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Impact of colour pigments on biochemical parameters of bivalve, *Lamellidens marginalis*

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Abstract

The present study was carried out to investigate the effect of colour pigments used for painting the decorative articles like idols, on the freshwater bivalve, *Lamellidens marginalis*. The effects of sub-chronic exposure were studied by the changes in the biochemical constituents like total protein, glycogen and lactic acid, in different tissues like muscle, mantle, gills, foot, hepatopancreas and gonads for 10 and 20 days period. The glycogen contents in the muscle, mantle and gonads were significantly decreased with increase in concentration of colour pigments. It decreased from 26.77 mg gm^{-1} in control to 19.17 mg gm^{-1} at 900 ppm after 20 days of exposure; whereas protein contents in the tissues studied decreased significantly from 22.5 mg gm^{-1} in control to 15.5 mg gm^{-1} at 900 ppm after 10 days of exposure. The increase in lactic acid content in all the tissues except gills and gonads may be due to acute hypoxia.

Key words

Colour pigments, Bivalve, *Lamellidens marginalis*

Introduction

The Ganesha idols are made either of Plaster of Paris or typical clay called 'Shadoo' with size ranging from ½ feet to 25 feet or sometimes more. The colours used for decorating the idols contain different shades with mica for luster. The decorative colours (pigments) contain heavy metals (Dhote *et al*., 2001). The fate of these idols is always sinking at the bottom of the water bodies. Different chemical compounds get dissolved in the water affecting the aquatic ecosystem (Reddy and Vijay Kumar, 2001). When dissolved in water these chemical compounds remain suspended in water bodies for many days and then settles down.

Trace metal concentrations in soft tissue samples of bivalve molluscs have been used worldwide in "Mussel watch" programs in order to assess pollution in coastal and estuarine environments (Sokolowski *et al*., 2004). Monitoring by means of exposed biological "sentinels" is performed based on the assumption that levels of trace contaminants accumulated in mussel tissues represent the time and space integrated value of these contaminants in the surrounding water (Bellotto and Miekely, 2007). Mussels can readily accumulate metals from freshwater ecosystems by direct transport of water across gills and from ingestion of suspended particles and bottom sediments (Solowski *et al*., 2004). They are sedentary, long-lived, widely distributed and tolerant to high heavy metal concentrations. As a result, their metal body burdens can reflect the contamination history of a certain environment (Jamil *et al*., 1999). Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of the inhabitants (Ferrando, 1991). Biochemical constituents like protein, glycogen, cholesterol and lactic acid are considered sensitive indicators of stress as they act as key substrates during stress metabolism (Jadhav *et al*., 1995). The idol immersion alters the water quality of the immersion sites (Dhote *et al*., 2001; Patil and Dongare, 2006) along with heavy metal concentrations (Reddy and Vijaykumar, 2001). During Ganesh and other festivals the idols immersed in the aquatic bodies with

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colour pigments and metallic compounds leads to change biochemical contents and other metabolic process of the aquatic organism. Therefore, it has become important to study the effects of these colour pigments of metallic compounds on the aquatic organism, bivalve, *Lamellidens marginalis*.

Materials and Methods

Bivalves, *Lamellidens marginalis* (75 ± 5 gm) were collected from Panchganga River at Kolhapur District, Maharashtra, India and transported to the departmental laboratory under suitable conditions. Animals were acclimatized to the laboratory conditions for 15 days using untreated lake water with change at alternate days. Bivalves were exposed to the sub-chronic concentrations 400 and 900 ppm of combination of colour pigment Pthalocyanine Green, S. F. Yellow R 135, Toluidine Red 405 along with Sumica Pearl donated by Sudarshan Chemical Industries, Pune, Maharashtra, India for 10 and 20 days. A group of bivalves unexposed to color pigment served as control.

Biochemical analysis : After the completion of the exposure period, the animals were sacrificed and different tissues like muscles, gills, hepatopancreas, gonads, mantle and foot were taken out. Total proteins were estimated by Folin-Phenol reagent method using bovine serum albumin as standard (Lowry *et al.*, 1951). To 1 ml tissue homogenate (1:10 W/V) 5 ml of alkaline solution was added. After 10 min of incubation at room temperature, 0.5 ml of diluted Folin-Phenol reagent was added, and the contents were thoroughly mixed. The color developed was read after 30 min using spectro photometer (Systronic India.) at a wavelength of 750 nm.

Glycogen from the selected tissues was estimated by Anthrone reagent method as described by Seifter *et al*. (1950). Freshly excised tissues of bivalves were digested in 30 % KOH. After digestion, the neutralization was done by glacial acetic acid. Each KOH digest was diluted to 100 ml with distilled water. To a 5 ml aliquot of this solution, 10 ml of freshly prepared Anthrone reagent (0.2 % Anthrone in concentrated H_2SO_4) was added gradually. Similarly blank and standard tubes were also prepared using distilled water and glucose solution respectively. The mixture was then boiled for 8 min by keeping the tubes in boiling water bath. After cooling the optical density of the mixture was read at 625 nm using spectrophotometer (Systronic, India.). Standard calibration curve was prepared for calculating glucose values. The glucose values thus obtained were converted into glycogen using Morrison's factor (1.11).

Lactic acid content in the tissues of bivalves were estimated following the method of Hawk and Oser (1965). The excised tissues were accurately weighed and homogenized in 10% TCA. After centrifugation, the clear supernatant was used for estimating lactic acid. Two ml aliquot of the protein free filtrate was mixed with 1 ml of 20% copper sulphate solution. The mixture was diluted to 10 ml and 1 gm of calcium hydroxide pellets was added to it. It was mixed on cyclometer for 1 min till the powder was uniformly dispersed and then allowed to stand for half an hour. The mixture was then centrifuged and 1 ml of clear supernatant from each tube was transferred to wide mouthed test tube to which 0.05 ml of 4 % copper sulphate solution was added followed by 6 ml of concentrated sulphuric acid. Then the tubes were kept in a boiling water bath for 5 min. After cooling, 0.1 ml of p-hydroxydiphenyl reagent was added drop by drop in each tube. The tubes were kept at 30º C for half an hour. After placing the tubes in boiling water bath for 90 sec and cooling, the optical density of the solution was measured at 560 nm.

Statistical analysis : Data presented in this study are mean of 5 samples with SD calculated on Microsoft Excel by standard statistical methods. The students t-test was used for determining the significance of difference between mean value of control and experimental groups.

Results and Discussion

In the present study, the bivalves exposed to 400 and 900 ppm colour pigments for 10 and 20 days showed significant decrease in glycogen content in all the tissues as compared to control (Table 1). Mechanisms of accumulation, storage and loss of polluting substances in bivalves consist of several alternative pathways, varying with species, and chemical form of substance and mode of assimilation (Gokhale and Mane, 1992). At the concentration of 400 ppm, after 10 days of exposure no significant decrease in glycogen was found in mantle, gills, foot and hepatopancreas. Muscles showed significant decrease in glycogen content from 26.77 to 23.81 mg gm^{-1} weight. Also glycogen content in gonads indicated a significant decrease from 44.95 to 40.33 mg gm^{-1} weight. After 20 days of exposure at 400 ppm no significant decrease was found in foot and hepatopancreas but all the other tissues showed significant decrease in glycogen. Glycogen content in gills were found to be decreased from 23.95 to 19.84 mg gm^{-1} weight while in gonads it decreased from 44.95 to 32.12 mg gm^{-1} weight. Similar results were found in muscles and mantle at 400 ppm after 20 days of exposure. At the sub-lethal concentration of 900 ppm after 10 days of exposure muscle, mantle and gonad indicated a significant decrease in glycogen while in other tissues it decreased insignificantly. Muscles showed decrease in glycogen content from 26.77 to 20.73 mg gm^{-1} weight. Mantle and gonads showed decrease from 48.79 and 44.95 to 33.66 and 31.78 mg gm^{-1} weight respectively. The long term exposure to 900 ppm showed significant decrease in glycogen content in all the tissues except foot.

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Table 1 : Glycogen (mg gm^{-1}) content in tissue of bivalve, *Lamellidens marginalis* after exposure to different concentration of colour pigments

Tissue	Control	400ppm		900ppm	
		10days	20 days	10days	20days
Muscle	26.77±2.25	23.81*±1.9	21.10*±0.32	20.73*±0.30	19.17*±1.4
Mantle	48.79±7.98	48.57±0.24	38.99*±1.5	33.66*±0.47	31.82*±4.14
Gills	23.95±3.11	22.76±1.4	19.84*±1.4	21.73±1.39	17.37*±0.41
Foot	23.33±6.07	23.09±0.3	18.56±1.1	17.90±0.70	16.49*±0.62
Hepatopancreas	54.98±17.34	52.72±2.2	35.77±2.0	35.58±3.19	30.57*±2.44
Gonads	44.95±3.65	40.33*±0.70	32.12*±2.3	31.78*±1.86	30.95*±2.81

Values are mean of five replicates ± SD *Significantly different from control ($P<0.05$)

A marked decrease in hepatopancreas glycogen in the present study indicates an extensive utilization of stored glycogen to meet the extra demands of energy necessitated under toxic stress. Decrease in glycogen was due to the high energy demands of the organism under toxic stress which leads to hyperglycemia. Decrease in glycogen content have been reported in different tissues of *Labeo rohita* exposed to Hg and *Catla catla* to Cr (Vincent *et al*., 1995). Decrease in glycogen and increase in lactic acid contents has been also reported by Ramalingam and Indra (2002) and Tilak (2002) due to $CuSO_4$ and nitrate toxicity respectively. Significant decrease in glycogen profiles in ovary, hepatopancreas, gill and muscles were reported by Kharat (2009).

Protein which serves as energy source during stress condition decreased from control as the concentration of the toxicant and the exposure period increased. More energy to mitigate any stress conditions may be obtained from carbohydrates, proteins and lipids. Organisms try to detoxify the toxicant by spending more energy and thereby showed reduction in protein level (Chaudhari, 1998). At 400 ppm after 10 days protein content of all the tissues decreased significantly except muscles and hepatopancreas. Gills showed a decrease in protein content from 28.75 to 24.5 mg gm^{-1} weight. Protein content in gonads were found to be decreased from 44.5 to 39.87 mg gm^{-1} weight. Mantle and foot showed decreased protein content from 31.87 and 21.75 to 26.37 and 18.87 mg gm^{-1} weight respectively (Table 2). At the same concentration for 20 days of exposure all the tissues showed significant reduction in their protein content. At 900 ppm for both the exposure periods, protein content showed significant decrease in all the tissues. The progressive decrease in protein content of the tissue with duration of exposure indicates a gradual accumulation of toxicant resulting in gradual increase in toxicity (Tilak, 2002). Proteins may be channalized into TCA cycle through aminotransferase system to cope up with excess demand of energy during toxic stress (Jadhav *et al*., 1995). *Lamellidens marginalis* shows shifts in its metabolism for survival and maintenance and additional demand of energy leads to accelerated catabolism of proteins (Muley and Mane, 1995). Altered protein synthetic mechanism may be the cause of its depletion.Several investigators have reported depletion in proteins in fishes exposed to various toxicants. This affects the nutritive value of edible organisms (Meenakshi, 1998 a). Some adverse effect of heavy metal exposure were also observed by Kristen (2007). Chronic exposure to Ni showed decrease in protein contents in fresh water bivalve, *Lammellidens marginalis* (Andhale, 2011).

Lactic acid content in mantle, foot and hepatopancreas showed significant increase in 400 ppm concentration while muscle, gills and gonads increased insignificantly after 10 days. Hepatopancreas showed increase in lactic acid content from 0.47 to 0.52 mg gm^{-1} weigh. Foot and mantle indicated rise in lactic acid levels from 0.40 and 0.38 to 0.67 and 0.56 respectively. After 20 days of exposure at 400 ppm lactic acid content of all the tissues except gills and gonads increased significantly. At 900 ppm lactic acid was increased significantly except gills and gonads after 10 days. After 20 days of exposure period at 900 ppm lactic acid content in muscles increased up to 0.83 mg gm^{-1} weight which is significant. Mantle showed increase in lactic acid up to 0.81 from 0.38 mg gm^{-1} weight. Gills and foot indicated increase in lactic acid from 0.75 and 0.40 to 1.13 and 0.96 mg gm^{-1} weight respectively. Lactic acid contents in hepatopancreas were found to be increased up to 0.76 from 0.47 mg gm^{-1} weight. Gonads showed increase up to 0.88 from 0.62 mg gm^{-1} weight (Table 3).

The enhanced glycolytic mechanism would indicate the animal's dependency on the anaerobic breakdown of glucose to alternate the increased anoxic condition. Conversion of glucose to lactic acid leads to increase in lactic acid content (Karuppasamy, 2000). The increase in lactic acid content possibly due to glycogenolysis and operation of glycolytic pathway in liver and muscle accounting for hyperglycemia. Excess utilization of these metabolites indicates the possible arrival of anoxic

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Table 2 : Protein (mg gm^{-1}) content in tissues of bivalve, *Lamellidens marginalis* after exposure to different concentration of colour pigments

Tissue	Control	400ppm		900ppm	
		10days	20 days	10days	20days
Muscle	22.5±1.58	20.25±1.9	16.75*±2.1	15.5*±1.08	16.62*±1.8
Mantle	31.87±1.7	26.37*±1.9	22.5*±2.12	17.0*±1.2	13.37*±1.75
Gills	28.75±2.3	24.5*±3.4	19.37*±3.5	17.75*±1.5	15.37*±2.13
Foot	21.75±1.8	18.87*±1.3	15.62*±1.7	12.5*±1.2	10.25*±1.3
Hepatopancreas	30.0±1.6	26.37±3.7	13.37*±2.2	13.87*±1.6	14.12*±1.6
Gonads	44.5±3.3	39.87*±2.5	26.5*±2.4	25.62*±1.7	19.5*±1.2

Values are mean ± SD of 5 samples, * Significantly different from control (P<0.05)

Table 3 : Lactic acid (mg gm^{-1}) content in tissue of bivalve, *Lamellidens marginalis* after exposure to different concentration of colour pigments

Tissue	Control	400ppm		900ppm	
		10days	20 days	10days	20days
Muscle	0.48±0.02	0.53±0.04	0.56*±0.007	0.72*±0.04	0.83*±0.003
Mantle	0.38±0.01	0.56*±0.04	0.67*±0.04	0.75*±0.04	0.81*±0.01
Gills	0.75±0.23	0.79±0.03	0.97±0.07	1.026±0.02	1.13*±0.07
Foot	0.40±0.05	0.67*±0.19	0.82*±0.02	0.80*±0.018	0.96*±0.005
Hepatopancreas	0.47±0.02	0.52*±0.03	0.56*±0.03	0.69*±0.008	0.76*±0.008
Gonads	0.62±0.16	0.66±0.05	0.66±0.03	0.705±0.05	0.88*±0.06

Values are mean ± SD of 5 samples, * Significantly different from control (P<0.05)

conditions (Meenakshi, 1998 a, b). Glycogenolysis was also reported during exposure to various pollutants considered stressful due to hypoxic situation (Gupta, 2005; Bhavan and Geraldine, 2002).

The results obtained in this study indicate that the colour pigments which get dissolved in water affect the metabolism in fresh water bivalve, *Lamellidens marginalis* by reducing glycogen due to stress conditions and protein levels due to proteolysis and impairment in synthesis. Lactic acid contents increased due to anoxic conditions forced on the animals. Thus, colour pigments used in colouring of idols with different heavy metals shows synergistic effects on the physiology of the bivalve, *Lamellidens marginalis* at sub lethal concentration after long term exposure.

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References

Andhale, A.V.: Effect of nickel induced biochemical alterations in fresh water bivalve, *Lamellidens marginalis*. *J. Ecobiotechnol.*, **3**, 18-24 (2011).

Bellotto, V.R. and N. Miekeley: Trace metals in mussel shells and corresponding soft tissue samples: A validation experiment for the use of *Perna perna* shells in pollution monitoring. *Anal. Bioanal. Chem.*, **389**, 769-776. (2007).

Bhavan, S.P. and P. Geraldine: Carbaryl induced alterations in biochemical metabolism of the prawn, *Macrobrachium malcolmsonii*. *J. Environ. Biol.*, **23**, 157-162 (2002).

Chaudhari, T.R., P.N. Patil, K.R. Rao and M. Subhash: Effect of sodium pentachlorophenate on protein metabolism of the Gastropod, *Indoplanorbis exustus. Uttar Pradesh J. Zool.*, **18**, 87-90 (1998).

Dhote, S., B. Varughese and S.M. Misra: Impact on idol immersion on water quality of Twin Lakes of Bhopal. *Indian J. Environ. Prote.*, **21**, 998-1005 (2001).

Durairaj, S. and V.R. Selvarajan: Influence of quinolphos, an organo phosphorus pesticide on the biochemical constituents of tissue of fish *Oreochronus mossambicus*. *J. Environ. Biol.*, **13**, 181-185 (1992).

Ferrando, M.D. and Andrew-Moliner: The effect of time on physiological changes in eel, *Anguilla anguilla* induced by Lindane. *Comp. Biochem. Physiol.*, **100**, 95-98 (1991).

Ghosh, R. and R.V. Shrotri: Blood glucose and tissue glycogen interrelationship in *Scylla serrata* (Forskal), chronically exposed to Thiodan. *J. Environ. Biol.*, **13**, 233-237 (1992).

Gokhale, A.A. and U.H. Mane: Acute fluoride toxicity to the freshwater bivalve mollusc, *Lamellidens marginalis* (Lamarck). *Ind. J. Inv. Zool. Aqua. Biol.*, **2**, 11-14 (1990).

Gupta, K. and A. Sharma: Macroinvertebrates as indicators of pollution. *J. Environ. Biol.*, **26**, 205-211 (2005).

Harper, H.A., V.W. Rodwell and P.A. Mayers: A review of physiological chemistry. 16th Edn., Long Medical Publication, California, pp. 289-325 (1978).

Hawk, P.B. and B.L. Oser: Hawk's Physiological Chemistry .14th Edn., McGraw-Hill, New York (1965).

Heath, A.B. and A.W. Pritchard: Effects of severe hypoxiaon

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carbohydrate energy stores and metabolism in two species of freshwater fish. *Physiol. Zool*., **88**, 325 (1965).

Jadhav, S., Y.B. Sontakke and V.S. Lomte: Effect of carbaryl on protein content of freshwater bivalve *Corbcula striatella*. *Indian J. Comp. Ani. Physiol.*, **13**, 33-35 (1995).

Jamil, A., K. Lajtha, S. Radan, G. Ruzsa, S. Cristofor and C. Postolache: Mussels as bioindicators of trace metal pollution in the Danube Delta of Romania. *Hydrobiologia*, **392**, 143-158 (1999).

Karuppasamy, R.: Effect of phenyl mercuric acetate on carbohydrate content of *Channa punctatus*. *Uttar Pradesh J. Zool*., **20**, 219-225 (2000).

Kasthuri, J. and M.R. Chandran: Sub lethal effect of lead on feeding energetics, growth performance, biochemical composition and accumulation of the estuarine catfish, *Mystus gulio* (Hamilton). *J. Environ. Biol*., **18**, 95-101 (1997).

Kharat, P.S.: Effect of TBTCL on glycogen profile in freshwater prawn, *Macrobrachium kistnensis*. *World App. Sci. J.*, **7**, 1534-1539 (2009).

Kristen, H.: Effect of some heavy metals on protein and collagen biosynthesis in rabbit dental pulp *in-vitro*. *Europian J. Oral Sci.,* **85**, 266-271 (2007)

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Rendel: Protein estimation with folin-phenol reagent. *J. Biol. Chem*., **193**, 265-278 (1951).

Mane, V.H., S.R. Akarte and D.A. Kulkarni: Acute toxicity to fenithion to freshwater Lamellibranch mollusk, *Indonaia caeruleus* from Godavari river at Paithan- A biochemical approach. *Bull. Environ. Contam. Toxicol*., **37**, 622-628 (1986).

Meenakshi, V. and K. Pugazhendhi: Toxicity of mercuric chloride to the fingerlings of freshwater fish *Cyprinus carpio* (Linn.). *Uttar Pradesh J. Zool*., **18**, 145-148 (1998a).

Meenakshi, V., N. Indira and K. Pugazhendhi: Influence of zinc sulphate on the freshwater edible fish, *Labeo rohita* (Hamilton) fingerlings. *Uttar Pradesh J. Zool*., **18**, 181-183 (1998b).

Muley, D.V. and U.H. Mane: Endosulfan toxicity to freshwater mussel *Lamellidens marginalis* and pH induced changes- A biochemical approach. *Ind. J. Comp. Ani. Physiol*., **13**, 21-26. (1995).

Nakano, T. and N. Thomson: Catecholamines and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physiological disturbance. *J. Fish Res. Board,* **24**, 1701-1704 (1967).

Patil, D. and M. Dongare: Effect of Ganesh idol immersion on water quality of some lakes of Kolhapur (Maharashtra). *J. Ecobiol*., **19**, 253-256 (2006).

Radhakrishnaiah, K.: Accumulation of copper in the organs of freshwater fish, *Labeo rohita* (Hamilton) on exposure to lethal and sublethal concentrations of copper. *J. Environ. Biol*., **9**, 319-326 (1988).

Rajasubramaniam, V.: Differential toxicity of tetravalent and hexavalent selenium in the freshwater teleost fish. *J. Ecobiol*., **19**, 219-224 (2006).

Ramlingam, K. and D. Indra: Copper sulphate ($CuSO_4$) toxicity on tissue phoshatases activity and carbohydrates turnover in *Achatina fulica*. *J. Environ. Biol*., **23**, 181-188 (2002).

Reddy, M.V. and A. Vijaykumar: Effects of Ganesh idol immersion on some water quality parameters of Hussainsagar Lake. *Curr. Sci*., **81**, 1412-1413 (2001).

Seifter, S., S. Dayton, B. Novic and M. Woylen: Estimation of glycogen with anthrone reagents. *Arch. Biochem.*, **25**, 191-200 (1950).

Sokolowski, A., A.S. Salem Bawazir and M. Wolowicz: Trace metals in the brown mussel *Perna perna* from the coastal waters off Yemen (Gulf of Aden): How concentrations are affected by weight, sex, and seasonal cycle. *Arch. Environ. Contam. Toxicol.,* **46**, 67-80 (2004).

Syversen, T.L.M.: Effects of methyl mecury on protein synthesis *in vitro*. *Acta. Pharmacol. Ed. Toxicol*., **49**, 422-426 (1981).

Tilak, K.S., K. Veeraiah and S. Lakshmi Jhanshi: Studies of some biochemical changes in the tissues of *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to NH_3-N, NO_2-N and NO_3-N. *J. Environ. Biol.*, **23**, 377-381 (2002).

Vasanthi, R., P. Baskaran and S. Palanichamy: Influence of carbofuron on growth and protein conversion efficiency in some freshwater fishes. *J. Ecobiol*., **2**, 85-88 (1990).

Villilan, P., K.R. Narayanan, S. Ajmalkhan and R. Natarajan: Proximate composition of muscle, hepatopancreas and the gill in the copper exposed estuarine crab, *Thalamita crenata* (Latreilla). *Proc. IInd Nat. Symp. Ecotoxicol*. Department of Zoology, Annamalai University, India. (1988).

Vincent, S., T. Ambrose, L.C. Arunkumar and M. Selvanagam: Biochemical response of the Indian major carp *Catla catla* (Ham.) to chromium toxicity. *Ind. J. Environ. Hlth*., **37**, 192-196 (1995).

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