Poll Res. 40 (May Suppl. Issue) : S153-S162 (2021)

ISSN 0257–8050

ACUTE TOXICITY OF OIL EEFLUENT AND ITS METABOLISM IN DIGESTIVE GLAND TISSUES OF FRESHWATER MUSSEL, *LAMELLIDENS MARGINALIS*

BALAMURUGAN S. [1]* AND SUBRAMANIAN P.[2]

[1] *P.G and Research Department of Zoology, Arignar Anna Govt Arts College, Musiri 621 211, Tiruchirappalli -District, Tamilnadu, South India*
[2] *Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamilnadu, South India*

(Received 30 October, 2020; accepted 23 December, 2020)

ABSTRACT

Acute toxicity test using the freshwater mussel, *Lamellidens marginalis* were conducted on oil effluent. Tentative preliminary test were conducted to fix the minimum concentration of the oil effluent. The test revealed that 96-hr LC_{50} total hydrocarbons was (upper level) 11.88 ppt and (lower level) 8.55 ppt. Based on this test, two sub-lethal concentrations 1/4th and 1/10th of LC_{50} were prepared and exposed to mussels. Hydrocarbon showed gradual increasing trends from Ist day (8.47±0.43 µg g^{-}l) and maximum attained at 30th day (43.7±0.67 µg g^{-}l) in both concentrations. More than 5 fold higher accumulation level of hydrocarbons were observed on 30th day, when compared to first day. Positive and significant regression coefficient (P<0.05) were observed. Approximately 90% of accumulated hydrocarbons were eliminated in 22nd days during depuration study. Significantly decreased (P<0.05) and negative regression coefficient observed in depuration study. Evidence indicates that concentration of hydrocarbon, duration of exposure; may influence patterns of accumulation and release of hydrocarbons in mussel.

KEY WORDS : Acute toxicity, Hydrocarbon metabolism, *Lamellidens marginalis*, *A*ccumulation, depuration, Digestive gland tissue.

INTRODUCTION

Aquatic pollution by petroleum hydrocarbons has become a global concern. Consequence of anthropogenic activities, organic chemical pollutant and various industrial pollutant released into the aquatic environment has been growing attention (Fig 1). Bioaccumulation, which involves the absorption, internal distribution, biotransformation, and excretion of chemical substances by biological organisms, has the potential to cause concentrations of chemicals in organisms that are suffificiently high to cause adverse biological responses. Among which, polycyclic aromatic hydrocarbons (PAHs) polychlorinated biphenyls (PCBs) are one of the more significant classes of pollutant chemicals, created great awareness. Industrial activities resulting in the production of PAH include: preparation of acetylene from natural gas; pyrolysis of kerosene to form benzene, toluene and other organic solvents; pyrolysis of wood to form charcoal, wood tars and carbon blocks, gas production from petroleum, coal gasification and oil refinery operations (Andelman and Snodgrass, 1972). Toxic by-products of environmental chemicals have been studied at various subcellular levels in aquatic organisms (Peters *et al.,* 1996). Cytochrome P450-dependent monooxygenase systems are involved in the biotransformation of environmental chemicals and the protection of aquatic organisms from the adverse effect of these hazardous substances (Livingstone, 1991; Everaarts *et al.,* 1998).

The increase of oil-derived hydrocarbons (PAH) in the environment has caused specific concern for the adverse effect of these lipophilic compounds in the aquatic system. Concerns arose that these

hydrocarbons could pose a health hazard to local residents and livestock because surface and subsurface water in the drainage system are used for domestic and livestock consumption. Several studies have reported the toxicity of oil-derived hydrocarbons on aquatic invertebrates. A great deal of research has been performed in recent years concerning acute and chronic effects of petroleum and specific petroleum hydrocarbons on aquatic organisms and ecosystems. Much of this research has been reviewed by several researchers, (Carthy and Arthur, 1968; American Institute of Biological Science, 1976; Braunstein *et al*., 1977; Wolfe, 1977; Malins, 1977a; McIntyre and Whittle, 1977). Among the invertebrates, in particular, crustacean species has been increasingly used to evaluate the toxicity of chemicals crude oil and effluents in an aquatic environment (US-EPA, 1987; Cerhardt *et al*., 1998). Filter-feeding molluscs, e.g. freshwater mussels, are often commercially and biologically important in the riverine system and are used as bioindicator organisms to foretell aquatic pollution. Bivalves, particularly mussels readily accumulate polynuclear aromatic hydrocarbons from the environment and are widely used in environmental monitoring programmes (Moore *et al*., 1982; Livingstone, 1984). several of the toxic responses elicited by dioxins and related compounds are mediated by common initial event i.e. the binding of the hydrocarbons to the cytosolic Ah-receptor (Poland and Knutson, 1982). PAH were investigated in mussel (*Mytilus edulis* L.) digestive gland cell mixtures using the model compounds benzo [a] pyrene and nitroaromatics (Mitchelmore *et al*., 1998). Mitchelmore *et al*., (1998) have studied the PAH, such as benzo [a] pyrene metabolism and mechanisms (pathways involved in the metabolism of benzo [a] pyrene) in *Mytilus edulis*. A large amount of research has been conducted in recent years on accumulation and release of petroleum hydrocarbons by aquatic (mainly marine) organisms (Anderson *et al*., 1974; Varanasi and Malins, 1977). Digestive gland is also the main tissue for biotransformation in the mussel (Livingstone and Pipe, 1992) is characterized by higher phase I activities due to the presence of both a mixed-function oxygenase (MFO) system and a microsomal flavin monooxygenase (FMO) system. The 96-hr exposure period was normally recommended in the literature to evaluate the LC_{50} (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Based on this information, the present investigation was undertaken to (i) find out the acute toxicity of oil-derived hydrocarbons to report the 96-hr. LC_{50} of oil effluent and (ii) to reveal the level of oil derived aromatic hydrocarbons in the digestive gland of mussel, during accumulation and depuration period of 96 hr LC_{50} in *Lamellidens marginalis*.

MATERIALS AND METHODS

Animals

Almost uniform size fresh water 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 experiment

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 LC50 concentration. In addition, a control was also maintained. The 96-hr LC50 values with 95% confidence limits were calculated (NCPC 1986; Finney 1971) .

Detection of oil-derived hydrocarbons

Total hydrocarbons in the tissues of digestive gland were determined by (Law *et al.,* 1988). Samples were extracted with hexane by using cylindrical glass separating funnel. The content of total hydrocarbon was measured with a fluorescence spectrophotometer (Ex. 310 NM; Em. 360 NM) using chrysene as a standard, since the pure aromatic compound chrysene strongly absorbs UV fluorescence intensity at 310 NM. Results are expressed as lg/g tissue in terms of chrysene equivalents.

Exposure Experiment

Based on the 96-hrLC_{50} value of oil effluent

sublethal concentration such as 1/4th 11.88 ppt (24.25mg hydrocarbon) and 1/10th 8.55 ppt (9.7mg hydrocarbon) of LC_{50} were prepared and used for the exposure study. In the accumulation and subsequent depuration / elimination study, triplicate sets of ten animals each in 10-litre plastic tubs have experimented. The exposure medium was replaced in alternative days with the same concentration of freshly prepared medium, to overcome the excretory contamination and degradation of pollutant exposed. At every seven days intervals, mussels from each exposure were sacrificed for the evaluation of hydrocarbon content analysis. After 30 days, exposures of oil effluent treated mussels, were released into hydrocarbon poverished freshwater and continue the depuration study. It was also extended for 30 days and at every seven days interval, the mussels representing different exposure were sacrificed for the hydrocarbon content analysis. A control was also run simultaneously without the addition of oil effluent. The results are recorded as mean ± SEM. Differences between the days of hydrocarbon values were analyzed by regression coefficient and $P<0.05$ was recorded as statistically significant.

Tissue and sub-cellular fraction preparation

Digestive gland tissues of mussels were dissected out for subcellular fractions. 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. Subcellular fractions (cytosol and microsomes) were prepared by the procedure of Livingstone and Farrar (1984). All the preparation procedures were carried out at 4 °C. The homogenization samples were centrifuged at first on 600 g for 10 min to sediment nuclei and cell fragments. Then, without transferring these fragments, samples were re-centrifuged at 12,000 g for 45 min. Consequently, the supernatant was collected and used as a mitochondrial fraction. The resultant pellet was re-suspended in homogenizing buffer and re-centrifuged at 100,000 g for 90 min, then, the supernatant was collected and used as a cytosolic fraction. Re-suspension 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 re-suspended pellet was used

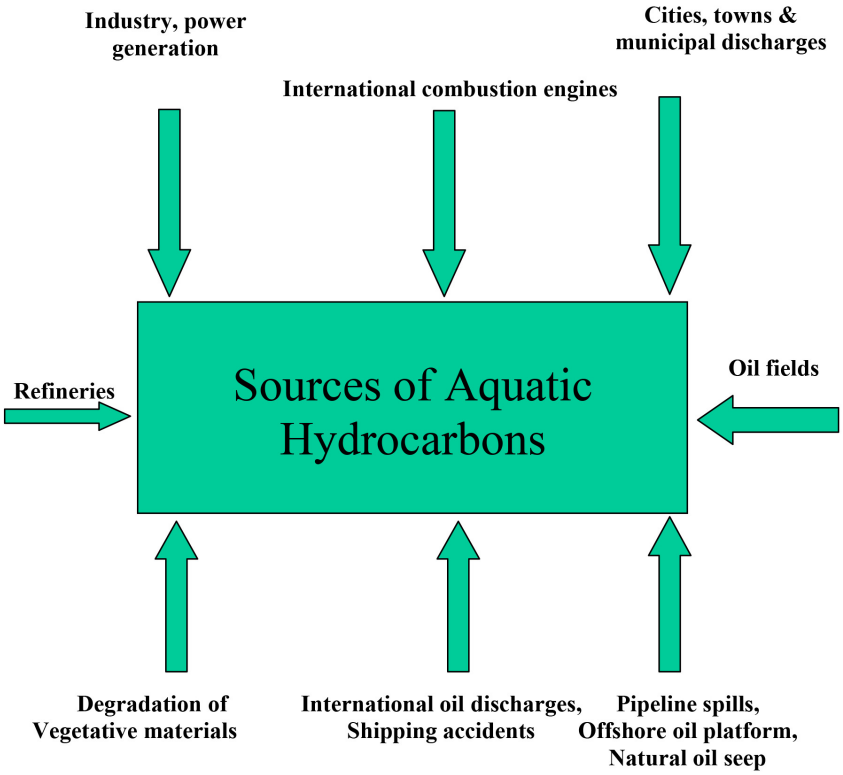

Fig. 1. Sources of Polycyclic Aromatic Hydrocarbons (PAHs) in the Aquatic environment Industry, power generation International combustion engines Cities, towns and municipal discharges Degradation of Vegetative materials International oil discharges, Shipping accidents Pipeline spills, Offshore oil platform, Natural oil seep Sources of Aquatic Hydrocarbons Refineries Oil fields

as a microsomal fraction. 2.6.Statistical analysis The results are recorded as mean ± SEM. Differences between the days of hydrocarbon values were analyzed by regression coefficient and P<0.05 was recorded as statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity

The acute toxicity studies reviled that the 96hr LC_{50} value of oil effluent on mussel *Lamellidens marginalis* was 10.08 ppt. In the present experiment, no mortality was observed in the control tubs for the entire duration of the experiment. The upper limit of the 96hr LC_{50} value was 11.88 ppt, whereas the lower limit was 8.55 ppt. The slope functions with a 95% confidence limit were calculated to be 6.12. The level of total hydrocarbon in the 10.08 ppt of oil effluent was ~97 mg Table 1. Figure 2 shows the % of mortality (converted to probits) after 96hr of oil effluent exposure on the freshwater mussel *Lamellidens marginalis*.

Acute toxicity during the experimental period, secretion of mucus in the gill chambers may be due to the lesions in gills of mussels. This observation corroborates with the findings of Khan (1991) who observed the excessive secretion of mucus in the gill of longhorn sculpin fish when exposed to oil-contaminated sediment. Sensitivity of the gills towards crude oil toxicity has also been related to the mode of uptake of petroleum fractions to the fish. Petroleum fractions enter the fish tissue via the gills (Lee *et al.*, 1976). The 96 hr. median lethal concentration (96 hr. LC_{50}) of oil effluents was found to be 10.08 ppt for *Lamellidens marginalis*. Arun (2000) have studied acute toxicity of oil effluent (120 hr LC_{50}) on *Macrobrachium malcolmsoni*i, was 9.12 ppt and it was 5.88 ppt for *Macrobrachium lamarrei lamarrei*. Generally, crustacean species are more sensitive to oil pollutants than other aquatic animals. Mussels even at low concentration of hydrocarbon exposure rapidly took up hydrocarbons from oil effluent. For example, 29 ppb of diesel oil exposed mussels *Littorina littorea* accumulate 60µg of hydrocarbons. Similarly, *Mytilus edulis* accumulated 14.7µg of hydrocarbons in their tissues (Livingstone *et al.*, 1985). Ninety-six hr-LC_{50} concentration of benzene to grass shrimp and bay shrimp was 27 ppm and 20 ppm respectively (Neff *et al*., 1976; Benville and Korn, 1977). Ehrhardt (1972) analyzed the total hydrocarbons (containing236 ppm (mg/kg w/w) in oyster tissues (*Crassostrea virginica*) in Galveston Bay. Miramand and Unsal (1978) studied the acute toxicity of vanadium to some marine benthic species and they have found 9-day LC_{50} values of 10, 35 and 65 ppm vanadium for *Nereis diversicolor*, *Carcinus maenas* and *Mytilus galloprovincialis* respectively. The 96-hr LC_{50} of vanadium to adult American flagfish was 11.2 mg L-1 in very hard water, while the threshold for chronic toxicity was judged to be about 0.08 mg L-1 (Holdway and Sprague, 1979). Beusen and Neven (1987) have studied acute toxicity of vanadium on different freshwater organisms, *Daphnia magna*, Zebra fishes (*Brachydanio rerio*) and guppies (*Poecilia reticulata*). Aromatic components of the oil effluent are much more toxic

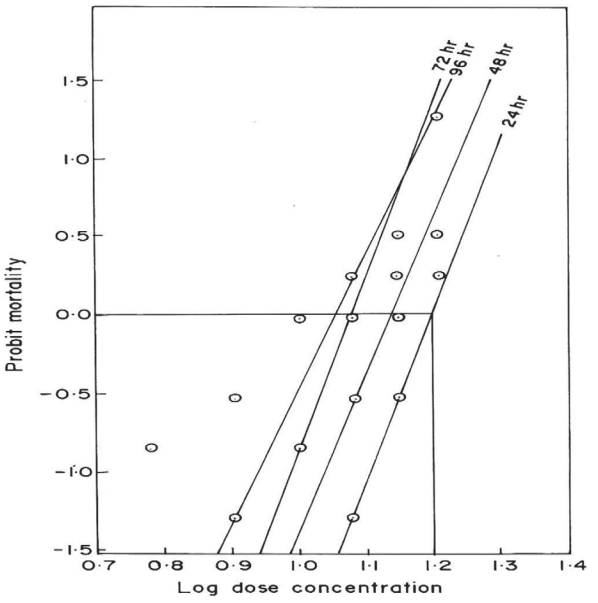

Fig. 2. Acute toxicity of oil effluent on the fresh water mussel *Lamellidens marginalis* and the resulting LC 50 for different duration

Table 1. 96 hr LC 50 values of oil effluent for *Lamellidens marginalis.*

Species	LC 50 (ppt)	Upper (ppt)	Lower (ppt)	Slope function at 95% confidence
Lamellidens marginalis	10.08 ppt (~97mg of total hydrocarbon)	11.88	8.55	6.12

than the aliphatic components and are readily taken up by the tissues of aquatic organisms. Foster and Crosby (1987) reported that high accumulation of these xenobiotics compounds further goes to oxidative activation in the freshwater mussels, and products of these metabolisms are more toxic than its parent compound.

Hydrocarbon Metabolism

The level of oil derived total hydrocarbon in the digestive gland of freshwater mussel *Lamellidens marginalis* during accumulation and depuration of sublethal exposure of oil effluent concentration was depicted in Table 2 and Figure 3. During the accumulation of 1/4th sublethal concentration, the level of total hydrocarbons was gradually increased from Ist (8.47 ± 0.43 µg g-1 tissue) day to 30th day and attained maximum level of hydrocarbon 43.7±0.67 µg g-1 tissue at 30 days. Similarly, during the 1/10th sublethal exposure, the level of total hydrocarbons reached 31.3±0.64-µg g-1 tissue at 30th day. Significant level of regression coefficient (P<0.05) and positive regression coefficient were registered in both sublethal concentrations (Table 2a). During the recovery period, this enhanced accumulated hydrocarbon are gradually decreased in the digestive gland and obtained minimum level of hydrocarbons 8.13±0.61µg g- 1; 6.30±0.32 µg g-1 in 1/4th 1/10th respectively. Level of hydrocarbon content was decreased significantly (P<0.05) and found negative regression coefficient in the depuration period (Table 2a).

Table 2. The level of total hydrocarbonsa in the digestive gland tissues of fresh water mussel, *Lamellidens marginalis* during accumulation of sublethal concentrations of oil effluent and depuration period.

Concentration/Days	Accumulation	Depuration
1/4th Conc.		
Ist day	8.47±0.43	42.3±0.64
8th day	16.7±0.55	32.4±0.32
15th day	27.7±0.76	24.57±0.56
22nd day	35.6±0.52	19.5±0.70
30th day	43.7±0.67	8.13±0.61
1/10th Conc.		
Ist day	6.84±0.60	26.57±0.67
8th day	12.4±0.51	21.53±0.47
15th day	19.57±0.69	16.67±0.58
22nd day	26.33±0.64	9.40±0.58
30th day	31.3±0.64	6.30±0.32

a-µg g^{-1} tissues
Each value represents mean ±SEM for 3 determination

Hydrocarbon metabolism many papers have been published on toxicological and chemical aspects of Halifax Harbour (Cook and Wells, 1996; Nicholls 1989; Tay *et al.,* 1992; Hellou *et al.*, 2002). Some organic contaminants have been examined in lobster and clams (King *et al.,* 1993). Aquatic organisms accumulate organic contaminants from water or food and relative importance of these routes of bioaccumulation has been discussed in several investigations (Wang and Simpson, 1996). Potentially dangerous chemicals that are released into the aquatic environment due to anthropogenic activity are real threats to the ecosystem (Gange and Blaise, 1995). For instance, high level of various pollutants have been reported in aquatic animals from the Baltic Sea (Rappe *et al.,* 1989; Bergqvist *et al.,* 1989; Asplund *et al.,* 1990) from Great lakes of United States (Kuehl *et al.,* 1989) and lake Jarnsjon of Southeastern Sweden (Bremle and Iwald, 1995). Lysosomes are present in high numbers in the digestive gland of mussel; readily accumulate xenobiotics such as PAHs (Winston, *et al.,* 1991). Patterns of concentration-dependent rate of accumulation and release of PAH by different body regions of oil-exposed aquatic animals also vary. Accumulation of hydrocarbons, in the digestive gland of mussel, was gradually increased and significantly attained maximum value at 30 days when exposed to both 1/4th and 1/10th of 96 hrs LC_{50}

Table 2a. Simple regression analysis showing the level of total hydrocarbons in the digestive gland tissues of fresh water mussel, *Lamellidens marginalis* during accumulation of sublethal concentration of oil effluent and equal depuration period.

Concentrations	Regression coefficients	Level of significance	Adjusted R square	SE
1/4th Accumulation	1.240	P<0.05	0.96	1.392
1/10thAccumulation	0.871	P<0.05	0.98	1.222
1/4th Depuration	-1.167	P<0.05	0.98	1.657
1/10th Depuration	-.815	P<0.05	0.98	1.004

concentrations (Table 2). This enhanced accumulation level of hydrocarbons observed on the day of 30 were more than 5 fold higher when compared to first day values. Stegeman and Teal (1973) observed many-fold increased accumulation of hydrocarbons in the oysters after 35 days exposure of fuel oil. Identically Stockes (1979) was also observed higher accumulation of copper in the gills than in hepatopancreas and muscles. Ninety days is approximately the time required under field conditions for the release of hydrocarbons after the acute exposure of mussels to oil (Porte *et al.*, 2000). Other studies carried out in temperate waters have pointed out that most of the petrogenic hydrocarbons have similarly been reduced after the period of 3 to 6 months (Farrington *et al*., 1980; Boehm *et al*., 1982). Although the mussel *Elliptio complanata* can utilize cytochrome P450 enzymes to metabolize PAHs and other xenobiotic organic compounds, the very slow metabolic rates allow bioaccumulation and therefore an adequate measurement of the parent compound (Bruner *et al*., 1994). When exposed the diesel oil/copper mixture to whole tissues of mussel (*Mytilus edulis*), increased the level of accumulation rate (Livingstone 1988). Stegeman and Teal (1973) exposed oysters (*Crassostrea virginica*) to No.2 fuel oil for 50 days and attained 334 µg total hydrocarbons / g w/w in 49 days. During depuration, the oysters rapidly released most of the accumulated hydrocarbons. During the depuration period, mussel *Mytilus edulis* approximately 90 per cent of the accumulated (11 weeks) hydrocarbons were released in 5 weeks. A gradient of PAH tissue concentration have been observed in mussels from the field sites (Livingstone, 1988). Although bivalves exhibit low ability to metabolize hydrocarbons (Livingstone, 1991) it is interesting to notice that the polycyclic aromatic hydrocarbon derivatives are predominant components only in bivalves but not in sediments, a selective enrichment in the organisms being possibly advisable by molecular constraints of these compounds (e.g. size and / or lipophilicity) that may facilitate their passage through the cell membranes and /or their uptake by the organisms. Sole *et al.* (1994) have shown that among bivalves, clams exhibit lower accumulation of hydrophobic pollutants compared to mussels. Bivalves are filtering organisms, but mussels will preferentially accumulate the compounds present in the water column, whereas clams, (*Tapes semidecussata*) and cockles (*Cardium edule*) are more associated with the sediment, that may act as a sink for these compounds and render them less bio-available. On the other hand, clams are less rich in lipids and have lower feeding rates, two factors that certainly influence bioaccumulation. The quantum of accumulation was more in $1/4^{th}$ of LC_{50} concentration than 10^{th} of LC_{50} concentration. It would indicate the process of accumulation in *Lamellidens marginalis* is dose-dependent. Accumulation of Polychlorinated biphenyls (PCBs) and total chlorinated pesticides in the Asiatic clam, *Corbicula fluminea* from the Rio de la Plata Estuary,

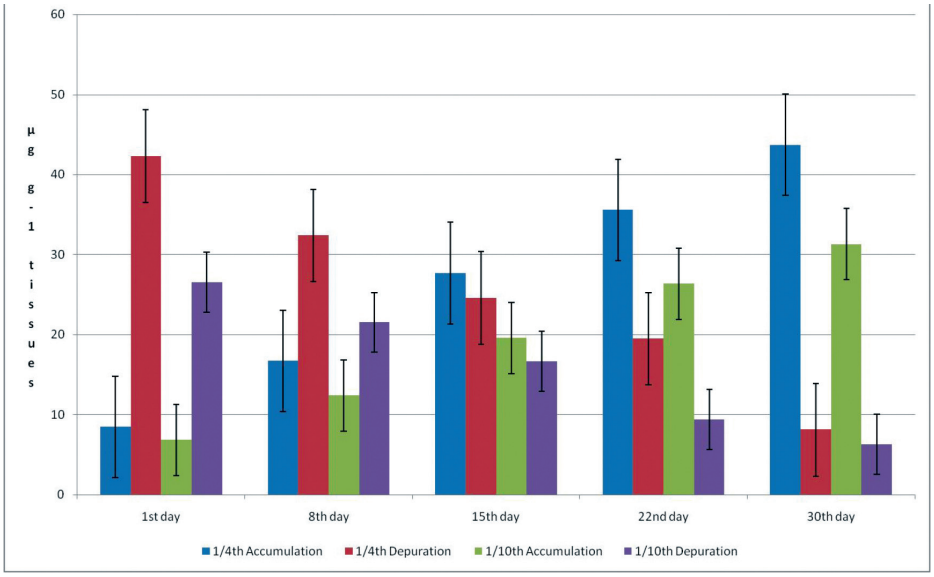

Fig. 3. The level of total hydrocarbons in the digestive gland tissues of freshwater mussel, *Lamellidens marginalis* during accumulation of sublethal concentration of oil effluent and depuration period

Argentina, ranged from 0.5-12.0µg/g and from 0.4-6µg/g respectively (Colombo *et al.,* 1995). When mussels (*Mytilus edulis*) were transferred to the oil-polluted station (aromatic hydrocarbons) in San Francisco, California, for 11 weeks, both aromatic and aliphatic hydrocarbons were taken up (Di Salvo *et al*., 1975). When returned to unpolluted water, approximately 90 % of the accumulated hydrocarbons were released in 5 weeks. In freshwater mussel *Lamellidens marginalis* during the present depuration period, the accumulated hydrocarbon levels gradually eliminated and after 30th day it attained 8.13 ± 0.61µg g-1 tissues and 6.30 ± 0.32 µg g-1 tissues in respective $1/4^{th}$ and $1/10^{th}$ sublethal concentrations. This result would extrapolate that approximately 50 to 60 % of the accumulated hydrocarbons were removed in 15 days, in the sublethal concentrations. This result corroborates with the findings of Lee *et al.* (1976) who found that *Callinectes sapidus* (Blue Crabs) could excrete/remove 50% of accumulated hydrocarbons in 6 days of depuration. Arun (2000) have found that *Macrobrachium malcolmsonii* excrete 90% of the accumulated hydrocarbons in 30 days. In the present study *Lamellidens marginalis* approximately 90% of the accumulated hydrocarbons were eliminated in 22^{nd} days (Table 3). Similar response on *Mytilus edulis* also stated the release of 90% hydrocarbons after 35 days (Di Salvo *et al.,* 1975). Further, in the process of reputation, the hydrocarbon level becomes undetectable after 45 days in PAH contaminated *Mytilus edulis* (Dunn and Stich, 1976). Thus these results would indicate that concentration of hydrocarbon, duration of exposure to hydrocarbons; induction of biotransformation enzymes may influence patterns of accumulation and release of hydrocarbons in a freshwater mussel. The bioaccumulation of organic contaminants by aquatic organisms is a balance between essentially passive processes of uptake and depuration of the xenobiotic via biotransformation pathways (Livingstone 1991; 1998). Benzo [a] pyrene hydroxylase (B (a) PH) activity of flounder *Platichthys flesus* were also higher at field sites than in experimental, control animals (Addison and Edwards 1988). High levels of BaP hydroxylase activity were found in fish, Echinoderm, Crustacean, and Mollusc. Level of the monooxygenase activity were higher in fish than aquatic invertebrates. Wide variability were observed in different fish species by xenobiotics (Livingstone, 1998). Despite reported success with some biochemical measurements in bivalves (Livingstone, 1988; Porte *et al.,* 1991) none has yet emerged as a widely used biomarker for organic pollution in molluscs. However, encouraging findings, i.e. a good correlation between benzo [a] pyrene hydroxylase in mussels from the French Mediterranean coast have been reported by Garrigues *et al.* (1990) and Narbonne *et al.* (1991). Hence, it is presumed that metabolites of this oxidative activation may bring mortality in mussel, *Lamellidens marginalis* when exposed to oil effluents. These acute toxicity tests suggest that mussel *Lamellidens marginalis* is more sensitive to oil effluent. These results, as well as those several similar studies, seem to indicate that composition of the hydrocarbon mixture and duration of exposure to the pollutant hydrocarbons, as well as the nutritional status of the animals, may all influence the patterns of accumulation and release of hydrocarbons. Furthermore, it leads to study the adverse effect of oil effluent on mussel and these results would warrant a further, detailed study to scrutinize the ability of hydrocarbon detoxification mechanism in freshwater mussels.

Table 3. Levels of total hydrocarbons in the hepatopancreas of *M. Malcolmsonii* and *M. lamarrei lamarrei* and digestive gland tissues of freshwater mussel, *Lamellidens marginalis* during oil efflfluent exposure.

Prawn	*M. malcolmsonii*		*M. lamarrei lamarrei*	
	Control	30th Day	Control	30th Day
Hydrocarbon levels				
2.3 ppt	ND	32.5 – 2.1	ND	25.4 – 2.4
0.9 ppt	ND	20.4– 1.6	ND	19.3– 1.8
Mussel	*Lamellidens marginalis*			
11.88 ppt	ND	43.7–0.67		
8.55 ppt	ND	31.3– 0.64		

-µg g^{-1} tissues

ACKNOWLEDGEMENT

Authors wish to thank, Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamilnadu, India, for providing necessary facilities for completion of research work.

REFERENCES

Addiso, R.F. and Edwards, A.J. 1988. Hepatic microsomal mono-oxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. *Mar. Ecol. Prog. Ser*. 46 : 51-54.

American Institute of Biological Sciences, 1976. Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment.Washington, D.C. *AIBS*.578pp

Andelman, J.B. and Snodgrass, J.E. 1972. Incidence and significance of polynuclear aromatic hydrocarbons in the water environment. *CRC Crit. Rev. Environ. Cont*. 4 (1) : 69-83.

Anderson, J.W., Neff, J.M. and Cox, B.A. 1974. The effects of oil on Estuarine Animals: Toxicity, uptake and Depuration, Respiration. In: Vernberg, F.J. and Vernberg, W.B. (Ed). *Pollution of Marine Organisms*. Academic Press, New York. P. 288.

Arun, S. 2000. *Biotransformation and antioxidant enzymes in the fresh water prawn Macrobrachium malcolmsonii* and *Malcolmsonii lamarrei lamarrei*. Ph.D., Thesis, Bharathidasan University, Tiruchirappalli, India.

Asplund, L., Grafstrom, A.K. and Haglund, P. 1990. Analysis of non-ortho polychlorinated biphenyls and polychlorinated naphthalens in Swedish dioxin survey samples. *Chemosphere*. 20 : 1481-1488.

Benville, P.E. and Jr. Korn, S. 1977. The acute toxicity of six monocyclic aromatic crude oil components to striped bass (*Marena saxatilis*) and Bay shrimp (*Grago franciscorum*). *Calif Fish and Game*. 63 : 204-209.

Bergqvist, P.A., Bergek, S., Hallback, H., Rappe, C. and Slorach, S.A. 1989. Dioxins in cod and herring from the seas around Sweden. *Chemosphere*. 19 : 513-518.

Beusen, J.M. and Neven, B. 1987. Toxicity of vanadium to different fresh water organisms. *Bull. Environ. Contam. Toxicol*. 39 : 194-201.

Boehm, P.D., Barak, J.E. and Fiest, D.L.1982. Elskus AA.A chemical investigation into the transport and fate of petroleum hydrocarbons in littoral and benthic environments: the Thetis oil spill. *Marine Environ Res*. 6 : 157-188.

Braunstein, H.M., Copenhaver, E.D. and Pfuderer, H.A. (eds.) 1977. *Environmental, Health, and Control Aspects of Coal Conversion: an Information Overview*. Oak Ridge, Tennessee: Oak Ridge National Laboratory. ORNL/EIS-94-5. 1600 pp.

Bremle, G. and Iward, G. 1995. Bioaccumulation of polychlorinated biphenyls (PCBs) in chironomid larvae, oligocheats worms and fish from contaminated lake sediment. *Mar. Fresh. Res*. 46 : 267-273.

Bruner, K.A. Fisher, S.W. 1994. Landrum PF.The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling:1. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. *J. Greak. Lakes Res*. 20(4) : 725-734.

Carthy, J.D. and Arthur, D.R. (eds.) 1968. The Biological effects of Oil Pollution on Littoral Communities. London: *Field Studies Council*. 198 pp.

Cerhardt, A., Carlsson, A., Ressemann, C. and Stich, K.P. 1998. New online Biomonitoring system for *Gammaris pulex* (L) (Crustacean) in situ test below a copper effluent in south Sweden. *Environ. Sci. Technol*. 32 : 150-156.

Colombo, J.C. Bilos, C. Campanaro, M. Presa, M.J.R. 1995. Catoggio JA. Bioaccmulation of polychlorinated biphenyls and chlorinated pesticides by the Asiatic clam, *Corbicula fluminae*: Its use as sentinel organisms in the Rio de la Plata Estuary, Argentina. *Environ. Sci.Technol*. 29 : 914-927.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods of acute toxicity tests with fish, macroinvertebrates and amphibians. Ecol Res. Report No. EPA-660/3-75-009. Environmental Protection Agency. USA. Compounds. 20 : 21-38.

Cook, N.H. and Wells, P.G. 1996.Toxicity of Halifax harbour sediments: an evaluation of the Microtox solid-phase test. *Wat. Qual. Res. J. Canada*. 31 : 673-708.

Di Salvo, L.H. and Guard, H.E. 1975. Hunter L. Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. *Environ. Sci. Technol*. 9 : 247-251.

Dunn, B.P. and Stich, H.F. 1976. Release of carcinogen benzo (a) pyrene from environmentally contaminated mussels. *Bull. Environ. Contam. Toxicol*. 15 : 398-401.

Erhardt, M. 1972. Petroleum hydrocarbons in oysters from Galveston Bay. *Environ. Pollut*. 3 : 257-271.

Everarts, J.M., Sleiderink, H.M., Den Besten, P.J. Halbrook, R.S. and Shugart, L.R.1994. Molecular responses as indicators of marine pollution. DNA damage and enzyme induction in *Limanda limanda* and *Asterias rubens*. *Environ. Health Pers*. 102 : 37-43.

Farrington, J,W. 1989. Bioaccumulation of hydrophobic organic pollutant compounds. In Levin S.A., Harwell, M.A., Keley, J.R. Kimball, J.D., eds. Ecotoxicology*: Problems and Approaches.Springer-Verlag, Berlin, Germany*. 279-313.

Finney, D.J. 1971. *Probit Analysis,* Cambridge University Press. London.

Foster, G.D. and Crosby, D.G. 1987.Comparative metabolism of nitroaromatic compounds in freshwater, brackish water and marine decapod crustaceans. *Xenobiotica*. 17 : 1393-1404.

Gange, F. and Blaise, C. 1995. Evaluation of the genotoxicity of environmental contaminations in sediments to rainbow trout hepatocytes. *Environ. Toxicol. Water Quality*. 10 : 217-229.

Garrigues, P., Raoux, C., Lemaire, P., Ribera, D., Mathieu, A., Narbonne, J.F. and Lafaurie, M. 1990. *In situ* correlations between polycyclic aromatic hydrocarbons (PAH) and PAH metabolizing system activities in mussels and fish in the Mediterranean Sea: preliminary results. *Int. J. Environ. Anal. Chem*. 38: 379-387.

Hellou, J. and King, T.L. Willis. 2000. Seasonal and geographical distribution of PAHs in mussels, *Mytilus edulis*, collected from an urban harbour. *Int. J. Polycyclic Aromatic Compounds*. 20 : 21-38.

Hellou, J., King, T.L., Steller, S.E. and Yeats, P. 2002. Trends in the distribution of PCBs compared to PACs in sediments and mussels of Halifax Harbour. *Wat. Qual. Res. J. Canada*. 37(2): 413-428.

Holdway, D.A. and Sprague, J.W. 1979. Chronic toxicity of vanadium to flagfish. *Water Res*. 13 : 905-910.

Khan, M.A.Q., Kamal, A., Wolin, R. and Runnels, J. 1972. *In vivo* and *in vitro* epoxidation of aldrin by aquatic food chain organisms. *Bull. Environ. Contam. Toxicol*. 8 : 219-228.

King,T.L., Uthe, J.F. and Musial, C.J. 1993. Polycyclic aromatic hydrocarbons in the digestive glands of the American lobster *Homarus americanus* captured in the proximity of a coal-coking plant. *Bull. Environ. Contam. Toxicol*. 50 : 907-914.

Kuehl, D.W., Butterworth, B.C., McBride, A. and Kroner, Bahnick, D.1989. Concentration of fish by 2,3,7,8-tetrachlorodibenzo-P-dioxin: a survey of fish from major watersheds in the United States. *Chemosphere*. 19 : 513-518.

Law, R.J. Fileman, Portmann, J.E. 1988. Aquatic environmental protection. Analytical methods. MAFF Direct Fish Res.*Lowestoft*. 2 : 25.

Lee, R,F. Ryan, C. and Neuhauser, M.L. 1976. Fate of petroleum hydrocarbons taken up from food and water by the blue crab *Callinectes sapidus*. *Mar. Biol*. 37 : 363-370.

Livingstone, D.R. 1971. Organic xenobiotic metabolism in marine invertebrates. In: Gilles, R. (Ed.), Advances in Comparative Environmental Physiology, Vol. 7. Springer-Verlag, Berlin. 46-185.

Livingstone, D,R. and Pipe, R.K. 1992.'Mussels and environmental contaminants: molecular and cellular aspects', in Gosling, E. (Editor) *The Mussel Mytilus*: *Ecology, Physiology, Genetics and Culture*, Vol.25 of *Developments in Aquaculture and Fisheries Science*, Elsevier Science Publishers, Amsterdam. 425-464.

Livingstone, D.R. 1984. Biochemical differences in field populations of the common mussel *Mytilus edulis* L. exposed to hydrocarbons: some considerations of biochemical monitoring. Toxins. Drugs and Pollutants in Marine Animals, edited by Bolis, L. Zadunaisky, J and Gilles, R. (Berlin: Springer-Verlag).161-175.

Livingstone, D.R., Farra, S.V., Fossi, C., Kirchin, M.A. and Moore, M.N. 1998. Responses of the digestive gland cytochrome P450 monooxygenase system of the common mussel, (*Mytilus edulis*) to 3-methylcholanthrene and sodium phenobarbita. *Mar. Environ. Res*. 24 : 118-119.

Livingstone, D.R. and Goldfrab, S.P. 1998. Biomonitoring in the aquatic environment: use of cytochrome P450 1A and other molecular biomarkers in fish and mussels. In: Environment Biomonitoring: the biotechnology ecotoxicology interface. (Lynch, J.M. and Wiseman A. Eds) University Press, Cambridge. 101-129.

Livingstone, D.R. 1985. Responses of the detoxification/ toxication enzyme systems of molluscs to organic pollutants and xenobiotics. *Mar. Pollut. Bull*. 16 : 158-164.

Malins, D.C. (ed.,). 1977a. Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms. Vol.1. Nature and Fate of Petroleum. 321 pp. Vol. II. Biological Effects. New York: Academic Press.500 pp.

McIntyre, A.D. Whittle, (eds.) 1977. Petroleum Hydrocarbons in the Marine Environment. Charlottenlund Slot, Denmark: Cons. *Intern. Explor. Mer*. 171 : PP, 230.

Miramad, P. and Unsa, I.M. 1978. Toxicite aigue du vanadium vis-à-vis de quelques especes benthiques et phytoplanctoniques marines. *Chemosphere*. 10 : 827-832.

Michelmore, C,L., Birmelin, C., Chipman, J.K. and Livingstone, D.R. 1998. Evidence for cytochrome P450 catalysis and free radical involvement in the production of DNA strand breaks by benzo[a]pyrene and nitroaromatics in mussel (*Mytilus edulis* L.) digestive gland cells. *Aquat. Toxicol*. 41: 193-212.

Moore, M.N., Pipe, R.K. and Farrar, S.V. 1982. Lysosomal and microsomal responses to environmental factors in *Littorina littorea* from Sullom Voe. *Mar. Pollut. Bull*. 13 : 340-345.

Narbonne, J,F., Garrigues, P., Ribera, D., Raoux, C., Mathieu, A., Lemaire, P., Salaun, J.P. and Lafaurie, M. 1991. Mixed-function oxygenase enzymes as tools for pollution monitoring; field studies on the French coast of the Mediterranean sea. *Comp. Biochem. Physiol*. 100C : 37-42.

National Crop Production Centre (NCPC). 1986. NCPC Technical Bulletin No: 1. Basic computer programmes for the study of populations and other applications (E.Benigo Ed.).

Neff,J,M., Anderson, J.W., Cox, B.A., Laughlin, R.B. Jr. Ross, S,S. and Tatem, H.E. 1976. Effects of petroleum on survival, respiration and growth of marine animals. In Sources and Sinks of hydrocarbons in the Aquatic Environment. Washington DC, *American Institute of Biological Sciences*. 515-540.

Nicholls, H.B (Ed.). 1989. Investigations of marine environmental quality in Halifax Harbour. Can. Tech. Report. *Fish. Aquat. Sci*. 1693. 83 PP.

Peters, L.D., O' Hara, S.C.M. and Livingstone, D.R. 1996. Benzo(a) pyrene metabolism and xenobiotic stimulated reactive oxygen species generation by sub-cellular fraction of larvae of turbot (*Scopthalmus maximus* L.). *Comp Biochem Physiol*. 114C : 221-225.

Poland, A. and Knutson, J.C. 1982. 2,3,7,8-tetrachlorodibenzo-P-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol*. 22 : 517-554.

Porte, C., Biosca, M., Sole, M. and Albaiges, L. 2000. The Agegean Sea oil spill on the Galician Coast (NW Spain). III: The assessment of long-term sublethal effects on mussels. *Biomarkers*. 5 (6): 436-446.

Rappe, C., Swansson, S., Glas, B., Kringstad, K., Johansso, L. and Abe, Z. 1989. On the formation of PCDDs and PCDFs in the bleaching of Pulp Pap. *Canada*, 90 (8) : T273-T278.

Sole, M., Porte, C. and Albaiges, J. 1994. The use of biomarker for assessing the effects of organic pollution in mussels. *Sci Total Environ*. 159 : 147-153.

Stegeman, J.J. and Teal, J.M. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster *Crassostrea virginica*. *Mar. Biol*. 22 : 37-44.

Stokes, P.M. 1979. Copper accumulation in freshwater biota. Cooper in the environment Part-I.Ecological cycling. Nriagu, J.O. (Ed.,). New York, John Willey and Sons. 357-382.

Tay, K.L., Doe, K.G., Wade, S.J., Vaughan, D.A., Berrigan, R.E. and Moore, M.J. 1992. Sediment bioassessment in Halifax Harbour. *Environ. Toxicol. Chem*. 11: 1567-1581.

US Environmental Protection Agency. 1987. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA 600/4-87-028 DRAFT. USPEA, Cincinnati, OH.

Varanasi, U. and Malins, D.C. 1977. Metabolism of petroleum hydrocarbons: accumulation and biotransformation in marine organisms. PP.175-270. In: Malins, D.C. (ed.). *Effects of Petroleum on Artic and Subarctic Marine Environments and Organisms*. Vol. II. *Biological effects*. New York. Academic Press.

Wang, J.S. and Simpson, K.L. 1996. Accumulation and depuration of DDTs in the food chain from Artemia to Brook Trout (*Salvelinus fontinalis*). *Bull. Environ. Contam. Toxicol*. 56 : 888-895.

Winston, G.W. Di Giulio, R.T. 1991. Pro-oxidant and antioxidant mechanisms in aquatic organisms. *Aquat Toxicol*. 19 : 137-161.

Wolfe, D.A. (Ed). 1977. *Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems.* New York: Pergamon Press. 478 pp.