1.005±2.38E-03	1.037±1.21E-03	1.053±1.71E-03	1.091±1.72E-03

*Lipid values (mg/g) are mean ± SEM of five determinations

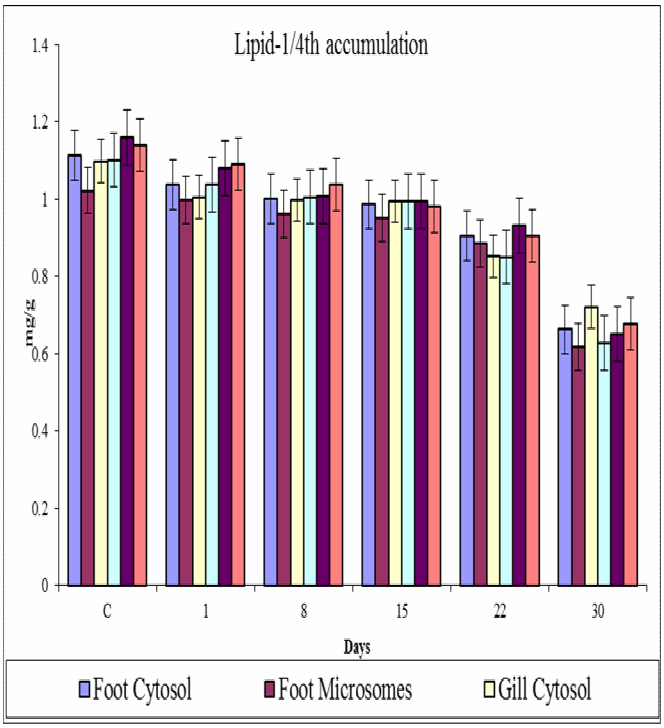

Fig. 3.A : Lipid content variations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4thexposure period .

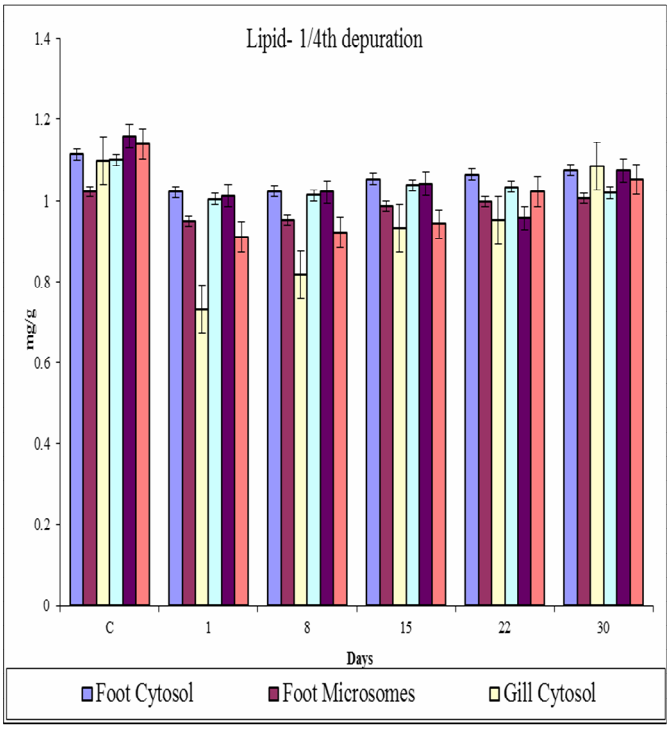

Fig. 3.C: Lipid content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/4th depuration period.

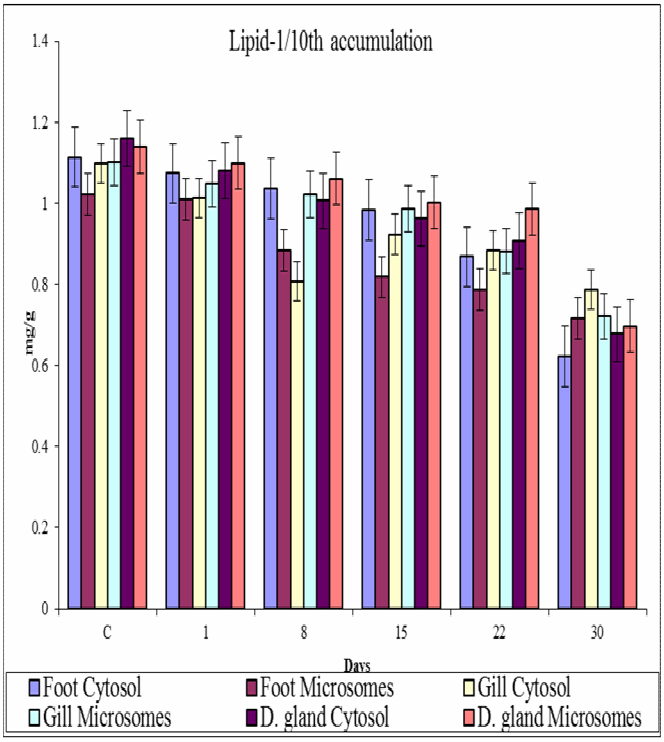

Fig. 3.B : Lipid content variations in the subcellular fractions of various tissues of fresh watermussel *Lamellidens marginalis* during /10thexposure period of oil effluent .

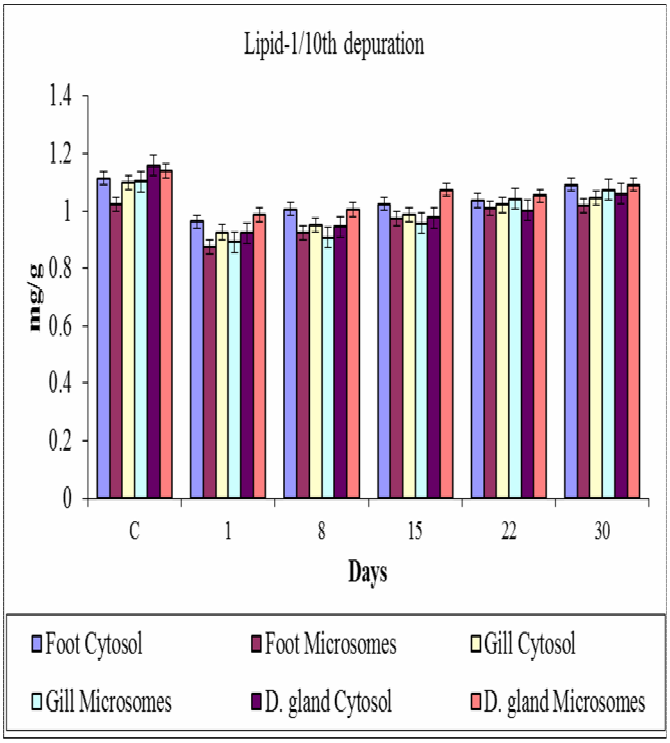

Fig. 3.D: Lipid content alterations in the subcellular fractions of various tissues of fresh water mussel *Lamellidens marginalis* during 1/10th depuration period.

DISCUSSION

The present study data show that approximately 90% of the oil effluent are eliminated in 30 days. Similarly *Mytilus edulis* released 90% hydrocarbon after 35 days (Di Salvo *et al*., 1975) and hydrocarbons are undetectable after 45 days in PAH exposed mussels (Dunn and Stich, 1976). The decreased response of biochemical constituents was associated with the increased accumulation of oil effluent (hydrocarbons).The present results indicate that concentration and duration of oil effluent patterns of exposure may influence release of oil effluent in freshwater mussels. Besides, the bivalve secretes an organic membrane to protect itself and in doing so, expends a great amount of energy (Kent 1979). Similar findings have been noted in the freshwater prawn *Macrobrachium kistensis* on exposure to naphthalene and pesticides (Jaiswal *et al*., 1991). Krishnamoorthy and Subramanian (1995) have observed the decreasing trend of biochemical constituents (protein, carbohydrate, and lipid) in different tissues of the exposed prawn during the copper accumulation period. The decline in protein content may also be related to the increased energy cost of homeostasis, tissue repair, and detoxification during stress (Neff 1985). This may be due to the interference of copper with the physiology of the organisms. Similarly, Vijayalakshmi and Ramana Rao (1985) have observed the decreased protein level in *Metapenaeus monoceros* when exposed to phosphamidon. The observed low level of protein contents in different tissues indicate that environmental stress reduces the rate of protein synthesis or increase the proteolysis to cope with the high energy demands under toxicants stress (Vincent *et al*., 1995; Waykar and Lomte, 2001). Total protein levels are reduced in the body fluids of *Anodonta cygnea* (L.) exposed to heavy metals (Mour *et al*., 2000) and decreasing protein content is probably due to higher energy demands caused by the stress situation; stress and starvation or exposure to intoxicant. These indications may be rapid utilization of energy stores to meet the energy demands warranted by the environment. The observed depletion in various tissue protein on exposure with sub-lethal concentrations of oil effluent is suggestive of proteolytic activity, possibly to meet the excess energy demands in toxic /stress conditions. In addition to that, the higher depletion of protein in the digestive gland might be due to the high metabolic potency and efficiency of the gland when compared to other tissues like foot and gills tissue of the bivalve. The digestive gland is the site of action of pollutants in the body of the bivalve or digestive gland seems to be the main site of degradation and detoxification of oil pollution and hence has the largest demand for energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein in the digestive gland provided a better indication of the extent of oil effluent toxicity. A marked fall in the protein level in all the tissues indicates a rapid initiation of the breakdown of protein. Above these observations conform with the reports on the fall in subcellular-fractions of organ protein level in *L. marginalis*, when exposed to oil effluent. Simultaneously during the recovery period, Approximately 80-90 % of protein content was recovered in both concentrations of exposure days and tissues. The present study agrees with the findings of Chitradevi (1990) who was reported during the depuration period the total protein content level was gradually regained in *Oreochromis mossambicus*.

In the present study, the carbohydrate content level gradually decreased from the 1st day to the 30th day in the sub-cellular fraction of various tissue of *L. marginalis* when exposed to oil effluent. Our present study showed a carbohydrate level strongly depleted in the microsomes fractions of digestive gland tissue at the 30th day when compared to other fractions and tissues of mussel in both exposure period. The carbohydrate of aquatic organisms comprised mainly glycogen and total free sugars and the fluctuations in the carbohydrate content may be due to accumulation and utilization of glycogen and sugars at different reproductive stages. Similar findings have been reported the decreasing carbohydrate level in different parts of prawn when exposed to copper (Krishnamoorthy and Subramanian 1995). Similarly, a reduction in carbohydrate level has also observed a considerable decrease in total carbohydrate levels due to phosphamidon toxicity in *Penaeus indicus* (Srinivasalu Reddy and Ramana Rao (1985). Nagabhushanam and Kulkarni (1981) suggested that the fall in carbohydrate levels after prolonged exposure to the heavy metal polluted water might be due to the inactivation of the enzyme involved in the carbohydrate synthesis and metabolism. Signifies their utility possibly to meet the higher energy demands. Vincent *et al*. (1995) have reported a decrease of carbohydrates content in liver, gill, and intestine after exposure to chromium in Indian major carp *Catla catla*. Villalan *et al.* (1990) have reported a decrease in the carbohydrate content in gill tissues of *Thalamita crenata* when exposed to copper. Glycogen content differences were studied in *Sphaerium corneum* from the outlet ditch and the lake (Holopainen 1987).

Lipid content is an important in bivalve tissue, which plays a prime role in energy metabolism, maturing gonadal tissues and constitute a major component of reproductive material, cellular and sub-cellular membranes. In the present study, the lipid content level gradually decreased from the 1st day to the 30th day in the sub-cellular fraction of various tissue of *L. marginalis* when exposed to sub-lethal concentrations of oil effluent. Anusha *et al*. (1996) also suggested that the decrease in lipid content in *Cyprinus carpio* may be either due to the uptake of lipid by the tissue for utilization at cellular levels or may be due to increasing lipolysis or mitochondrial injury, which affect the fatty acid oxidation mechanism. Krishnamoorthy and Subramanian (1995) have observed a marked decrease in lipid content in different parts of prawn tissues during the accumulation of copper. Nagabhushanam *et al*. (1972) have reported the lipid level reduction in hepatopancreas alone during acute and in all tissues during chronic exposure of some pesticides. Similarly, Amudhavalli *et al*. (1988) have reported the dwindling of lipid content in muscle and liver in *Tilapia mossambica* due to the exposure in the sublethal concentration of zinc. Besides, Villalan *et al*. (1990) have also observed the reduced lipid content in muscle due to Cadmium stress in *Macrobrachium idella*. Toxic effects occur after excretory, metabolic, storage, and detoxification mechanisms are no longer able to match the uptake rate (Canli *et al*., 2001). These results agree with the already established concept, which proposes that the lipids reflect the biochemical composition of the environmental conditions

where bivalve development occurs (Fernandez-Reiring *et al*., 1996). The present study showed the predominant recovery of lipid content from digestive gland microsomes fraction, compare to cytosol fractions on the 30th day. Consequently from the above findings it was clearly revealed that, in freshwater mussel, microsomal electron transport component and monooxygenase enzymes (cytochrome b5, NADPH-cytochrome -c- reductase and CYP) may be involved in the removal of accumulated oil effluent. In addition, during depuration, alteration that occurred in biochemical these enzyme activities reflect the ability of detoxification. The exposure of treated mussels to oil-derived hydrocarbons impoverished water, biochemical constituents level also gradually regained. Approximately 80 - 90 % of lipid content was recovered during the period depuration in both concentrations of exposure days and tissues. It presents work it's reveals that biochemical changes in freshwater bivalve, *Lamellidens marginalis* mainly affected by pattern of concentration and duration of exposure of oil effluent. According to obtained results greatly stored energy in the form of glycogen, lipid and protein degraded by the oil effluent exposures and during depuration phase attained closer to control level.

CONCLUSIONS

The present investigation concluded that the higher depletion of biochemical constituents compares to sub-cellular fractions of a foot, gill tissue, the microsomal fractions of digestive glands tissue might be due to high metabolic potency and efficiency of the gland under oil pollution (hydrocarbon) stress. The digestive gland is the main site of degradation and detoxification of oil pollution and hence resulting in increased utilization of protein, carbohydrate, lipid to meet energy demand. The potential for its development as a specific index of oil pollution and biochemical impact therefore exist and future research can now be directed to the MFO system. Hence, it is concluded that the freshwater mussel, *Lamellidens marginalis* could be utilized as a test organism/pollution indicator organism for monitoring long-term hydrocarbon pollution in freshwater environments.

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