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Quantification of Lipid in different organs of Mercuric Chloride treated Fresh water crab, *Barytelphusa guerini* (Milne Edwards, 1853).

Sujata Magdum[1*] and Ms. Gauri Shahane[2]

[1]P.G. Department of Zoology, [1]K.R.T.Arts, B.H.Commerce, A.M. Science College, Nashik.
[2]GMD Arts BW Commerce and Science College, Sinnar, Nashik
*Corresponding Author : sujata_magdum@yahoo.com

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Abstract

Non-degradable heavy metals are hazardous to aquatic ecosystem. Its persistence and ability to accumulate in living world is a matter of concern. It enters the aquatic medium in its inorganic form where it may undergo transformation depending upon biotic and abiotic factors of the ecosystem. An attempt is made to understand the effect of $HgCl_2$ on lipid content in various tissues of freshwater crabs *Barytelphusa guerini*. Lipids are an important energy source for both the developing stages and adults in the decapod crustaceans. The LC_{50} was found to be 0.93 ppm of $HgCl_2$ and the sublethal concentration 0.35 and 0.85ppm of $HgCl_2$ was used for experimentation which was set for 4 days. A set of control group was also maintained for comparison. The trend of effect of $HgCl_2$ was Gills < Muscles < Gonads < Hepatopancreas. Further, female gonads (ovaries) showed more effect when compared with males. Time-dependent and concentration -dependent reduction was noted.

Key words: Non-degradable, heavy metals, ecosystem, biotic, abiotic, sublethal, *Barytelphusa guerini* , Lipids ,Gills, Muscles, Gonads, Hepatopancreas

Introduction

Contaminated environment due to daily addition of various kinds of non-degradable pollutants is unwanted gift to ecosystem. These pollutants includes certain particulate matter, dissolved gases, agricultural waste in the form of pesticides/

runoff fertilizers, industrial effluents, sewage, household non degradable waste or many such anthropological contributions. Heavy metals are also one form of contaminant which are added to environment by natural means or through human activities. Heavy metals are a set of metals or metalloids that possess densities 5 times higher than that of water [1,2].

These metals are natural constituents of the earth's crust, and natural phenomena such as weathering and volcanic eruptions causing litigation and mitigation contribute significantly to heavy metal pollution. Anthropogenic activities such as mining, smelting, industrial, and agricultural use increases the abundance of heavy metals in the environment by either releasing or concentrating them into the environment [2,3,4].

Heavy metals are widely used in industries, agriculture, pharmaceuticals', and many of the other technological applications. Studies have revealed that very low levels of exposure of these heavy metals can be toxic to living organisms. Extensive studies on excessive usage of heavy metals have raised concerns for human health over time, and their impacts on the overall environment [4]. Based on their immediate threat to human health and the environment, arsenic, lead, mercury, and cadmium are listed as top most chemicals of major public concern by the World Health Organization [2].

Terrestrial animals are generally exposed to metals through their diets, or through the air they breathe while aquatic organisms are exposed to dissolved and particulate metals in their ambient medium. Being non-degradable many Molluscs and Crustaceans have been known to accumulate a wide range of these heavy metals.

Different heavy metals are accumulated by aquatic biota, sometimes at levels far above those found in the surrounding medium, thereby enabling the use of selected organisms for monitoring the levels of the metals in water bodies. These small aquatic organism helps in understanding the availability of pollutant in their natural environment and helps in quantifying it directly. Thus, these animals produce a time-averaged index of pollution. The much higher concentrations of pollutants in the body, compared with those in the surrounding water, make it easier to analyse the samples, and assess the more evident biochemical, histopathological, ionic balance, and enzymatic changes caused by pollutants.

Heavy metal pollution of fresh water bodies has become the major concern in recent years. Organisms living in ponds, rivers, streams etc. are subjected to natural periodical fluctuations in the environment and generally get adapted to these environmental stresses [5]. However, residents of Mukane village are involved into agriculture and run off from theses agricultural lands in the form of used fertilizer, insecticides, pesticides, other wastes, and sewage has prove to be a threat to aquatic biota in nearby area. There is always a threat that these pollutants due to unavoidable circumstances combines together creating a stressful environmental to its inhabitants.

A number of studies have been conducted on the effect of heavy metal toxicity on physiology of freshwater crabs. But the available literature does not provide sufficient information regarding the effect of $HgCl_2$ upon physiology of *Barytelphusa guerini* from Mulane Village. Majority of tribal people from this region use these crabs as their source of food. So, it has a great economic value. For this reason, present investigation was undertaken to fulfil the existing gap to study the effect of $HgCl_2$ pollutants upon the physiology of crab.

Material and Methods

Collection of crab:

Freshwater crabs, *Barytelphusa guerini* were collected from Mulane village, near Shapthshrigi ghad, Kalwan Tal, Dist Nashik (Fig 1). After collection, crabs were carefully transported, with least disturbance, to the laboratory in live condition. The crabs were maintained for 20 days in sufficient amount of plain tap waters in aquarium so that these crabs could be submerge. Water from aquarium was changed on alternate days to remove the waste materials. This was to acclimatized them at laboratory conditions (12L: 12D) at 27 ± 2° C. Care are was taken to place only 3-4 crabs in container ensuring that there was no 'crowding effect'.

Ethics:

The place where crabs were caught is privately owned field. The permission from the owner of the land, was seeked prior to catch. Care was taken confirmed that the current studies did not involve endangered or protected species

Sexual dimorphism:

In crabs the sex of the individual was determined by observing the abdomen and the segments. In males abdomen is narrow V shaped and the segments are slightly concave where as in females abdomen is broad, wide and U shaped.

Stock solution:

54.3gm of $HgCl_2$ in 1L of water making a concentration of 0.2 M was prepared. Later this stock solution was diluted to make the working solution of 0.35 and 0.85 ppm

Treatment:

Each of five males and females of approximately same size and weight were selected. Males with carapace width 42±2 mm and body weight 52±2gm and females with carapace 44±2mm and body weight 54±2 gm were selected. Two crabs(male and female)were placed in serial dilution 0.1, 0.15, 0.2 till 0.95 ppm solution to study acute toxicity for 96 hours .LC_{50} was found to be 0.93ppm. Two of the sublethal concentration 0.35 ppm and 0.85 ppm solution of $HgCl_2$ were used for experimentation. For biochemical analysis2 groups comprising of Control, $HgCl_2$treated were made. Three males and three female crabs in each of the treated (0.35 and 0.85ppm respectively) control group were monitored for analysis for 96 hours.

Biochemical analysis:

Biochemical analysis for estimation of total lipids was done using gills, muscles, hepatopancreas and gonads of freshwater crabs, *Barytelphusa guerini* . These were sacrificed on fourth day of treatment.

Figure 1. Site map of collection area(courtesy Google map)

Estimation of lipids:

Lipids were extracted as described by Folch [6], and estimated by the method of Barnes and Blackstock [7].

Sample preparation:

2 mg of each tissues were homogenized (5% w/v) separately in a tissue homogenizer in the chloroform-methanol mixture (2:1). The homogenates were filtered through Whatman No.1 filter paper. The non-lipid matter from the pooled filtrate were removed by shaking vigorously with 0.9 % NaCl. Then the mixture was shaken and transferred to small separating funnel, and allowed to stand overnight at 0°C, to obtain a clear biphasic layer. The lower phase was used for estimation of lipids. The lower phase after replacing in Tarson tubes were adjusted to the volume of 10 ml by addition of chloroform.

Data Analysis:

One-way Analysis of Variance was used to evaluate differences between control and exposed crabs. Probability values less than 0.05 were considered significant. Statistical computations were performed with SPSS 15.0.

Results and Discussions

0.35 and 0.85ppm $HgCl_2$ exposed male and female *Barytelphusa guerini* from 0.35 and 0.85ppm solution were sacrificed on fourth day. Tissue was processed as mentioned above in materials and method and lipids were analysed. The trend of effect of $HgCl_2$ in both the doses was Gills< Muscles <Gonads <Hepatopancreas. Further, all these were dose dependent changes which is also represented in **Figure 2**.

Gills: Exposure to 0.35ppm $HgCl_2$ for four days, 76.68% of total lipids were measured in gills of male *Barytelphusa guerini* whereas in females 65.66% lipids were found when compared with control group. Similarly, the second treated group (0.85ppm) showed presence of 67.32% and 59.03% of total lipids in gills of $HgCl_2$ exposed male and female *Barytelphusa guerini.*

From these findings it can be seen that females are more affected than males upon exposure to 0.35 and 0.85 ppm of $HgCl_2$. The gills of aquatic organisms are the first organ which comes in direct contact with aquatic medium. Thus, they are highly vulnerable to toxic chemicals if any in the medium. Further because of their large surface area they facilitate the interaction with the toxins and helps in absorption. In the present study it is found that gill are the most affected organs amongst all. These results are in accordance with studies done by Pandey *et al* [9].

Gonads:

The next organ into studies were gonads. Both testis and ovaries were considered for estimation of lipids. From the experiment it was observed that testis of *Barytelphusa guerini* showed 95.85% of lipids on fourth day when males were exposed to 0.35ppm of $HgCl_2$. The ovaries of crabs exposed to same concentration of $HgCl_2$ showed 77.76% of lipids. This was 18% less than males within same dosage. Further with higher dose of 0.85ppm of $HgCl_2$ the lipids in testis further reduces to 90.67% and in ovaries it was found to be 64.84%.

These observations lead to the conclusion that the testicular proteins are less affected when compared to ovaries, muscles, gills and hepatopancreas (Figure 2). This could be due to no interactive changes in Fatty acid binding proteins (FABPs). These are small cytosolic proteins which are useful in the uptake and utilization of fatty acids [10,11,12,13]. These FABPs are extensively studied in higher vertebrates while invertebrates have received little attention in spite of its similar nutritional requirements during periods of reproductive activity [14]. From these observation it can be noted that less effect on testis may be due to lower interaction with FABPs which causes proper uptake and utilization of fatty acids. The author thus proposes to take up studies on FABP to confirm the results.

From the present studies it's found that lipid content in ovaries of both the treated groups (0.35 and 0.85ppm) is lower than control group as well as testis of males exposed to same concentration of $HgCl_2$. In normal conditions, ovaries, especially during yolk accumulation stores large amount of lipid [15,16]. This stored lipid is mobilized for energy production during embryogenesis. Therefore, lower level of lipids in 0.35 and 0.85ppm of $HgCl_2$ exposed females can probably lower the fecundity in future and therefore from the conservation point of view, studies should be taken up to reduce exposure of $HgCl_2$ in crabs. According to Lautier *et a*l [17] and Abava *et a*l [18], crustacean fecundity, survival and egg growth are associated with lipid content and composition of lipids in ovaries.

Muscles:

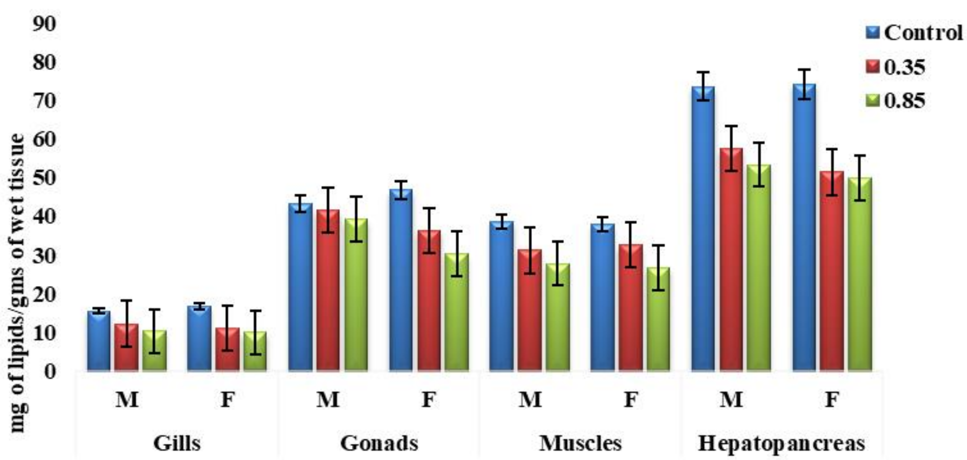
The effect of $HgCl_2$ on male and female muscles was also studied. 80.65% of lipids was found in males while females showed 85.88% when exposed to 0.35ppm of $HgCl_2$. 55 difference was found in the lipids content in muscles of both males and female *Barytelphusa guerini* on exposure to 0.85ppm of $HgCl_2$

The muscles of the crab are the chief edible portion for human consumption. Of the four organs (Gills, Gonads, Muscles and Hepatopancreas) taken into consideration, muscles did not show much effect of $HgCl_2$. Figure 2 gives the spatial distribution of effect of heavy metal concentrations on the muscles of male and female crabs. Lower level of muscle lipids could be due to less exposure and accumulation of $HgCl_2$ into blood as these reach the muscle mainly through blood supply [20]. Generally, muscle have much lower concentrations of heavy metals than gills and hepatopancreas. These results are in accordance with the studies reported by Batvari *et al* [21] and Liu *et al* [22].

Hepatopancreas:

Hepatopancreas were least affected organ in both the concentrations of $HgCl_2$ exposed males and females *Barytelphusa guerini.* In males, 78.19% of lipids was found when exposed to 0.35ppm of $HgCl_2$ whereas females exposed to same concentration showed drop in lipid content. It was found to be 69.41%. With the higher concentration i.e., 0.85 ppm the lipid was found to be 72.54% and 67.84% respectively in male and female *Barytelphusa guerini.*

The hepatopancreas of crab is the primary metabolically active organ which synthesizes high levels of metallothioneins. This protein is concerned with heavy metal detoxification and excretion [23,24]. Heavy metals accumulated in the hepatopancreas may be due to their intake by crab from water and diet. As hepatopancreas helps in excretion of heavy metal probably due to these reasons there is less effect seen in hepatopancreas in present study. The results can be confirmed by understanding the relevant experiments related to synthesis of metallothioneins in *Barytelphusa guerini*



Graph 2: Composition of lipid concentration in different organs of control and treated fresh water crab, *Barytelphusa guerini*. Means and S.E. (n = 3) are represented.

Table 2: Two way Anova analysis for lipid content in gills, gonads, muscles and hepatopancreas of $HgCl_2$ exposed and control group of *Barytelphusa guerini*

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Male	Between Groups	215.083	2	107.541	.257	.041
	Within Groups	3771.841	9	419.093		
	Total	3986.924	11			
Female	Between Groups	468.957	2	234.479	.636	.032
	Within Groups	3320.655	9	368.962		
	Total	3789.612	11			

Data Analysis

From the result it was clear that the most affected organ was gills and hepatopancreas affected the least. The data was statistically analysed in SPSS 15.0 and was found that the data was significant at 0.05(table 2).This indicates that there is significant difference seen in the lipid contents in different organs into consideration and also between the organs of two sexes. From the above results and discussion it can be stated that fresh water crabs are affected by HgCl2.

Conclusion

The effect of HgCl2 on crab tissue in present study indicates that there is accumulation of these heavy metals from the surrounding environment through assimilation or absorption from food, water and/or sediment. Being considered as ecosystem engineer due to their dynamic role as a borrower in mangrove, estuarine environment or any other water bodies it's very important to conserve them. In spite of their such an significant role in maintaining the environment the importance, only a few studies have been reported on the bioaccumulation of heavy metals in fresh water crab. Efforts should be taken to reduce the heavy metal pollution caused by anthropogenic activities so that crabs will be conversed and there will be sustainable development.

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