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TISSUE GLYCOLYTIC POTENTIAL OF PELECYPOD MOLLUSC, *LAMELLIDENS MARGINALIS* (LAM.) UNDER STRESS OF A COMMERCIAL CLOTH-WASHING DETERGENT

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ABSTRACT

Detergents are multiple chemical formulations with cleansing properties in dilute solutions, synonymously referred to as surfactants, syndets and tensides etc. However, detergent is a blanket name for many different compounds. Detergents are extensively used in different chores of life, both domestic and industrial, from where they make their way into rivers, lakes, streams, ponds and seas causing aquatic pollution, and finally entering the food chains at various trophic levels causing catastrophic effects. 'Wheel' is one such synthetic anionic detergent commonly used in and around Mumbai. Changes in tissue glycolytic potential of mollusc *Lamellidens marginalis* (Lam.) following its exposure to sublethal concentrations of detergent 'Wheel' were studied. A decrease in glycogen level was observed in tissues like hepatopancreas, gill, gonad, mantle and foot of the pelecypod mollusc under stress of the detergent. Among the vital organs, hepatopancreas is the chief metabolic organ in pelecypods and is involved in the regulation of general metabolism by storage and release of reserves. Results of the present study indicate that hepatopancreas suffered maximum damage followed by gill, gonad, mantle and foot in *Lamellidens marginalis* exposed to sublethal concentrations of the detergent.

INTRODUCTION

Detergents are defined as "a mixture containing one or more surfactants having cleansing properties in dilute solutions". Detergents are non-soap preparations intended for washing clothes and cleansing vessels. They are widely used for various cleaning processes. Synthetic detergent industry in India is about 25 years old. Some 3,00,000 tonnes of soaps and detergents have been produced and almost 99% of them are synthetic detergents (Krishnan Kanan 1997). Detergents generally consist of a surfactant or a surface-active agent and many 'builders'. Surfactants displace dirt on the surface by preferential adsorption whereas builder chemicals make the water soft and prevent the redeposition of dirt onto the clothes by complexing Ca^{++} and Mg^{++} ions in solution, preventing their interference with the surfactant and help to maintain level of alkalinity in the solution. The builders used in detergent preparations include borates, carbonates, phosphates, silicates and sulphates of sodium.

Surfactants or detergents, based on the nature of ions or molecules, can be classified into anionic, cationic, amphoteric and non-ionic. In India 90% of the detergents are of anionic type. One such commercially available anionic detergent is 'Wheel'. Tetrapropylene sodium alkyl benzene sulphonate (ABS) is active ingredient of the anionic detergents produced and used in India. These ABS-type detergents are not easily biodegradable, and as a result, high concentrations buildup in the environment. A survey conducted by the National Environmental Engineering Research Institute (NEERI), Nagpur found variable concentrations of ABS (1-100 mg/L) in household wastes in New Delhi. Studies proved that the branching of the hydrocarbon side-chain offered ABS resistance to microbial degradation hence, compounds with linear side-chains were introduced. These surfactants are known as linear alkyl benzene sulphonates (LABS). Acute toxicity of various LABS varies with alkyl chain

length and position of the benzene ring. Both ABS and LABS are toxic (Abel 1974), and effect is similar in fishes, invertebrates and algae (Hirsch 1963, Lindahl & Cabridence 1978, Maki & Bishop 1979). Synthetic detergents are responsible for about 16-35% of all phosphates in wastewaters (Krishnan Kanan 1997), which lead to eutrophication and depletion of dissolved oxygen favouring production of foul odours by anaerobic bacteria. The surfactants are toxic because they lower the surface tension, causing an increase in cellular permeability and, therefore, greater penetration of both the surfactant itself and also other substances present in the environment.

Energy metabolism has a key role as the animal is forced to spend more energy to mitigate the augmented toxic stress. With this perspective background in the present investigation, an attempt was made to probe into the sublethal impact of 'Wheel', a widely used synthetic detergent, on the tissue glycolytic potential of the pelecypod mollusc, *Lamellidens marginalis*. The filter feeding pelecypod molluscs are often selected and can be used for reclamation of the intoxicated aquatic environments due to their immense biofilter potential (Das & Jana 2003). Nevertheless, pelecypod molluscs undergo modifications in their functional response as well as biochemical characteristics of tissue in response to ambient pollutant level. Among freshwater mussels, *Lamellidens marginalis* is most commonly available and distributed all over the country, forming important species for pearl culture operation in India (Gopalkrishna et al. 2003, Surajit Das 2005).

MATERIALS AND METHODS

The test animals, freshwater pelecypod mollusk, *Lamellidens marginalis* were collected from the Vaitarna lake situated in the outskirts of the City Mumbai. They were acclimatized to laboratory conditions for a period of one week prior to the experimentation. During acclimatization they were fed with artificial pellet food ground to fine powder and made into paste. Tanks were aerated with oil free oxygen and exposed to natural day and night rhythm. Feeding was stopped 24 hrs prior to and also during the course of the experiments. Short-term tests of acute toxicity over a period of 96 hrs were performed on *Lamellidens marginalis* following renewal bioassay using the method of Litchfield & Wilcoxon (1949). Only healthy molluscs were used for the tests. Glass aquaria of twelve liters capacity were used as test chambers and in each chamber eight molluscs of uniform shell length (8.5-9.5 cm) were kept.

The physico-chemical characteristics of water used during the test period were as follows:

Temperature	:	27-30°C
pH	:	7.2-7.6
Dissolved oxygen	:	6.8-7.4 mg/L
Free CO_2	:	Nil
Total Hardness (as $CaCO_3$)	:	175-182 mg/L
Alkalinity	:	155-172 mg/L

On the basis of predetermined LC_{50} value, which was 800 ppm at the end of 24 hrs exposure to synthetic detergent 'Wheel' (Shingadia & Sakthivel 2003), the static bioassay was conducted using three sublethal concentrations of the detergent namely 200, 133 and 100 ppm that were $1/4^{th}$, $1/6^{th}$ and $1/8^{th}$ values of LC_{50}. Toxic medium was renewed after every 24 hrs. Control without toxicant was also maintained simultaneously. At the end of the test period of 48 hrs and 96 hrs the treated and untreated molluscs were sacrificed separately and vital tissues like hepatopancreas, gill, gonad, mantle and foot were collected for estimation of glycogen. Glycogen was estimated by anthrone reagent method. Experiments were repeated thrice and only arithmetic mean of the three experiments at each concen-

tration was taken to express the results. Student 't' test was applied for statistical analysis of the data. The level of significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

Pollutants cause stress to the aquatic organisms (Newell 1973), change their metabolic activity and cause physiological disarrangement in the living system. Toxicants due to their potential toxicity, produce biochemical changes in the tissues and organs of the exposed animals (Sastry & Sharma 1979). Glycogen, a polysaccharide, is often referred to as 'animal starch'. Stress conditions in the environment induced by the pollutants cause depletion in the glycogen reserves to meet the energy demands (Tazeen et al. 1996). Glycogen is the immediate source of energy during stress (Reddy et al. 1986), with hepatopancreas acting as a sink for toxicants and glycogen playing a significant role in glucose turnover and the mobilization of liver glycogen. In the present investigation, hepatopancreas was observed to suffer maximum damage (Table 1, Figs. 1 & 2). When compared to control value of 10.13 g%, test animals exposed to concentrations of 100, 133 and 200 ppm, showed decrease in values of glycogen content *viz*., 9.82, 9.36 and 8.74 g% respectively at the end of 48 hrs exposure period. At the same concentrations for 96 hrs the values further decreased to 8.23, 7.68 and 7.05 g%. At the end of 48 hrs exposure period glycogen values for gills were 4.22, 4.03 and 3.85 g% in concentrations of 100, 133 and 200 ppm respectively as compared to the control value of 4.65 g%. For the same concentrations at the end of 96 hrs exposure period the values of glycogen decreased further to 3.72, 3.51 and 3.38 g%. The glycogen content in gonad was 13.54, 13.11 and 12.79 g% in concentrations of 100, 133 and 200 ppm respectively as compared to control value of 13.86 g%. For the same concentrations glycogen content decreased to 12.04, 11.67 and 10.24 g% after 96 hrs. The glycogen values of mantle in 100, 133 and 200 ppm were 12.93, 12.69 and 12.14 g% respectively at

Table 1: Glycogen content in g % of different tissues of ***Lamellidens marginalis*** exposed to sublethal concentrations of detergent 'Wheel'.

Tissues	Control	48 hrs Exposure Group Test Concentrations			96 hrs Exposure Group Test Concentrations		
		100 ppm	133 ppm	200 ppm	100 ppm	133 ppm	200 ppm
Hepatopancreas	10.13 ± 0.01	9.82 ± 0.008 (-3.06)	9.36 ± 0.012 (-7.6)	8.74 ± 0.009 (-13.72)	8.23 ± 0.012 (-18.78)	7.68 ± 0.015 (-24.18)	7.05 ± 0.018 (-30.4)
Gills	4.65 ± 0.011	4.22 ± 0.008 (-9.24)	4.03 ± 0.009 (-13.33)	3.85 ± 0.012 (-17.2)	3.72 ± 0.018 (-20.00)	3.51 ± 0.011 (-24.51)	3.38 ± 0.015 (-27.31)
Gonad	13.86 ± 0.015	13.54 ± 0.011 (-2.3)	13.11 ± 0.014 (-5.41)	12.79 ± 0.012 (-7.72)	12.04 ± 0.009 (-13.13)	11.67 ± 0.011 (-15.8)	10.24 ± 0.012 (-26.11)
Mantle	13.44 ± 0.011	12.93 ± 0.011 (-3.79)	12.69 ± 0.013 (-5.58)	12.14 ± 0.011 (-9.67)	11.95 ± 0.012 (-11.08)	11.48 ± 0.008 (-14.58)	10.16 ± 0.012 (-24.4)
Foot	2.09 ± 0.012	2.02 ± 0.012 (-3.34)	1.94 ± 0.011 (-7.71)	1.86 ± 0.014 (-11.0)	1.79 ± 0.015 (-14.35)	1.70 ± 0.009 (-18.66)	1.65 ± 0.011 (-21.05)

Mean values of six observations. ± Standard deviation; Figures in parenthesis indicate significant % depletion (-) in the glycogen content as compared to control.

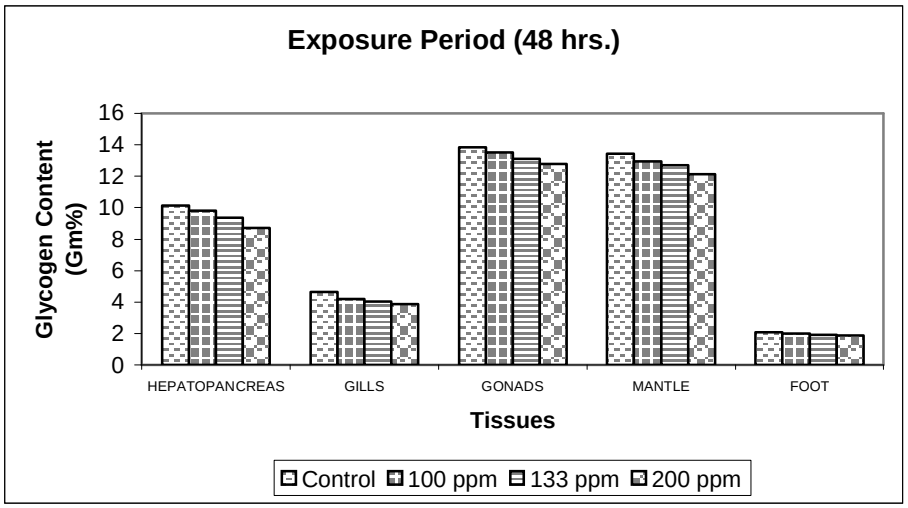

Fig. 1: Glycogen content in g% of different tissues of *Lamellidens marginalis* exposed to sublethal concentrations of detergent 'Wheel' for 48 hrs.

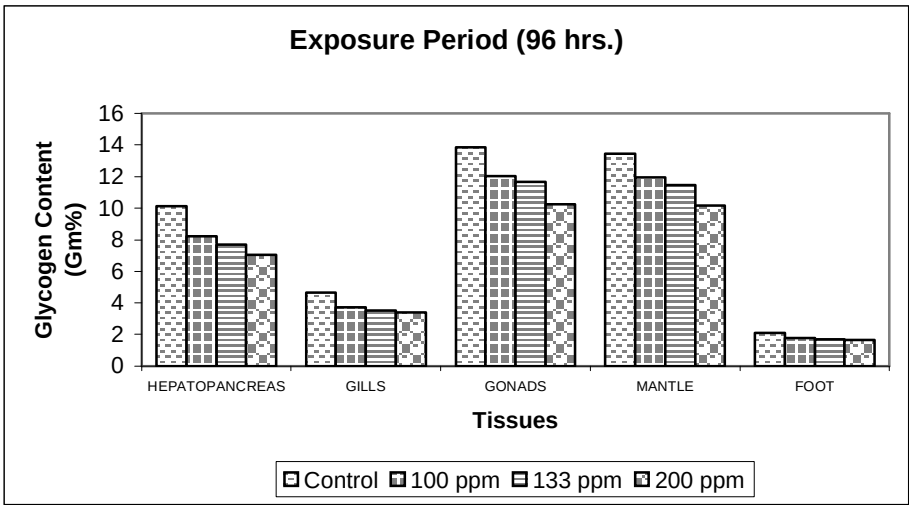

Fig. 2: Glycogen content in g% of different tissues of *Lamellidens marginalis* exposed to sublethal concentrations of detergent 'Wheel' for 96 hrs.

the end of 48 hrs exposure period as compared to the control value of 13.44 g%. In the same concentrations the values decreased further to 11.95, 11.48 and 10.16 g% after 96 hrs. The values of glycogen in foot at the end of 48 hrs exposure were 2.02, 1.94 and 1.86 g% in concentrations of 100, 133 and 200 ppm respectively as compared to the control value of 2.09 g%. In 96 hrs exposure period glycogen content for the same concentrations were 1.79, 1.7 and 1.65 g%.

Hepatopancreas is the organ of storage of glycogen in molluscs and also the site for detoxification of pollutants. Nevertheless pollutants stress due to detergent 'Wheel' might have increased the demand for energy, exhausting glycogen reserves of the body especially in the hepatopancreas. It also

suggests that those carbohydrates that are convertible or related to glycogen were being trapped and actively mobilized towards the production of glucose to provide maximum energy during the stress caused by the detergent. Kulkarni et al. (2005) reported in *Lamellidens marginalis* higher glycogen content in hepatopancreas during monsoon while that of gonad was higher during winter.

Sornaraj & Singh (2002) observed maximum decrement in glycogen and SDH activity in liver during initial period of exposure of detergents on air-breathing fish, *Channa striatus*. Ramana Rao and Rammurthy (1980) found a significant decrease in the glycogen content of foot and hepatopancreas in *Pila globosa* under the impact of sumithion. Hypoxic and anoxic conditions increase the carbohydrate consumption leading to overall decrease in glycogen content of the tissue (Dezwan 1972). This strengthens the possibility that acceleration of glycogen degradation could be due to anoxic conditions caused by the detergent 'Wheel'. Kulkarni & Patil (1994) reported significant changes in glycogen content of hepatopancreas, gill, gonad and mantle of *Lamellidens marginalis* exposed to cobalt chloride and suggested that glycogenolysis to be increased with increase in toxicant concentrations. Nagabhushanam & Kulkarni (1981) suggested that fall in carbohydrate level after a prolonged exposure to the pollutant may be due to inactivation of the enzymes involved in carbohydrate synthesis and metabolism. Kulkarni et al. (2005) reported significant decrease in total sugar and glycogen content in *Lamellidens corianus* under sublethal impact of hildan and concluded it to be due to energy demand by the animal in stress conditions. The maximal reduction of glycogen in hepatopancreas of the pelecypod mollusc in the present study may be the result of detergent stress-induced hypoxia reflecting the elevation of glutamate dehydrogenase (GDH) activity in hepatopancreas for mobilization of glucose from tissue glycogen to meet the high-energy demand. Similar observations were made by Das & Jana (2003) on cadmium induced stress in *Lamellidens* corroborates the present findings.

CONCLUSION

The freshwater pelecypod molluscs are generally sold in the local markets in clusters along side with a variety of fish. A cluster may contain 12-15 mussels depending on the size and is comparatively low priced, thus forming a staple diet of the economically challenged section of the society. Unintentional presence of the detergents in the domestic sewage finds its way into the freshwater bodies by seepage. This is detrimental to the biota and adversely affects the nutritional value. Thus, the poor populace that feed on them is deprived of the same. Care should be taken for proper disposal of synthetic detergents. Alternatively use of biodetergents should be encouraged and replace the synthetic detergents that are harmful. *Lamellidens* species are being used in a big way for the manufacture of cultured pearls, which provides employment to many individuals. If the species is under threat due to pollution it will have cascading detrimental effects on the culture industry as well.

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REFERENCES

Abel P. D. 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. J. Fish. Biol., 6: 279-298.

Das Shamik and Jana B.B. 2003. Oxygen uptake and filtration rate as animal health biomarker in *Lamellidens marginalis* (Lamarck). Indian J. of Exptal. Biol., 42: 1306-1310.

David Calamari and Robert Marchetti 1973. The toxicity of mixtures of metals and surfactants to rainbow trout (*Salmo gairdneri* Rich). Water Research Pergamon Press, 7: 1453-1464.

Dezwan, A. 1972. Utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Perna edulis*. Comp. Biochem. Physiol., 47-54.

Gopalkrishna, A., Bhaskar V. S. and Kapoor D. 2003. Pearl forming freshwater mollusks of India. First Indian Pearl Congress and Exposition (Abstract), CMFRI, Kochi, 17.

Hirsch, E. 1963. Structure Lemente Von: Alkyl benzene sulfonaten und ihr Einflub aus das verhalten Von Fischen. Vom. Wass., 30: 249-259.

Krishnan, Kanan 1997. Fundamentals of Environmental Pollution. S. Chand and Co. Ltd., New Delhi.

Kulkarni, A.N., Kamble S.M. and Keshavan R. 2005. Studies on impact of hildan on biochemical constituents in the freshwater mussel, *Lamellidens corianus*. J. Aqua. Biol., 20(1): 101-104.

Kulkarni, D.A., Chati, R.S., Mohekar, A.D., Deshpande, P.A. and Pawar, A.H. 2005. Seasonal variation in biochemical content in freshwater mollusk, *Lamellidens marginalis* from Manjra, a tributary of Godavari River, Maharashtra. J. Aqua. Biol., 20(1): 67-69.

Kulkarni, S.D. and Patil, R.P. 1994. Cobalt chloride induced biochemical alteration in freshwater bivalve mollusk, *Lamellidens marginalis* (Lamarck). Proc. Indian Acad. Sci., 87(B): 377.

Lindahl, P. and Cabridence, R. 1978. Molecular structure biological properties relationship in anionic surface-active agents. Wat. Res., 12: 25-30.

Litchfield, J.T. and Wilcoxon, F. 1949. A simple method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.

Maki, A.W. and Bishop, W.E. 1979. Acute toxicity of surfactants to *Daphnia magna* and *D. pulex*. Environ. Contam. Toxicol., 8: 599-612.

Nagbhushanam, R. and Kulkarni, G. K. 1981. Freshwater prawn, *Macrobrachium kistnesis* (Tiwari). Effect of metal pollution. Proc. Indian Nat. Sci. Acad., B, 47: 380-386.

Newell, R.C. 1973. Factors affecting the respiration of intertidal invertebrates. American Zool., 13: 573.

Rao Ramana, M.V. and Rammurthy, R. 1980. Effects of sub-lethal concentrations on some biochemical constituents of snail *Pila globosa*. Geobios, 7: 247-250.

Reddy, R., Vijaykumar, N. and Chari, N. 1986. Effect of mercuric chloride on carbohydrate metabolism of mantle, foot and gill of freshwater mussel, *Perreysia rugosa* (Gmelin). J. Environ. Biol., 2: 225.

Sastry, K.V. and Sharma, K. 1979. Toxic effect of endrin on liver and kidney of the freshwater teleost. Proc. Symp. Environ. Biol., pp. 337-342.

Seifer, S. 1950. Estimation of glycogen with anthrone reagent. Arch. Biochem., 25: 191-200.

Shingadia, H.U. and Veena Sakthivel 2003. Estimation of LC_{50} values for *Lamellidens marginalis* (Lamarck) with detergent 'Wheel', a component of domestic sewage. J. Aqua. Biol., 18(2): 187-189.

Sornaraj, R. and Ranjit Singh, A.J.A. 2002. Influence of commercial detergents on biochemical and physiological responses of a freshwater air-breathing fish, *Channa striatus*. Ad. Bios., 2(1): 23-34.

Surajit Das 2005. Operational techniques for cultured pearl production in *Lamellidens marginalis* (Lamarck): A hands on experience. J. Aqua. Biol., 20(1): 163-165.

Tazeen Avasta, Bais V.S. and Thakur, P. 1996. Effects of nuvan on some biochemical parameters of Indian catfish, *Mystus vittatus*. J. Environ. Biol., 17(2): 167-169.