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Effect Of Heavy Metals $Hgcl_2$ And $Cdcl_2$ On Glycogen Activity Of Bivalve Lamellidens Marginalis.....

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Abstract: Heavy metal and pesticide pollutants cause stress to the aquatic organism and change its metabolic activity. The heavy metal pollutants give rise to alterations in the metabolic and physiological activity both after short and long term exposures. To investigate the physiological changes after pesticide and heavy metal treatment the most fundamental one would be the study of change in the biochemical constituents. Carbohydrates, proteins and lipids are the important metabolites which provide energy to different vital processes.

They glycogen content in freshwater bivalve, L. marginalis was altered indicating the effects of heavy metals. The average glycogen content in acute and chronic treatment by heavy metal $HgCl_2$ and $CdCl_2$ was decreased in the whole body. The depletion of glycogen content was greater in the digestive gland as compared to the foot and mantle of the bivalve, when exposed to pollutants. This indicates that the digestive gland is the principal metabolic center for various metabolic functions. During acute and chronic exposures a significant decrease in the glycogen content of the digestive gland suggests greater glycolytic activity in the gland than the mantle and foot. The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants.

Keywords: Heavy metals $HgCl_2$ and $CdCl_2$, L. marginalis, Glycogen, digestive gland, acute, chronic.

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I. INTRODUCTION

Most information about the effect of environmental pollutants on aquatic animals has been obtained from mortality studies. Often very little is known about damage to different internal organs or about disturbed physiological and biochemical processes within an organism following exposure to environmental poisons. Consequently knowledge about the mode of action of toxicants and causes of death in poisoned aquatic animals is often lacking. A better understanding of these mechanisms is necessary if we want to predict the potential harmfulness of various chemicals to the environment.

Since different environment pollutants are likely to affect biological systems in different ways according to their respective chemical properties, the sum of physiological changes created by a particular pollutant is likely to be characteristic of that pollutant. Thus by observing the effects of pollutants on a set of physiological parameters, it might be possible to establish specific responses of that pollutant, and may take it possible to identify a pollutant on the basis of its physiological effect pattern.

The higher concentrations of toxicants bring the adverse effects on aquatic organisms, at cellular level or molecular level and ultimately lead to disorder in biochemical composition which is useful in determining different toxicants and protective mechanism of the body to resist the toxic effects of the substances.

The toxic chemicals (pollutants) act as one kind of stress to organism and organism responds to it by developing necessary potential to counter act that stress. The biochemical changes occurring act that stress. The biochemical changes occurring in the body give first indication of stress. During stress the organism needs sufficient energy which is supplied from reserve materials (glycogen, lipid and protein). If the stress is mild then only stored glycogen is used, as a source of energy, but if the stress is strong then the energy stored in lipid and protein may be used.

The heavy metals cause metabolic dearrangement in the living system, when come in contact. Heavy metals due to their potential toxicity produce biochemical changes in the tissues of animals.

Eventhough there is much work on the toxic effects of pesticides and heavy metals on specific target animals, little attention was paid to study the physiological and biochemical changes on non target aquatic species. Since Lamellidens Marginalis are fresh water bivalves an attempt was made to study the changes in biochemical composition in different heavy metals like mercuric chloride and cadmium chloride. Carbohydrates

(glycogen), proteins and lipids constitute the vital organic substances playing an important role in energy metabolism. So changes in total glycogen is studied in the present investigation.

The biochemical constituents were estimated from whole body, foot, digestive gland and mantle. These tissues are vital and metabolic important and any stress on the animal is depicted by the changes in the constituent in these tissues. Carbohydrate plays structural role in every living organism and serves as a reservoir of the chemical energy and many decrease or increase according to needs of the organism. The tissue glycogen is a major source for energy for metabolic processes (Lather, 1975).

A change in glycogen content in various organisms after exposure to various pollutants was studied by many workers (Peter, 1973; Mayers, 1977; Koundinya and Ramamurthi, 1979; Bhagyalakshmi, 1981; Gill and Pant 1981; Patil 1986; and Choudhari, 1988). Baner and Ghosh (1978) has recorded a change in levels of serum glucose, liver glycogen and glucose-6-phosphate of fishes, Clarius batrachus and Tilapia mossambica when exposed to cadmium. Koundinya and Ramamurthi (1978) observed the effect of lethal concentration of sumithion on carbohydrate metabolism in Tilapia Mossambica. The acute effect of methyl parathion on carbohydrate metabolism of the Indian cat fish, Heteropheustes fossilis was determined by Srivastava and Singh (1981). The effect of various concentrations of zinc sulphate on glycogen content of liver, muscle, brain, kidney and grills of air breathing freshwater food fish, Anabad Scandes has been investigated by Natarajan (1982).

A little information is available about biochemical diversions due to heavy metals and pesticide pollutant stress in Molluscs. Mandal and Ghose (1970) studied the effect of cadmium arsenate on the snail Achatina Fulica has (Bawdich) on mobilization of digestive gland glycogen. Ramanarao and Ramamurthi (1980) studied the effect of sublethal concentration of sumethion on biochemical composition of the snail, Pila globosa. Chaudhari (1988) studied the effect of pesticides on the freshwater prosobranch snail, Bellamya bengalensis with respect to change in glycogen.

Therefore, in the present study the glycogen contents were estimated in the whole body, foot, digestive gland and mantle of the freshwater bivalve, Lamellidens marginalis after exposing to lethal and sublethal concentrations of heavy metals (mercury chloride and cadmium chloride) as acute and chronic treatments.

So far most of the studies have been performed on fish because of their large size and more detailed knowledge about normal functioning and behaviour of the animals compared to aquatic invertebrates.

II. MATERIAL AND METHODS

The bivalves, Lamellidens marginalis were collected from the Godavari river at Paithan. They were brought to the laboratory and maintained in plastic throughs. The bivalves were acclimatized to laboratory conditions for two-three days. The water in the troughs was changed every day. The healthy adult bivalves of approximately the same size and weight were selected for the experiment. During the chronic treatment the animals were fed on algae or Hydrilla.

To study the effects of heavy metals (mercuric chloride and cadmium chloride) on the biochemical composition of Lamellidens marginalis, the bivalves were exposed to median lethal concentration and sub lethal concentration of heavy metals as acute treatment and chronic treatment respectively.

Acute treatment : The acclimatized bivalves were divided into three groups. The first two groups were exposed to 0.6 ppm mercuric chloride and 3.9 ppm cadmium chloride for 72 hours. The third group of bivalves was kept as control.

At the end of 24, 48 and 72 hours treatment on different pollutants mercuric chloride and cadmium chloride, the control and treated bivalves were scarified to analyze the biochemical composition. The bivalve were dissected and their foot, digestive gland and mantle were separated. Then glycogen content in the tissues of treated and control bivalves was analyzed. The percentages of biochemical components in the tissues of treated and control bivalves were compared. **Chronic treatment** : The acclimatized bivalves were divided into three groups, one group of bivalves was kept as control and the remaining two groups of bivalves were exposed to 0.32 ppm mercuric chloride and 1.95 ppm cadmium chloride respectively. The chronic treatment was given up to 20 days. The control and treated bivalves were fed on freshwater algae or Hydrilla during exposure period.

At the end of the 5^{th}, 10^{th}, 15^{th} and 20^{th} day, the control and treated bivalves were sacrificed to analyze the biochemical composition. The control and treated bivalves were dissected and their foot, digestive gland and mantle were separated. The glycogen contents in the tissues (whole body, foot, digestive gland and mantle) of treated and control bivalves were analyzed. The percentages of biochemical components in the tissues of control and treated bivalves were compared.

Estimation of Biochemical component (Glycogen) from tissues :

The colorimetric estimation of glycogen present in the tissue was done by anthrone reagent method (Dezwaan and Zandee, 1972). 50 mg. of wet tissue was taken in 1 ml of 30% KOH solution. The mixture was boiled in water bath for 5-10 minutes, till the tissue was completely dissolved. The solution was cooled and to it 0.2 ml 2% Na_2SO_4 and 6 ml of absolute alcohol were added. This solution was kept in refrigerator for

overnight. It was then centrifuged for 15 minutes at 3000 rpm. The supernatant was discarded and the residue cake was dissolved in 10 ml of distilled water 0.1 ml of this solution was taken and to it 0.9 ml of distilled water were added. The solution was heated in boiling water bath for 5 minutes and then cooled. The intensity of the colour developed was measured with the colorimeter (Erma) at 620 mu (Red Filter) filter. Anthrone, reagent was prepared by dissolving 50 mg anthrone powder and 1 gm Thiourea in 100 ml of 72% H_2SO_4. The amount of glycogen was calculated by referring to a standard graph value, where glucose was used as a standard. The glycogen value was calculated by multiplying with the conversion factor 0.927 to glucose value. The amount of glycogen was expressed in terms of Mg. of glycogen/100 mg of wet tissue.

III. OBSERVATION AND RESULTS

Biochemical components such as carbohydrates (glycogen) was studied in the normal (control) and pollutant (mercuric chloride and cadmium chloride) treated whole body, foot, digestive gland and mantle of freshwater bivalve, L. marginalis., the results are summarized in Tables .

Acute treatment: The acute treatment was given upto 72 hours by heavy metal pollutants mercuric chloride and cadmium chloride. After the acute treatment by pollutant, biochemical composition of the bivalve was altered and the results are summarized in the tables.

The glycogen content of the whole body, foot digestive gland and mantle decreased after acute treatment by heavy metals. After 72 hours acute treatment of mercuric chloride, the glycogen content decreased in the whole body, foot, digestive gland and mantle from 2.05% to 1.00%, 2.55% to 1.13%, 2.85% to 1.40%, 1.01% to 0.058% respectively. After cadmium chloride acute treatment, the glycogen depleted in the whole body, foot, digestive gland and mantle from 2.08% to 1.15%, 2.52% to 1.82%, 2.89% to 2.00% and 1.01% to 0.96% respectively.

A significant change in the glycogen content was found in mercuric chloride treated freshwater mussels and it was followed in cadmium chloride treatments. The maximum depletion occurred in the digestive gland of L. Marginalis.

Chronic treatment: The biochemical component such as glycogen was observed after chronic treatment in control and treated freshwater mussels, Lamellidens marginalis. The biochemical components of the whole body foot, digestive gland and mantle were observed and recorded in the tables.

The glycogen content of the whole body, foot, digestive gland and mantle decreased in chronic (5, 10, 15 and 20 days) treatment by heavy metals After 20 days chronic treatment of mercuric chloride , the glycogen content decreased in the whole body, foot, digestive gland and mantle from 1.89% to 0.60%, 2.76% to 0.90%, 2.64% to 0.62% and 0.92% to 0.27% respectively. After cadmium chloride chronic treatment, the glycogen depleted in the whole body, foot, digestive gland and mantle from 1.89% to 1.12%, 2.76% to 1.00%, 2.64% to 1.68% and 0.93% to 0.92% respectively.

A significant change in the glycogen content was found in mercuric chloride treated freshwater mussels and it was followed in cadmium chloride treatments. The maximum depletion occurred in the digestive gland of Lamellidens marginalis.

A significant change in the lipid content of L. marginalis was noted after chronic treatment of the heavy metals ($HgCl_2$ and $CdCl_2$).

Table – 1 Glycogen content in selected tissues of the control and $HgCl_2$ exposed Lamellidens marginalis as a function of exposure period

Treatment	Sr. No.	Body Organ	Total glycogen content (%) + S.D.		
			24 hours	48 hours	72 hours
Control	1	Whole body	2.1874 ± 0.0713	2.1425 ± 0.002	2.0589 ± 0.0710
	2	Foot	2.9652 ± 0.00501	2.8659 ± 0.0576	2.5524 ± 0.0270
	3	Digestive gland	3.3045 ± 0.0036	3.0242 ± 0.0128	2.8594 ± 0.0778
	4	Mantle	1.5224 ± 0.0197	1.2542 ± 0.0472	1.0103 ± 0.0106
Acute treatment by $HgCl_2$	1	Whole body	1.6051 ± 0.0282 P<0.01	1.3042 ± 0.08052 NS	1.0052 ± 0.0020 P<0.001
	2	Foot	1.8458 ± 0.1221 P<0.001	1.4329 ± 0.0286 P<0.001	1.1342 ± 0.1196 P<0.001
	3	Digestive gland	1.8927 ± 0.0711 P<0.001	1.7057 ± 0.0877 P<0.001	1.4000 ± 0.0861 P<0.001
	4	Mantle	0.8945 ± 0.0771 P<0.001	0.7241 ± 0.0147 P<0.001	0.5823 ± 0.0617 P<0.001

Table – 2 Glycogen content in selected tissues of the control and $CdCl_2$ exposed Lamellidens marginalis as a function of exposure period

Treatment	Sr. No.	Body Organ	Total glycogen content (%) + S.D.		
			24 hours	48 hours	72 hours
Control	1	Whole body	2.1874 ± 0.0713	2.1245 ± 0.002	2.0859 ± 0.0701
	2	Foot	2.8625 ± 0.0510	2.9695 ± 0.0567	2.5254 ± 0.0201
	3	Digestive gland	3.3045 ± 0.0036	3.0224 ± 0.0182	2.8954 ± 0.0771
	4	Mantle	1.5242 ± 0.0197	1.2524 ± 0.0427	1.0103 ± 0.0106
Acute treatment by $CdCl_2$	1	Whole body	1.3015 ± 0.0828 P<0.001	1.0085 ± 0.0069 P<0.001	1.1520 ± 0.0424 P<0.001
	2	Foot	2.2248 ± 0.1018 P<0.01	2.1045 ± 0.0853 P<0.001	1.8219 ± 0.0178 P<0.001
	3	Mantle	1.3420 ± 0.1159 NS	1.0065 ± 0.0053 P<0.01	0.9632 ± 0.0516 NS

Table –3 Glycogen content in selected tissues of the control and $HgCl_2$ exposed Lamellidens marginalis as a function of exposure period

Treatment	Sr. No.	Body Organ	Total glycogen content (%) + S.D.			
			5 Days	10 Days	15 Days	20 Days
Control	1	Whole body	2.2105 ± 0.0134	2.1113 ± 0.0713	2.1603 ± 0.0002	1.8907 ± 0.0701
	2	Foot	3.2021 ± 0.0009	3.1257 ± 0.0306	2.8103 ± 0.0725	2.7692 ± 0.0513
	3	Digestive gland	3.7999 ± 0.0851	3.2214 ± 0.01231	2.9012 ± 0.0017	0.6402 ± 0.0324
	4	Mantle	1.6325 ±	1.2010 ±	1.0758 ±	0.9230 ±

			0.0524	0.08131	0.0769	0.0621
Chronic treatment by $HgCl_2$	1	Whole body	1.0421 ± 0.0671 P<0.001	0.9302 ± 0.0216 P<0.001	0.8202 ± 0.0159 P<0.001	0.6602 ± 0.0521 P<0.001
	2	Foot	1.3191 ± 0.0713 P<0.001	1.2112 ± 0.0173 P<0.001	1.0832 ± 0.0697 P<0.001	1.9018 ± 0.0056 P<0.001
	3	Digestive gland	1.4000 ± 0.0861 P<0.001	1.1351 ± 0.0214 P<0.001	0.9278 ± 0.0243 P<0.001	0.6251 ± 0.0221 P<0.001
	4	Mantle	0.5013 ± 0.0021 P<0.001	0.3121 ± 0.0754 P<0.001	0.2818 ± 0.0432 P<0.001	0.2710 ± 0.0602 P<0.001

Table –4 Glycogen content in selected tissues of the control and $CdCl_2$ exposed Lamellidens marginalis as a function of exposure period

Treatment	Sr. No.	Body Organ	Total glycogen content (%) + S.D.			
			5 Days	10 Days	15 Days	20 Days
Control	1	Whole body	2.2398 ± 0.0243	2.1879 ± 0.0732	2.1004 ± 0.0002	1.8907 ± 0.0731
	2	Foot	3.2021 ± 0.0009	3.1357 ± 0.0306	2.9820 ± 0.0715	2.7692 ± 0.0513
	3	Digestive gland	3.3213 ± 0.0831	3.1214 ± 0.0101	2.8012 ± 0.0071	2.6402 ± 0.0432
	4	Mantle	1.3456 ± 0.0524	1.3000 ± 0.0861	1.0957 ± 0.0763	0.9302 ± 0.0216
Chronic treatment by $CdCl_2$	1	Whole body	1.5042 ± 0.0850 P<0.001	1.4808 ± 0.0689 P<0.001	1.2250 ± 0.0204 P<0.001	1.1240 ± 0.01012 P<0.001
	2	Foot	3.3475 ± 0.0387 P<0.001	3.1401 ± 0.0327 P<0.001	1.9550 ± 0.0449 P<0.001	1.0024 ± 0.0019 P<0.001
	3	Digestive gland	-	-	-	-
	4	Mantle	1.4454 ± 0.1187 NS	1.1142 ± 0.0932 NS	1.1000 ± 0.0816 NS	0.9201 ± 0.0733 NS

IV. DISCUSSION

Heavy metal and pesticide pollutants cause stress to the aquatic organism and change its metabolic activity. The change in biochemical composition of tissues due to heavy metals and pesticides and the physiological state of metabolic activity of an organism reflect the utilization of their biochemical energy to counteract the toxic stress. The observed biochemical changes in bivalves representing adaptive or regulatory mechanism may be due to pathological effect. The animal by changing its metabolic processes tries to overcome the toxic effects as a protective measure. The heavy metal pollutants give rise to alterations in the metabolic and physiological activity both after short and long term exposures. To investigate the physiological changes after pesticide and heavy metal treatment the most fundamental one would be the study of change in the biochemical constituents. Carbohydrates, proteins and lipids are the important metabolites which provide energy to different vital processes.

Glycogen is the stored food material in animal tissue which is used as an immediate source of energy when required and is an essential feature of the normal organismal metabolism (Thunberg and Manchaster, 1972.)

They glycogen content in freshwater bivalve, L. marginalis was altered indicating the effects of heavy metals. The average glycogen content in acute and chronic treatment by heavy metals (mercury chloride, and cadmium chloride) was decreased in the whole body. The depletion of glycogen content was greater in the digestive gland as compared to the foot and mantle of the bivalve, when exposed to pollutants. This indicates that the digestive gland is the principal metabolic center for various metabolic functions. During acute and chronic exposures a significant decrease in the glycogen content of the digestive gland suggests greater

glycolytic activity in the gland than the mantle and foot. The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants. Depletion in glycogen level might be because of the anoxia and hypoxia caused due to stress conditions which are known to increase carbohydrate consumption (Dezwan and Zandee, 1972).

The decrease in glycogen content indicates shifting towards anaerobic metabolism. Carbohydrates are considered to be the first organic nutrient to be deputed and degraded in stress conditions imposed on animals (Clerke, 1975). According to Koundinya and Ramamurthi (1979), the decrease in glycogen may be due to enhanced breakdown of glycogen to glucose through glycolysis. The greater decrease in the glycogen level, in the digestive gland might be due to high potential of digestive gland for glycolysis, similar to that of the vertebrate liver as suggested by Kabeer et al., (1977).

The mode of action of pollutants may be responsible for cellular dis-organization offering the storage and metabolism of the glycogen. Decrease in glycogen content indicates disrupted carbohydrate metabolism. The pollutants give the heavy physical irritate stress causing rapid movement and increased respiration rate thus increased utilization of reserved glycogen to meet higher energy demand of body causing decrease in glycogen content (Bhagyalaxmi, 1981). Many worker support the above results in vertebrate and invertebrate animals. A change in serum protein and glycogen content of rainbow trout exposed to endrin was studied by Grant and Mahrle (1973). Koundinya and Ramamurthi (1978) observed the effect of lethal concentration of sumethion on carbohydrate metabolism in Tilapia mossambica. Baner and Ghosh (1978) have recorded the alterations in the levels of serum glucose, liver glycogen and glucose-6-phosphate of the fish, Clarius batrachus when exposed to cadmium. Banerjee et al. (1978) reported an increase in blood glucose level in the fish Tilapia mossambica when exposed to cadmium. Kabeer (1979) stated that decrease in glycogen content in Malathion exposed tissues can also be due to decrease in glycogen synthesis. Koundinya and Ramamurthi (1979) have observed that sumethion leads to an increase in blood glucose level in decrease in glycogen content. Rao and Rao (1979) studied the effect of methyl parathion on the fish Sarotherodon mossambica and noted a significant decrease in glycogen content, Gill and Pant (1981) studied the effect of Nickel intoxication of carbohydrate metabolism i.e. blood glucose and liver glycogen which were measure to assess the magnitude of biochemical stress. Bhagyalakshmi (1981) studied the levels of certain carbohydrate metabolites in the tissue of field crab, Oxiotelphusa senex senex after exposure it to an organophosphate pesticide sumithion, and recorded an increase in haemolymph glucose level and decrease in total carbohydrate level.

Nagabhushanam and Kulkarni (1981) observed increased haemolymph glucose and a decrease in midgut gland, when the prawns, Macrobrachium Kistensis were exposed to $ZnSO_4$ and $CuSO_4$. Srivastava and Singh (1981) studied the acute effect of methyl parathion on carbohydrate metabolism of the Indian carfish, heteropheustes fossilis. Forooqui (1982) observed a insignificant increase of glycogen in the ovary of Barytelphusa cunicularis after two days exposure to sevimol and a significant decrease after seven days exposure and concluded that glycogen breakdown provides the immediate energy source in stress condition. Natarajan (1982) studied the effect of various concentration of $ZnSO_4$ on glycogen content of river, muscle, brain, kidney and gills of the fish Anabas Scands. Bhagyalkshmi et al. (1984) observed a decrease in glycogen and elevated phosphorylase activity in the crab, Oxiotelphusa senex senex exposed to sumithion, suggesting onset of glycogenolysis forming free glucose and the possible exist of these glucose molecules in the haemolymph resulting in the hyperglycemia condition. Patil (1986) studied the effects of pesticides on the glycogen content of Mythimna (P) separate and found decreased glycogen content ater treatment.

Many workers studied the effects of pollutants on mollusks in Mandal and Ghose (1970) observed glycogen depletion in the digestive gland of the snail, Achatina fulica (Bawdich) when exposed to calcium arsenate. Ramana Rao and Ramamurthi (1980) studied the effect of sublethal concentration of sumithion on some biochemical constituents of the snail, Pila globosa and found a decrease in glycogen content. Lomte and Alam (1982) observed the stable level of glycogen in the foot and mantle but very sharp fall of glycogen in the digestive gland during sublethal exposure for 24 hours to organophosphate pesticide, malathion. Swami et al. (1983) found increased haemolymph glucose and decreased stored glycogen in Lamellindens marginalis when treated with Flodit and Metacid. Kulkarni et al. (1984) studied the impact of endosulfan on the apple snail, Pila globosa and found an elevation of blood glucose level after treatment. Chaudhari (1988) studied the effect of pesticides on biochemical composition of the snail Bellamya (viiparous) bengalensis and found decreased glycogen content after treatment.

V. SUMMARY

1. The biochemical composition of Lamellidens marginalis after acute and chronic treatment of heavy metals (mercuric chloride and cadmium chloride) was studied to better understand the mechanism of action of pollutant, by observing the time bound and tissue specific alterations of biochemical components glycogen in different organs of the body.

2. After acute treatment of heavy metals glycogen content of the whole body, foot, digestive gland and mantle was altered very prominently, glycogen depletion was more due to $HgCl_2$ when compared to $CdCl_2$.
3. The glycogen content of the whole body, foot, digestive gland and mantle decreased after chronic treatment of heavy metal pollutants, much glycogen content decreased in digestive gland which was followed by mantle and foot. Glycogen decrease due to $HgCl_2$ was followed by $CdCl_2$.

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