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CHANGES IN RESPIRATORY ENZYMES (SDH, LDH, MDH) AS STRESS INDICATORS IN SUBCELLULAR FRACTIONS OF LAMELLIDENS MARGINALIS EXPOSED TO OIL

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Abstract

We examined the changes in respiratory enzymes in response to oil effluent in freshwater mussels, *Lamellidens marginalis*. The mussels were exposed to two sublethal 1/4 (11.88 ppt) and 1/10 (8.55 ppt) LC50. After 30 days, the treated mussels were transferred to untreated fresh water and depuration (recovery) was followed for a further 30 days. At 7-day intervals, the respiratory enzymes SDH, LDH and MDH were analyzed in subcellular fractions (cytosol, microsome) from foot and gills and digestive gland of mussels. The results indicated that the oil effluent caused a disruption to the mussel's normal physiology. A decreasing trend in respiratory enzyme (SDH) activity in subcellular fractions of the digestive gland was observed predominantly in comparison to other fractions of mussel tissues for all oil concentrations tested, and 70-80% of respiratory enzymes were recovered from both recovery periods. From day 15, a decreasing trend in LDH was observed in the subcellular fractions of the digestive gland, foot and gill in all oil concentrations tested, and a 70-80% increasing trend was observed during both recovery periods. In parallel, a gradually decreasing trend was observed in MDH in the subcellular fractions of foot and digestive gland in all tested oil concentrations and 70-80% increasing trend was observed during both recovery periods. The results were analyzed for their significance.

Keywords: Oil Effluent, *Lamellidens Marginalis*, Repiratory Enzymes (SDH, LDH, MDH), Subcellular Fractions, Stress Indicators

1. INTRODUCTION

Various pollutants entering the aquatic bodies affect the normal functioning of the aquatic fauna and flora. The effects may be biochemical, physiological, histopathological or may be in the reproductive system affecting the total yield of aquatic animals. The organism when exposed to the xenobiotics undergoes inhibition or acceleration of the catalyzed reaction rate of the enzyme systems. The mechanism of these effects can involve changing the enzyme activity, biochemical processes or it may have a direct effect on the enzyme molecule (Heath, 1987; Livingstone et al., 1994).Several aquatic pollutants responsible for redox cycle processes are most potent for oxidative stress; however, contaminating compounds such as most oil effluents produce free radicals, which then reduce molecular oxygen to superoxide, thereby generating another free radical (Kappus and Sies 1981). Polyaromatic hydrocarbons (PAH) enters the aquatic environment via a

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number of sources viz, effluent discharges, oil mining refineries, oil spillage, etc. Oil effluent is a complex mixture of PAH among which benzo[a]pyrine is one of the predominant compound toxic to aquatic animals (Livingstone et al., 1994).The principal mechanism behind PAH toxicity is that they bind to the hydrophobic sites of macromolecules of the cell resulting in molecular and cellular damage and thereby carrying disturbances in normal functioning of the physiology. The PAH are lipophilic in nature and gets integrated into biological systems through uptake and accumulation by aquatic organisms with the potential to exert toxic actions.MFO activity in freshwater invertebrates has received little or no attention. In general, the lipophilic (fat-soluble) compounds are converted to more water-soluble (hydrophilic) forms by protective biotransformation enzymes found in all living organisms (Goldberg, et al., 1978; Neff, 1988; Balamurugan, 2005; Balamurugsan, 2021; Balamurugan and Subramanian, 2021).

The chemical concentration in the environment may not be enough to kill the organism. However sublethal concentrations often affect the biochemistry of an organism. The nature and extent of pollution depend on the dissolved oxygen (DO) in the aquatic bodies and these effects that deplete the DO can cause stress to animals living in it. The initial symptoms of sublethal poisoning are alteration or failure of respiratory metabolism (Spicer and weber, 1991). Since the oxygen consumption is an index of respiratory metabolism, fluctuations in the activities of the respiratory enzymes serves as an early marker to assess the extent of pollution in the exposed aquatic animals (McMahon, 2001 and Chinni et al., 2002).Therefore, the bioaccumulation of chemicals in biota may be a prerequisite for testing the adverse effects of xenobiotics on the ecosystem (Franke et al., 1994). It has also been suggested that the uptake rate of hydrophobic chemicals such as oil effluent depends on the stability of the membranes and the molecular weight of the chemical and it appears and is most concentrated and retained for long periods by marine animals (Neff, et al., 1976). Therefore, it is necessary to study the accumulation of pollutants in the commercially important aquatic animals. PAHs can interact with cells to elicit a toxic response by binding to lipophilic sites in cells and interfering with cellular processes (Long et al., 2003).

Mitochondria are the most essential cell organelle that plays an important role in the cell metabolism. One of the primary functions of mitochondria in the cell is the generation of ATP for cellular activities (Flower, et al., 1982). Since mitochondrial oxidative process plays a central role in the maintenance of cellular energy supply (Burcham and Harman, 1991) the deleterious effect of xenobiotics on mitochondrial respiration have a serious consequences on the normal physiological functions. Respiratory enzymes such as succinate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase etc. are involved in cellular respiration through glucose metabolism which yields the high energy compound adenosine triphosphate (ATP). Enzymes serve as indicators of tissue and molecular dysfunction. Depending on their function, enzymes are divided into oxidative, hydrolytic and transaminating enzymes. Any stress/toxicity the organisms are exposed to would alter the enzyme levels as the cells or tissues are directly damaged. Such changes have been reported in the activities of lactate dehydrogenase (LDH), succinate

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dehydrogenase (SDH) in animals exposed to toxicity (Payne and Payne, 1985). The changes in enzymatic activities directly reflect metabolic disorders and cell damage in certain organs (Casillas et al. 1983). Therefore, it is considered a sensitive biochemical indicator of hazardous effects on aquatic organisms, including molluscs, and these are important parameters for testing water in the presence of toxins (Velmurugan et al. 2008).

Succinate is one of the major metabolic end products in many facultative anaerobes such as parasitic helminths (Saz, 1971) and mussels (Holwerda and de Zwaan, 1979, 1980). The enzyme succinate dehydrogenase (SDH) is believed to catalyze the NADH-dependent reduction of fumarate to succinate under anaerobic conditions and thus acts as a fumarate reductase (Singer et al., 1972). This is an apparent reverse reaction of the succinate to fumarate aerobic oxidation step in the tricarboxylic acid cycle. Thus, in the absence of oxygen, fumarate would act as an electron acceptor. Kaundinya and Ramaurthi (1978) in *Tilapia mossambica*; Kabeer Ahmed (1979) in *Pila Globosa* and (Alam 1984) in *V. Bengalenis* discovered altered oxidative metabolism caused by organophosphate pesticides and metals, reported SDH depletion and increased LDH activity in their species, respectively. LDH enzyme response in *Lamellidens marginalis* (Pandey et al., 2018); MDH activity in subcellular tissues from four mussels (Paynter et al., 1985); SDH, MDH activities in freshwater mussel, *Lamellidens marginalis* (Santhya, 2017). Seasonal changes of two biomarkers of oxidative stress (LDH, MDA) in the edible mollusks *Donax trunculus* Sififi and Soltani, 2018; mercury-induced changes in the energetics (SDH, LDH) of the hepatopancreas of two freshwater molluscs, *Pila globosa* and *Pila nigra*; tissue metabolism of LDH and MDH in mollusks (Karam and Al-Wazzan, 2021; SDH, LDH, MDH metabolism in the tissues of three species of scallops (Soldatov, et al., 2010). Vijayavel and Balasubramanian (2006) found that changes in respiratory enzymes acted as stress markers in shrimp *Scylla serrata* exposed to naphthalene.

Lactate dehydrogenase (LDH), an enzyme that catalyzes the conversion of lactate to pyruvate and the oxidation of nicotinamide adenine dinucleotide (NAD) to NADH2 (reduced nicotinamide adenine dinucleotide), is a crucial part of the glycolysis cycle. Chemical stress has been represented by the presence of this enzyme (Diamantino et al., 2001). Lactate dehydrogenase catalyzes the reversible conversion of pyruvate to lactate with the simultaneous oxidation of NADH to NAD+. Under anaerobic conditions, LDH becomes an important enzyme due to its ability to regenerate NAD+ and allows for a continuous flow of carbon through the glycolytic pathway to support anaerobic ATP synthesis (Hochachka and Somero, 2002). This process can be particularly important in organisms exposed to prolonged hypoxic or anoxic conditions that require maintenance of energy balance solely through the functioning of glycolysis. Elevated lactate dehydrogenase (LDH) activity is a marker of tissue damage in fish (Ramesh et al., 1993) serves as a good diagnostic tool in toxicology. Biomarkers for exposure to petroleum hydrocarbons (LDH) have been detected in *Mytilus edulis* planulatus using respiratory enzymes (Sara, et al., 2003).

MDH is an enzyme that catalyzes the NAD/NADH-dependent conversion of malate and oxaloacetate as a substrate. This reaction plays a key role in the malate/aspartate shuttle

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across the mitochondrial membrane and in the tricarboxylic acid cycle within the mitochondrial matrix. The TCA cycle is complete when the oxidation of L-malic acid to oxaloacetic acid is achieved by the enzyme MDH. The biomarker malondialdehyde (MDA) for lipid peroxidation of polyunsaturated fatty acids in cell membranes is another that can be used to assess oxidative stress (Sarkar, et al., 2006). MDA is a byproduct of lipid peroxidation and a biomarker of free radical damage in lipid molecules. The effects of anaerobic and recovery periods on the expression of pyruvate kinase and lactate dehydrogenase in the foot muscle of the sea mussel *Mytilus galloprovincialis* have been studied (Lushchak,et al., 1997).The glycolytic enzymes (SDH, LDH, MDH) in the freshwater Unionidae family have been studied (Ieva Roznere et al., 2021). Comparative microbiological study of ascorbic acid and dehydrogenase (SDH, LDH and MDH) levels in two populations of green mussels (*Perna viridis*) (Indumathi, et al., 2015).

One of the major factors impeding conservation success is our lack of understanding of freshwater mussel health and disease (Waller and Cope, 2019). Because mussel physiology is understudied, the exact roles of potential causes of population declines (e.g., habitat destruction, pollution) are poorly understood. Captivity's effects on mussel physiology, as well as appropriate health assessment techniques, are also unknown. A preliminary study on the freshwater mussel *Lamellidens marginalis* was conducted to fill a knowledge gap about the tissue and subcellular distribution of oxidoreductase enzyme activity in mollusks. Due to the scarcity of data on respiratory enzymes in the subcellular fraction of invertebrates, particularly bivalves, this study seeks to understand the oxidoreductase/oxidative stress enzymes for the freshwater mussel, *Lamellidens marginalis*.

2. MATERIALS AND METHODS

2.1 Animals

In 2021, freshwater mussels of the species *Lamellidens marginalis* (Lamarck) were gathered from the Cauvery River in Tiruchirappalli, Tamil Nadu, India, and brought to the lab. Their total length was 6-7 cm, and their weight was 25-27 g. Five days were spent acclimating the mussels to their environment before any of the animals were used in the enzyme test.

2.2 Preparation of Sub-cellular fractions

Freshwater mussel gill, foot, and digestive gland tissues were processed according to the method of Livingstone and Farrar (1984) to isolate sub-cellular fractions (cytosol and microsomes). All of the prep work was done at a temperature of 4 degrees Celsius. Using 20 mM Tris-HCl (pH 7.6) (1 g/4 ml) containing 0.25 M sucrose, 0.15 M KCl, 1 mM EDTA, 1 mM DTT, and 100 lM PMSF, were homogenised a mixture of tissues. Everything was done in a temperature controlled environment of 4 degrees centigrade. Centrifugation at 600 xg for 10 minutes was used to separate nuclei and cell debris from the homogenised samples. The materials were re-centrifuged at 12,000 xg for 45 minutes, but this time the pieces weren't transferred. The recovered supernatant was then employed as a

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mitochondrial fraction. This pellet was resuspended in homogenising buffer, centrifuged at 100,000x g for 90 minutes, and the resulting supernatant was collected and utilised as the cytosolic fraction. The leftover pellet was resuspended in a solution containing 20 mM Tris pH 7.6, 1 mM Dithiothreitol, 1 mM EDTA, and 20% v/v glycerol. A microsomal fraction was prepared from this pellet after it was resuspended.

2.3 Exposure Experiment

Oil effluent was found to have an LC_{50} of 96 hours by Balamurugan (2005); using this information, sublethal doses of $1/4^{th}$ (11.88 ppt) and $1/10^{th}$ (8.55 ppt) of LC_{50} were generated and employed in a biochemical investigation. Two sets of plastic tubs, each holding 10 liters, were employed in this experiment. Mussels were placed in either 8.55 ppt or 11.88 ppt of oil effluent in each container. At the same time, a control run was conducted without the inclusion of oil wastewater. Mussels were killed every week for biochemical testing. The treated mussels were reintroduced into fresh water, and a depuration (recovery) study was done after 30 days. A total of eight mussels (four from each of two containers) were taken for examination.

2.4 Biochemical analysis

2.4.1 Succinic dehydrogenase (SDH)

Nicholas, et al., (1960) approaches was used to calculate SDH. There were a total of 40 µ moles of sodium succinate, 100 u moles of sodium phosphate buffer (pH 7.4), and 4 µ moles of INT in the reaction mixture, which had a final volume of 2.0 ml. With the addition of just 0.5 ml of homogenate, the reaction was set in motion. After 30 minutes of thermostatic incubation at 37°C, 5.0 ml of glacial acetic acid was injected to halt the enzyme process. Overnight at 50 degrees Celsius, 500 millilitres of toluene was used to extract the produced formazan. In a colorimeter, the formazan's hue was evaluated at 495 um against a toluene blank. Moles of formazan produced per milligramme of protein per hour was used as a measure of SDH activity.

2.4.2 Lactate dehydrogenase (LDH)

The specific activity of LDH was measured using the approach of Nicholas et al., (1960). There are a total of 10 moles of phosphate buffer (pH 7.4) and 40 µ moles of INT in the 2.0 ml of final volume of the reaction mixture. When 0.4 ml of homogenate was added, the reaction began. The reaction mixture was incubated at 36°C for 30 minutes, and the reaction was halted by the addition of 5.0 ml of glacial acetic acid. Five millilitres of toluene were used to extract the formazon, which was then compared to a toluene black control that had not been exposed to light. Moles of formazan produced per milligramme of protein per hour was used as a measure of the enzyme's activity.

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2.4.3 Malate dehydrogenase (MDH)

The approach of Nachlas et al., (1960), with a tweak given by Prameelamma and Swami, (1975) was used to calculate MDH activity. The enzyme was extracted from the supernatant (cytosol, microsomes). Final volume of the reaction mixture was 2.0 ml, and it comprised 100 µ moles of phosphate buffer (pH 7.4), 4 µ moles of INT, 50 µ moles of sodium malate, and 0.1 µ moles of NAD+ enzyme. This mixture was left to incubate at 37°C for 30 minutes before being neutralised with 5.0 ml of glacial acetic acid. Following an overnight incubation at 50 °C, the colour was extracted into 5.0 ml of toluene, and the resulting color's optical density at 495 nm was measured using a spectrophotometer. The same procedure was performed on a 0.5 ml sample of distilled water as a blank and on a 0.5 ml sample of the boiled enzyme as a control. INT benchmarks were developed for evaluation. Formazan micromoles per milligramme of protein per hour is the unit of enzyme activity. The amount of protein was measured using a standard of bovine serum albumin and the procedure described by Lowry et al., (1951).

2.5 Statistical Analysis

Values are shown as the mean plus standard error of the mean for the three calculations. Linear correlation and student's t-test was used to assess differences between datasets, and a significance level of $P<0.05$ was taken as indicating a significant difference.

3. RESULT

3.1 Succinic dehydrogenase (SDH)

SDH activity was measured at 0.285 in the cytosol and 0.278 in the microsomes of control mussels; 0.338 in cytosol and 0.337 in microsomes; and 0.534 in the cytosol and 0.516 in microsomes at 1/4 control; 0.289 in cytosol and 0.278 in microsomes; 0.345 in cytosol and 0.336 in microsomes; and 0.540 in the cytosol and 0.520 in microsomes (µmole formazan/mg protein/h) at 1/10 control in the foot, gills and digestive gland, respectively (Figures 1-4).

Mussel tissues exposed to both concentrations of oil showed a statistically significant (*P<0.05*) and progressive decrease in SDH activity relative to control in the subcellular fractions. Comparing control tissues from mussel subcellular fractions at both sublethal exposures, more than 50 percent of SDH depletion activity was observed from day 1 and reached a minimum by day 30. On the first day, at 0.161 in the cytosol and 0.156 in the microsomes, more than 50% of the SDH depletion activity was observed, and it decreased on the 30th day of the foot tissue to 0.059 in the cytosol and 0.057 in the microsomes on the first day at 0.285 im Cytosol, 0.273 in microsomes, more than 60% SDH depletion activity was observed and it decreased to 0.080 in cytosol, 0.068 in microsomes on day 30 of gill tissue, on day 1 at 0.331 in gill tissue cytosol, 0.324 in microsomes, more than 50 % of SDH depletion activity was observed and it decreased to 0.105 in cytosol, 0.081 on day 30 of digestive gland tissue of 1/4 exposure period (Figure-1); on the first day at 0.162 in cytosol, 0.159 in microsomes, more than 60% of

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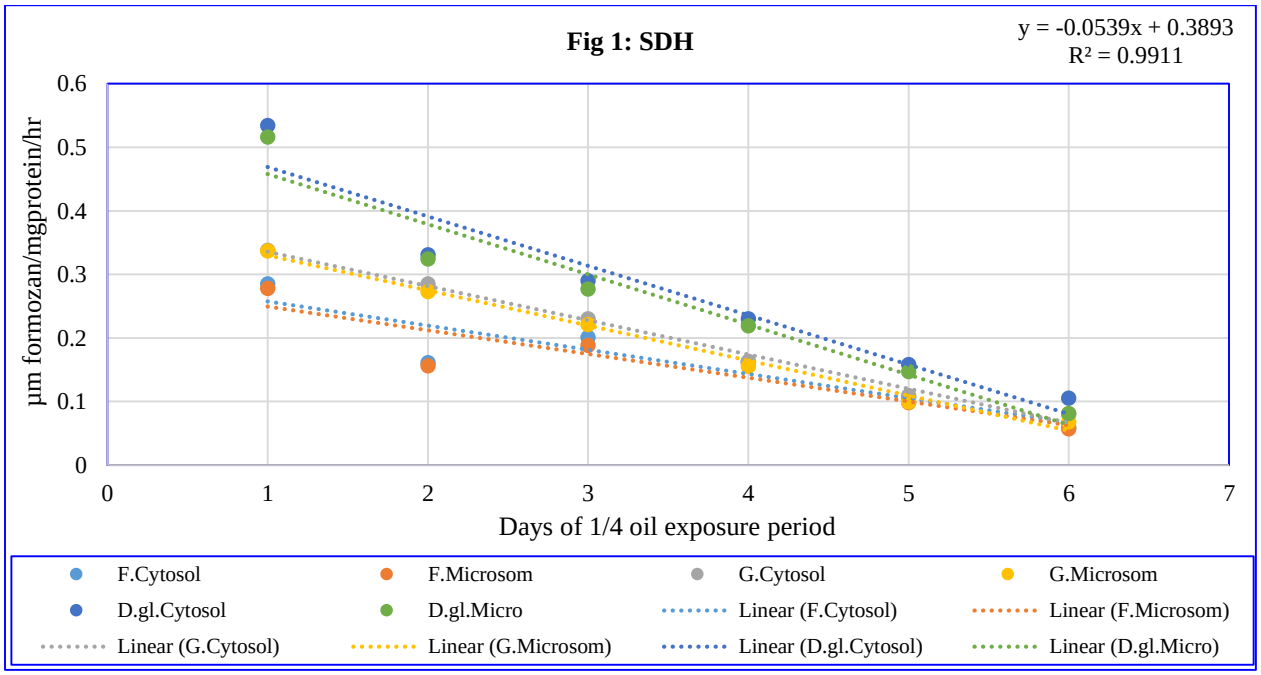
SDH depletion activity was observed and it decreased to 0.064 in cytosol, 0.063 in microsomes on day 30 of foot tissue, on day 1 at 0.295 in foot tissue cytosol and 0.290 in microsomes, more than 60% of SDH depletion activity was observed and it decreased to 0.089 in cytosol and 0.084 in microsomes on day 30 of gill tissue, to 0.350 in cytosol and 0.346 in microsomes on day 1, more than 60% of SDH -Depletion activity was observed and it decreased to 0.112 in cytosol and 0.108 in microsomes on day 30 of the digestive gland tissue of a 1/10 exposure period (Figure-2) .

Freshwater mussels showed a consistent decrease in SDH activity across all tissues and subcellular fractions from day 1 to day 30. By the 30th day, 80–90 percent of SDH activity was restored compared to controls in subcellular fractions of mussels. On the 30th day at 0.255 in the cytosol, 0.248 in the microsomes, more than 80-90 percent of SDH activity was recovered in the foot tissue, likewise,0.265 in the cytosol and 0.261 in the microsomes in the gill tissue,0.328 in the cytosol and 0.325 in the microsomes µmoles of formazan /mg protein/hr were recovered in the digestive gland tissue in 1/4th depuration respectively (Figure 3); on the 30th day at 0.285 in the cytosol, 0.277 in the microsomes in the foot tissue, 0.297 in the cytosol and 0.288 in the microsomes in the gill tissue,.336 in the cytosol and 0.327 in the microsomes µmoles of formazan/mg protein/hr were recovered in 1/10th of the depuration period of digestive gland tissue (Figure 4).



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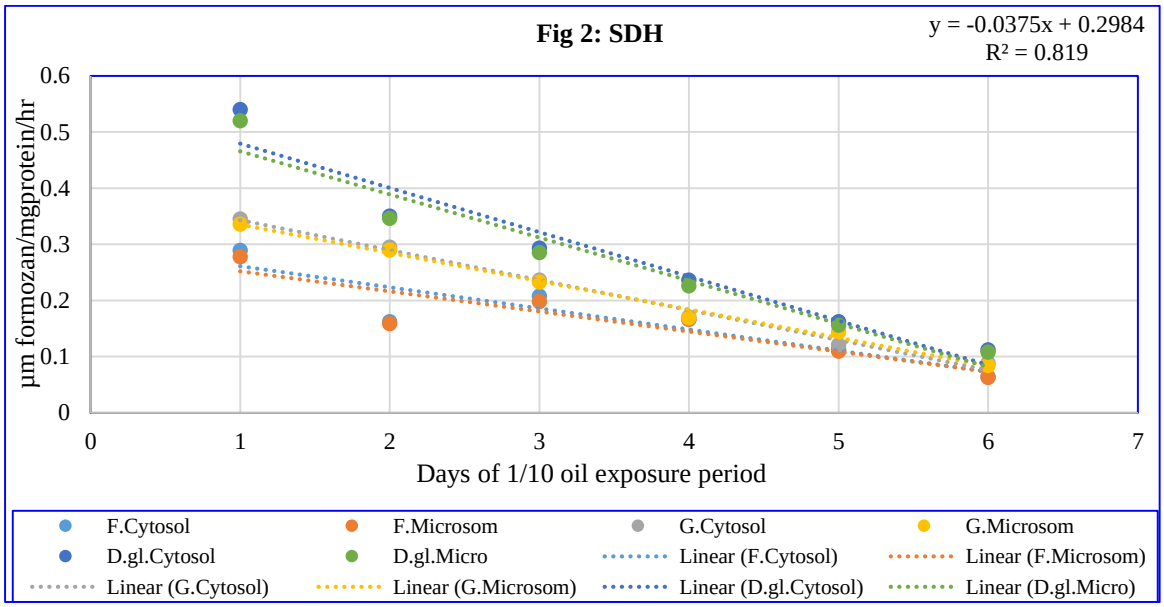

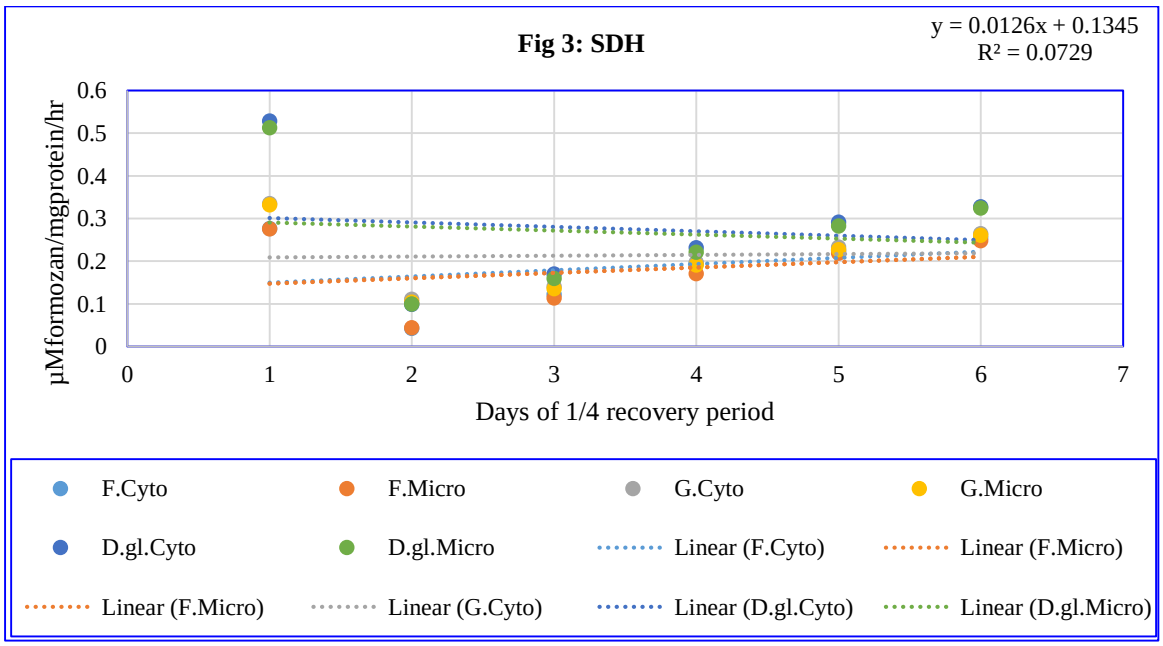

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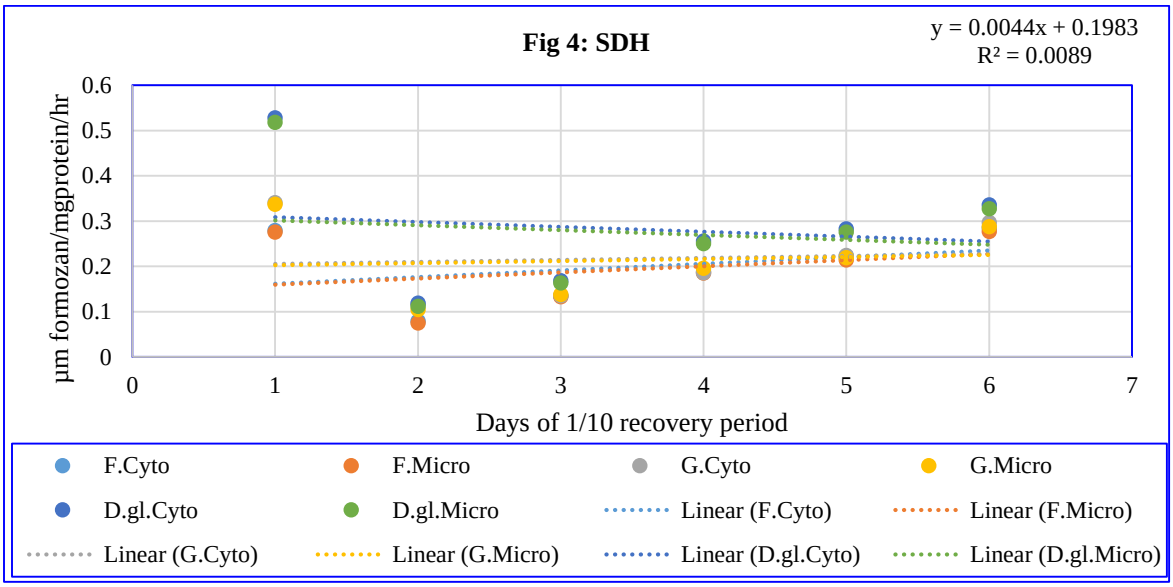

3.2 Lactate dehydrogenase (LDH)

For control bivalves, LDH activity was measured at 1.27 in the cytosol and 1.28 in the microsomes. 0.87 in the cytosol and 0.88 in the microsomes; and 1.89 in the cytosol and 1.81 in microsomes after 1/4th oil exposure in the foot, gill, and digestive gland tissue; 1.34 in the cytosol and 1.31 in the microsomes; 0.91 in the cytosol and 0.89 in the microsomes; and 1.91 in the cytosol and 1.84 in the microsomes µmoles of formazan/mg protein/hr) in 1/10th oil exposure in the foot, gill, and digestive gland tissue (Figures 5-8). In both concentrations of oil exposure, a significant (*P<0.05*) and gradual decrease in LDH activity was observed in the subcellular fractions of mussel tissues.

When comparing the control of mussel tissues from subcellular fractions in both sublethal exposures, more than 50% of LDH depletion activity was observed from the first day on and reached a maximum on the 30th day compared to the control. On the first day, at 1.05 in the cytosol and 1.03 in the microsome, more than 50 % of LDH depletion activity was observed, and it increased to1.55 in the cytosol and 1.3 in the microsome on the 30th day of the foot tissue, on the first day at 0.39 in the cytosol,0.38 in micro some,50% of LDH depletion activity was observed, and it increased to 0.80 in the cytosol, 0.73 in the microsome on the 30th day of gill tissue, on the first day at 2.22 in the cytosol, 2.17 in the microsome, more than control of LDH activity was observed, and it increased to 2.71 in the cytosol, 2.53 in micro some on the 30th day of digestive gland tissues of 1/4th exposure periods respectively (Figure-5);on the first day at 1.08 in the cytosol,1.06 in micro some, more than 50 % of LDH activity was observed and it reached to1.62 in the cytosol, 1.40 in the microsome on the 30th day of foot tissue,on the first day at 0.44 in the cytosol and 0.40 in the microsome, 50 % of LDH activity was observed and it reached to 0.77 in the cytosol and 0.73 in the microsome on the 30th day of 1/10th oil exposure,on the first day at 2.23 in the cytosol and 2.20 in microsome, more than control was observed

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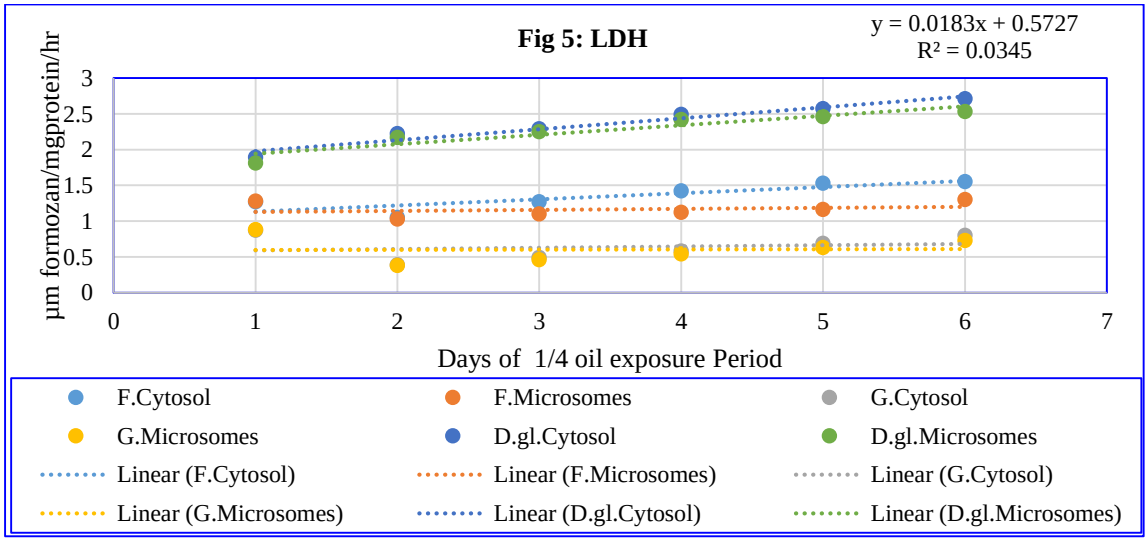
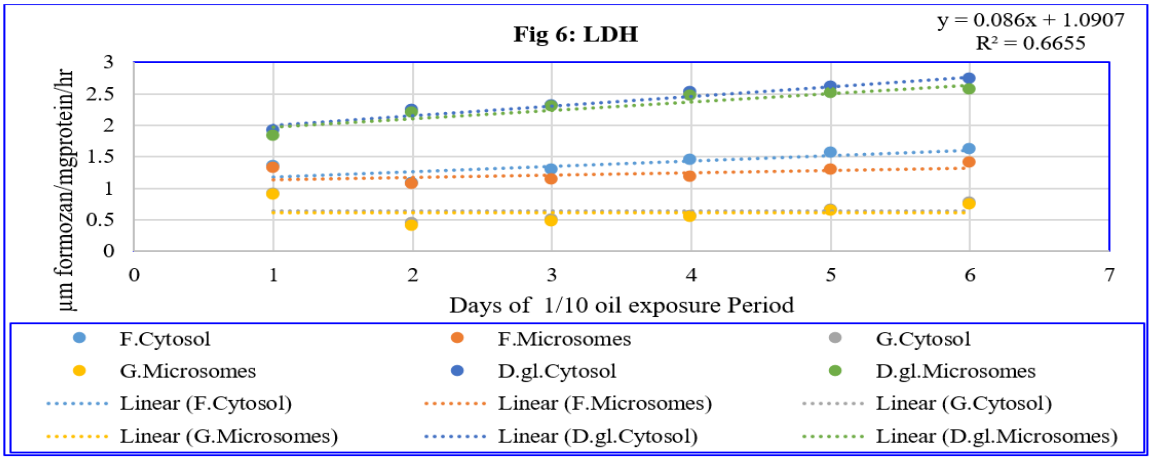
and it reached to 2.73 in the cytosol and 2.56 in the microsome on the 30th day of the digestive gland tissue of 1/10th exposure periods respectively (Figure-6).

From the first to the 30th day of both recovery periods, there was a gradual decrease in LDH activity in all tissues of the subcellular fraction in freshwater mussels. By the 30th day, 80-90 percent of LDH activity was restored compared to controls in subcellular fractions of mussels. More than 80-90 percent of LDH activity was recovered on the foot tissues on the 30th day at 0.82 in the cytosol and 0.81 in the microsome, similarly on the 30th day at 0.35 in the cytosol and 0.32 in the microsome on the gill tissues on the 30th day, on the 30th day at 1.60 in the cytosol and 1.56 in the microsome on the digestive gland tissue of 1/4th exposure periods, respectively (Figure 7); on the 30th day at 0. 80–90 percent of LDH activity was observed on the gill tissues, on the 30th day; at 1.55 in the cytosol and 1.48 in the microsome, 80–90 percent of LDH activity was recovered on the digestive gland tissue of 1/10th recovery periods, respectively (Figure 8).





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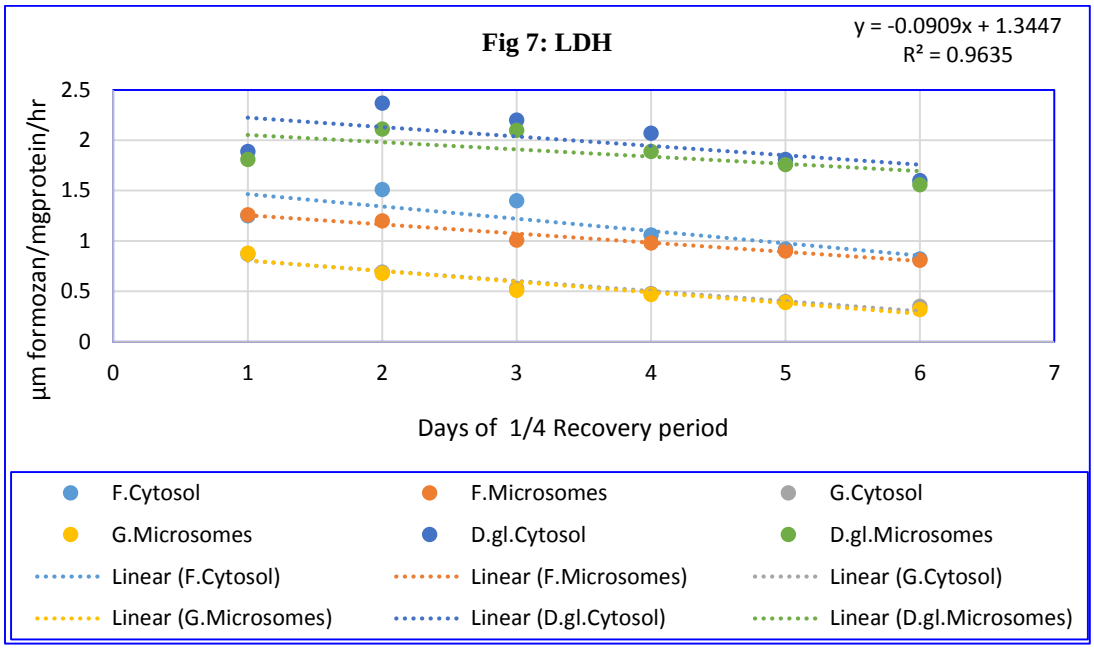

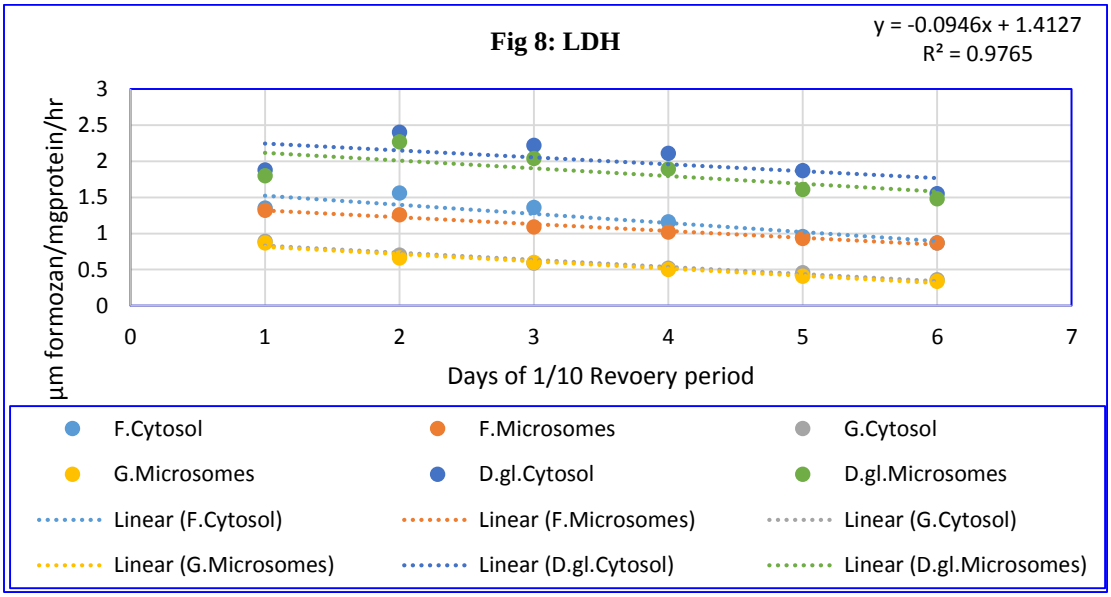

3.3 Malate dehydrogenase (MDH)

MDH activity in control mussels was found to be 2.93 in the cytosol and 2.90 in the microsome; 1.54 in the cytosol and 1.52 in the microsome; and 2.09 in the cytosol and 2.07 in the microsome at 1/4 oil exposure; 2.96 in the cytosol and 2.91 in the microsome; 2.08 in the cytosol and 2.06 in the microsome; and 2.95 in the cytosol and 2.90 in the microsomes moles formazan/mg protein/h at 1/10 oil exposure in the foot, gills and digestive gland, respectively (Figs. 9-12). Subcellular fractions of mussel tissues showed

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a significant (*P<0.05*) and time-dependent decrease in MDH activity after exposure to both oil concentrations. Comparing the regulation of tissues from subcellular fractions of mussels exposed to both sublethal exposures, more than 50% of MDH depletion activity was detected on day 1, with a trough on day 30. On day 1, MDH depletion activity was 2.32 in the cytosol and 2.30 in the microsome; by day 30 it had decreased to 1.49 in the cytosol and 1.44 in the microsome. Comparable MDH depletion activity was also observed in foot and gill tissues at day 1 at 1.47 in cytosol and 1.45 in microsome and again at day 30 at 0.96 in cytosol and 0.91 in microsome (Figure-10).

The MDH activity in control bivalves was found to be 2.93 in the cytosol and 2.90 in the microsome; 1.54 in the cytosol and 1.52 in the microsome; and 2.09 in the cytosol and 2.07 in the microsome in 1/4th oil exposure; 2.96 in the cytosol and 2.91 in the microsome; 2.08 in the cytosol and 2.06 in the microsome; and 2.95 in the cytosol and 2.90 in the microsome µmoles of formazan/mg protein/hr in 1/10th oil exposure of the foot, gill, and digestive gland in the foot, Subcellular fractions of mussel tissues showed a substantial (*P<0.05*) and time-dependent decrease in MDH activity following exposure to both concentrations of oil. When comparing the regulation of tissues from subcellular fractions of mussels subjected to both sublethal exposures, more than 50% of MDH depletion activity was detected from the first day on, with a low on the 30th day. On day 1, MDH depletion activity was 2.32 in the cytosol and 2.30 in the microsome; by day 30, it had decreased to 1.49 in the cytosol and 1.44 in the microsome. Comparable MDH depletion activity was also observed in foot and gill tissues on day 1 at 1.47 in the cytosol and 1.45 in the microsome, and again on day 30 at 0.96 in the cytosol and 0.91 in the microsome (Figure-10). After 30 days at 2.45 in the cytosol and 2.40 in the microsome, MDH activity was recovered on foot tissue; after 30 days at 0.96 in the cytosol and 0.91 in the microsome, MDH activity was recovered on gill tissue; and after 30 days at 2.14 in the cytosol and 2.12 in the microsome, 95% of MDH activity was recovered on the digestive gland tissue in a quarter of the depuration period (Figure 11-12). Neither the duration nor the concentration of oil exposure affected the levels of SDH, LDH, and MDH activity in the mussels' subcellular tissues (Figures 1–9). On day one of both exposure periods, enzyme activity increased, and then decreased, by day 30. Curiously, by day 30, following both the recovery and depuration periods, enzyme activity (SDH, LDH, and MDH) had returned to normal levels (80%–90%) (*P<0.05*).

For example, this study would confirm the dominance of SDH during accumulation and recovery from both sublethal concentrations, as well as the activity of LDH in the cytosol fraction of the digestive gland. Mussel foot tissue had the highest levels of MDH activity in the cytosol

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Fig 9: MDH

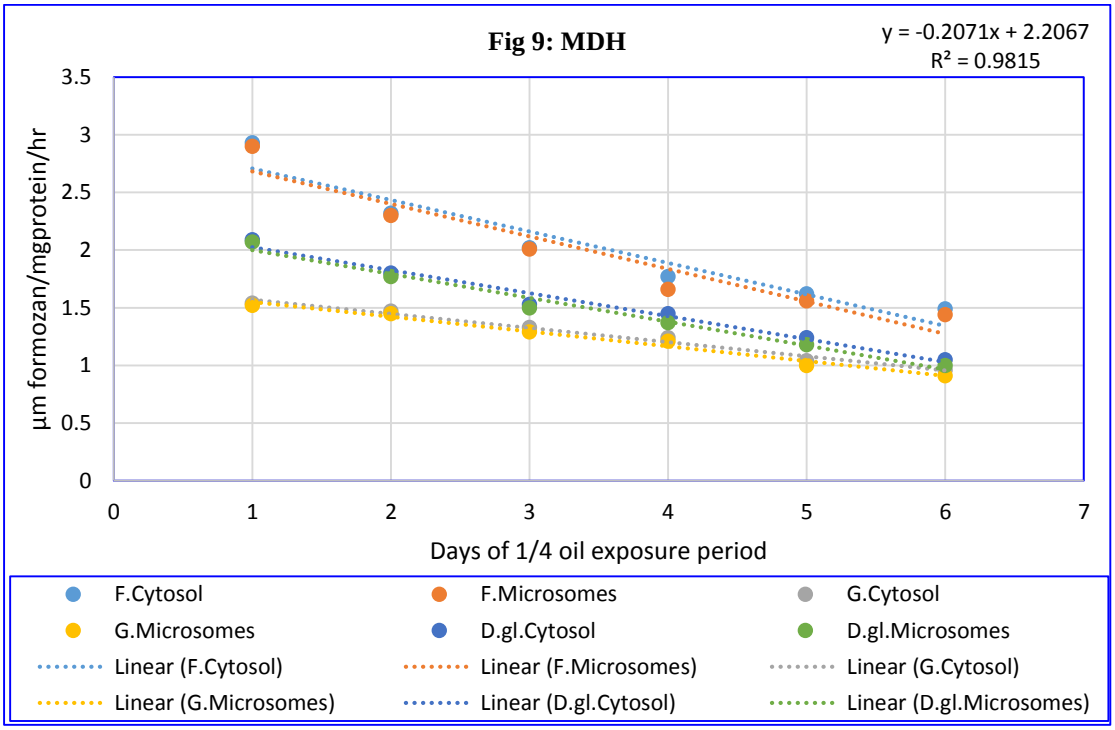
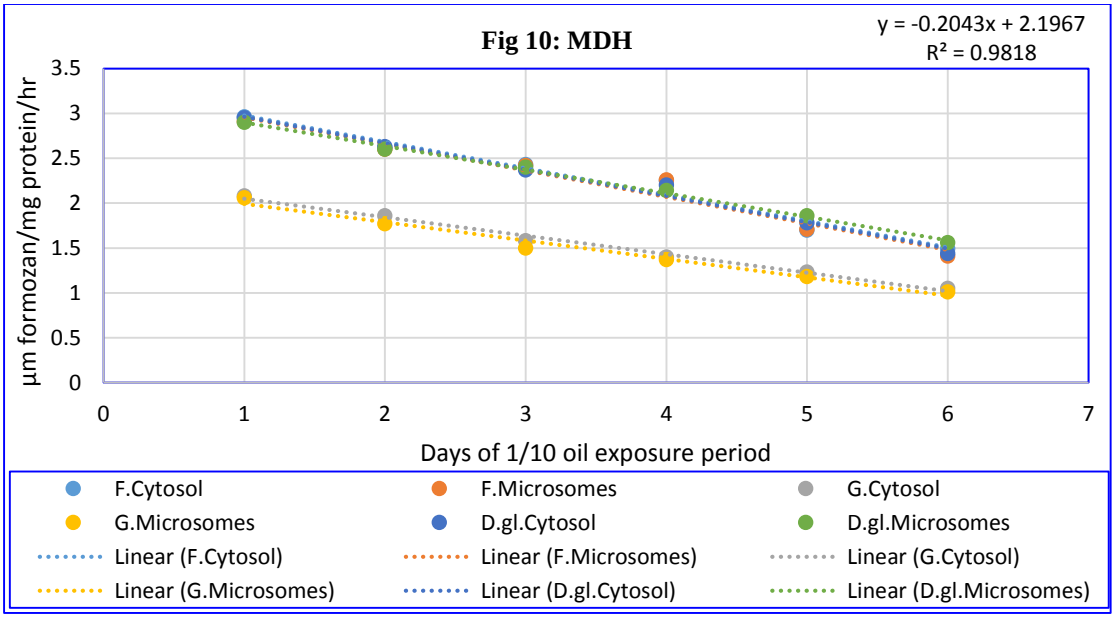


Fig 10: MDH



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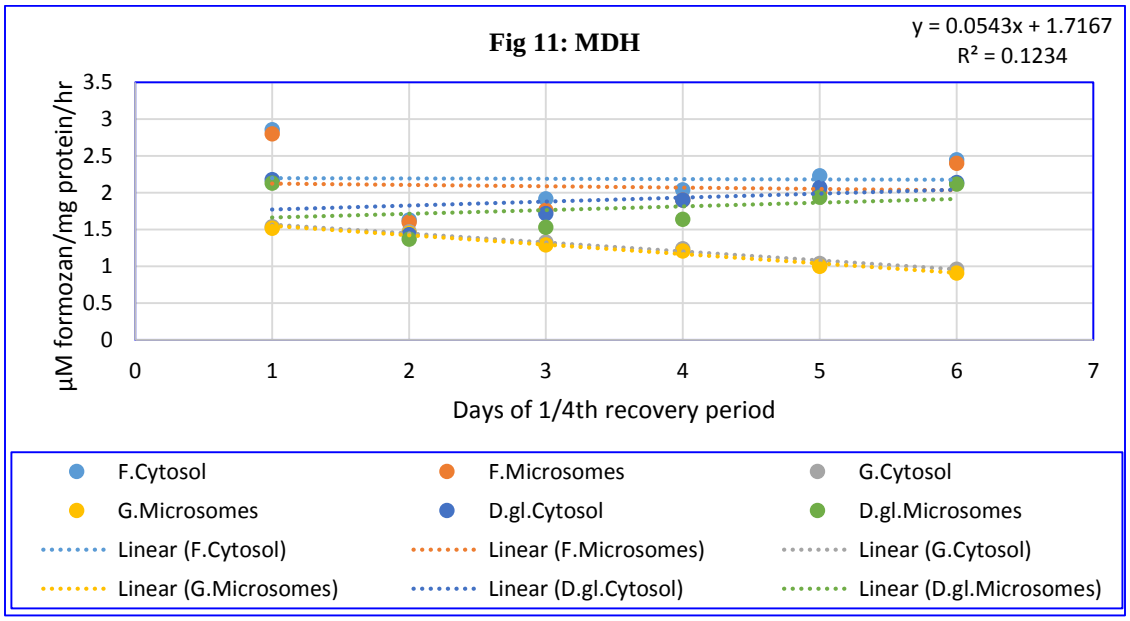

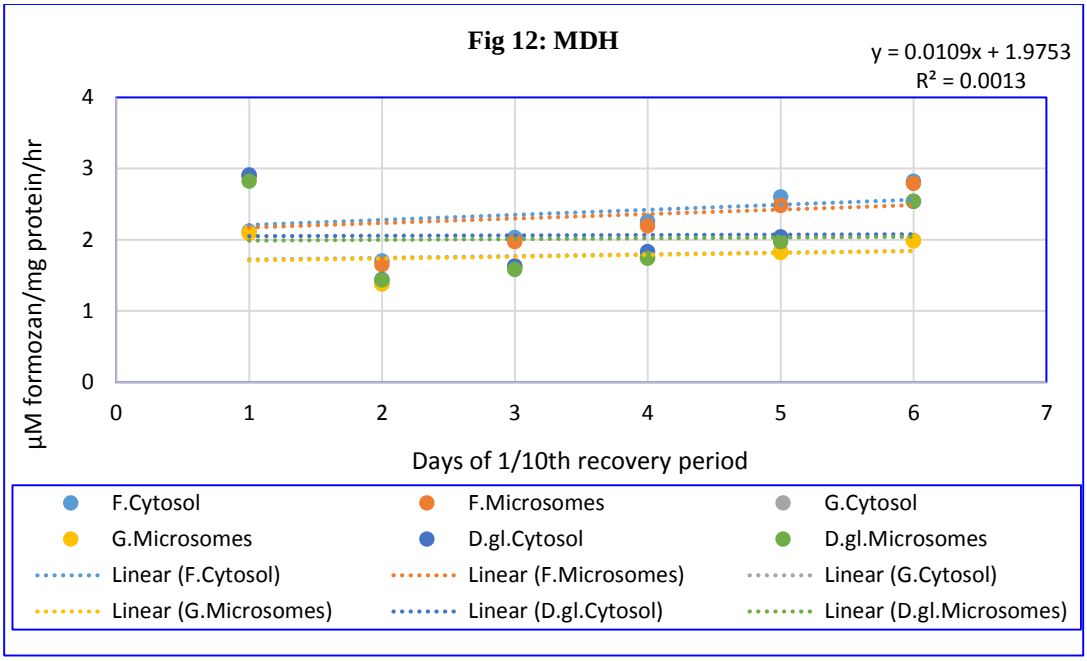

4. DISCUSSION

The idea behind the biomarker strategy is to study how an organism's internal systems change in response to oil effluent pollution. A major advantage of biomarkers over chemical residue analysis is that they quantify the stress level of the organism. *Lamellidens marginalis*, a freshwater mussel, is an excellent model to observe how pollution affects the body's physiological processes in the following order: >SDH in the

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cytosols, microsomes of the digestive gland, foot and gill at 1/4 and 1/10 exposure ; >LDH in the cytosols, microsomes of the digestive gland, foot and gill at 1/4 and 1/10 exposure; >MDH in the cytosols, microsomes of the foot, digestive gland and gill at 1/4 and 1/10 exposure, respectively.

TCA cycle enzymes such as SDH, LDH and MDH were found to be decreased and increased in the digestive gland, foot and gills exposed to oil discharge and recovery periods, respectively (Figures 1-12). The enzymes located in the mitochondria catalyze the oxidation of a number of substrates via the citric acid cycle to reduction equivalents. These reduction equivalents are channeled through oxidative phosphorylation, which provides the energy needed for many cellular functions. The inner and outer mitochondrial membranes contain unsaturated lipids and are more susceptible to attack by oxidants (Bironaite and Ollinger, 1997). Thus, damage to the mitochondrial membrane leads to inhibition of mitochondrial enzymes. Inhibition of these enzymes could further affect mitochondrial substrate oxidation, resulting in decreased oxidation of substrates, further reducing the transfer rate of reduction equivalents to molecular oxygen and the depletion of generated energy (Eaton and Gallagter, 1994).

SDH was reduced in the subcellular fractions of the digestive gland, foot and gill. Glutathione (GSH), which is not synthesized in mitochondria, is required for mitochondrial function and is therefore imported from the cytosol (Martensson et al., 1990). GSH therefore plays a critical role in cell viability by regulating mitochondrial inner membrane permeability by maintaining sulfhydryl groups in the reduced state (Femandes-Checa et al., 1980). This suggests that oxidative stress and GSH deficiency affect redox status in mitochondria, leading to protein inactivation through oxidation of protein thiols. Therefore, the decrease in mitochondrial enzyme activities is due to the decreased glutathione levels.

The decreased activities of the TCA cycle enzymes suggest that the state of use of pyruvate for the generation of the reducing equivalent in the TCA cycle is delayed during exposure to oil effluents. The present study also found that the SDH enzyme activities in the the oil effluent exposed mussels decreased. This reduced enzyme activity, delay in oxidative phosphorylation and ATP production in mitochondria may well be associated with the consequences of xenobiotic stress. The influence of environmental pollution on cellular respiration in mussels also found a shift from aerobic to anaerobic metabolism (Wang et al., 1992, DeZwaan et al., 1995, Eertman et al., 1996, Page et al., 1998, Bennett, 2000).Nevertheless, the current results are consistent with existing research. Satyaparameshwar et al., (2006) found that SDH occurred at 17,642.10 µmol formazan/g wet weight tissue/h in the labial palp and at 14,860.70 µmol formazan/ g wet weight tissue/h in gills exposed to sublethal concentrations of copper sulfate for 72 hours.

In the foot tissue of the sea mussel *Scapharca inequivalvis*, Livingstone, et al. (1983) reported a specific activity of SDH of 8.19 M/min/gr/fr.wt. The toxicity of methylparathion in *L. marginalis* was reviewed by Moorthy et al. (1985) at 0.0340.03 µmol formazan/mg/h on the hepatopancreas and at 0.0240.02 µmol formazan/mg/h on the gill tissue. Similarly,

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Mytilus edulis planulatus treated with petroleum hydrocarbon showed SDH activity (Sara et al., 2003), and heavy metal exposure in *L. marginalis* also elicited SDH activity (Sandhya, 2017). It is an example of a physiological and biochemical adaptation that cells exposed to oil effluents experience a reversal of their anaerobic activity stress during recovery from pollution. Similarly, in *Lamellidens marginalis* exposed to metals, it was reported that LDH activity in the digestive gland, foot and mantle decreased significantly and in a time-dependent manner (Sandhya, 2017).

LDH is widely used in ecotoxicity studies to diagnose damage to cells, tissues and organs (Diamantino et al., 2001). Mitochondrial damage leads to decreased respiration and partial uncoupling of oxidative phosphorylation. The activity of the cytoplasmic enzyme LDH showed a marked decrease in its activity in subcellular fractions of the digestive gland, foot and gill of mussels in the present study. LDH is generally associated with cellular metabolic activity. It functions as a central enzyme between the glycolytic pathways and the tricarboxylic acid cycle. Low LDH activity in the gills of *L. marginalis* during both recovery periods is consistent with the notion that the changes in LDH activity were caused by the reproductive stage, as energy would have been diverted to gametogenesis and conserved in other tissues.

The carbohydrate-metabolizing enzyme LDH is crucial. By blocking LDH function, lactic acid, a byproduct of anaerobic glycolysis, builds up in the body. Decreases in LDH activity suggest that cellular oxidation is blocked by oil exposure stress. Nevertheless, the current results are consistent with existing research results. Valarmathi and Azariah (2003) exposed *Sesarma quadratum*, the muzzle crab, to copper and found that the activity of LDH was increased. Muscle LDH increased by 166.2.8 U/L, gill LDH by 97.23 U/L and hepatopancreatic LDH by 146.1.4 U/L after treatment with chlorpyrifos compared to the control group (Amanullah et al., 2010). The specific activity of LDH in the foot of the freshwater mussels *Unio pictorum* and *Anodonta cygnea* was measured by Livingstone et al., (1983) to be 2.29 and 3.12 µmol/min/gr.fr.wt, respectively. The toxicity of methylparathion in *L. marginalis* was reviewed by Moorthy et al. (1985) assessed 0.0340.002 µmol formazan/mg/h on the hepatopancreas and 0.0180.001 µmol formazan/mg/h on the gill tissue.

The malate aspartate shuttle is regulated by the activity of malate dehydrogenase. Reducing their activities would reduce the flux of reducing equivalents and gluconeogenic intermediates across the mitochondrial barrier and thereby the consequent decrease in MDH activity (Fujii et al., 1994 and Mishra and Shukla, 2003). SDH is associated with the mitochondrial inner membrane while MDH is associated with the mitochondrial matrix. Oil accumulated by the mussel, which is incorporated into gill membranes, should have influenced the changes in the activity of mitochondrial enzyme activity, since it has been observed that under oil effluent stress, the organism increases its respiratory rate in an attempt to synthesize more energy to sustain life .

Due to the lipophilic nature of oil effluent and its metabolites, they can incorporate into biological membranes, affecting the fluidity of the cell membrane and thereby impairing

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its normal functions, further leading to fluctuations in enzyme activity (Vijayavel et al., 2004). These changes can affect the passage of high-energy molecules across the membranes and thus inhibit the citric acid cycle, as reported by Long et al., (2003) suggested. The current results are consistent with existing research. After 72-hour exposure to a sublethal dose of copper sulfate, Satyaparameshwar et al., (2006) recorded MDH activity of 14810.60 µmol formazan/g wet tissue weight/h in the labial palpus and 9490.60 µmol formazan/g wet tissue weight/h in the gills. The specific MDH activity in the foot of the freshwater mussels *Unio pictorum* and *Anodonta cygnea* was determined by Livingstone et al. (1983) measured 76.56 and 121.49 µmol/min/gr.fr.wt, respectively. The toxicity of methylparathion in *L. marginalis* was reviewed by Moorthy et al. (1985) with 0.470.04 µmol formazan/mg/h on the hepatopancreas and with 0.390.03 µmol formazan/mg/h on the gill tissue. Changes in the activity of cellular metabolic enzymes are used as sensitive biochemical indicators to regularly monitor the health status of aquatic organisms such as *G. mollusks* (Gul et al. 2004). Oxaloacetate is converted to malate in the mitochondria by MDH, and then the malate-succinate transporter transports malate into the mitochondria, where the enzymes of the Krebs cycle branch convert it to succinate. This metabolic alignment prevents lactate from building up to toxic levels and prevents tissue energy levels from dropping. It has been observed in a variety of hydrobionts and functions under severe hypoxia and anoxia (Shapiro and Bobkova, 1975; Almeida-Val et al., 2000).

5. CONCLUSIONS

The present results drew the conclusion from these findings that the energy metabolism was changed by the oil containment. Our results show that confinement-induced stress alters energy metabolism. The upregulation of metabolite-converting enzymes can be explained by the fact that the flow of these metabolites through the energy pathways has increased with a constant amount of metabolites in the organism. Sublethal oil effluent concentrations and recovery periods were studied in freshwater mussel subcellular tissues; however, other parameters such as temperature, natural food availability, reproductive status, concentration, and exposure duration may have affected energy metabolism.Microsomal electron transport components and monooxygenase enzymes are clearly involved in detoxification of oil effluent toxicity in freshwater (hydrocarbon) mussels. This study suggests that the freshwater mussel, *Lamellidens marginalis*, may have evolved an unusually high concentration of these oxidoreductase/respiratory enzymes as a form of self-protection against the toxic effects of oil effluent produced by oxygen metabolism.

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