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Histological Structure of Reproductive Organ of Freshwater Female Crab, Barytelphusa Cunicularis (West-Wood)

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ABSTRACT

Mercury concentrations were recorded in water and tissue of Barytelphusa cunicularis from Pimpalwadi site (Jaikwadi Dam) near Aurangabad. The level of heavy metals in the ovary and spermatheca of crabs was investigated using Atomic Absorption Spectrophotometer (AAS). The mean concentration of mercury in the crab was 0.9 ±0.001 µg/g. A histopathological alteration in ovary and spermatheca was also studied. Several histological changes were noted in the ovary tissue i. e. Distortion of yolk granules, vacuolization, slight necrosis in the oocytes in the ovary and vacuolization observe in lumen, granular substances, sperm mass and spermathecal fluid was evenly distributed in the crab was observed after exposed to sublethal concentration (24th of LC_{50}:1/5th 0.208 ppm) of mercuric chloride.

Key Words: Crab, histology, ovary, spermatheca

Introduction:

Agricultural activities also contributed to the pollution or the aquatic environment through run off which find their ways into the water bodies (Adams and Chapman, 2006). Increasing concentrations of the metals cause significant increases in the mortality in crabs and prawns. In general, considerations of metal bioavailability and bioaccumulation in aquatic media can be split into direct and indirect exposure and impacts. Direct exposure occurs via the water column where biotic and abiotic factors can influence metal bioavailability, and bioaccumulation may lead to toxic impacts on crustacean development [Dennis, *et al.*, 2005; Adeniyi, *et al.*, 2008, Soundarapandian, *et al.*, 2010].

To better understand not only physiological effects but also toxicological and hygienic organism great attention is paid to hazardous elements such as mercury, lead, cadmium and arsenic and their ability to accumulate in organs (Spurny, *et al.*, 2002; Yilmaz, 2006; Andreji, *et al.*, 2006b].

Histopathological studies are also useful in evaluating the pollution potential of heavy metal pesticides, since trace amount of these chemicals which do not bring animal mortality over a given period, were capable of producing considerable organ damage. Despite much information available on the histopathological changes caused by heavy metal pesticides, the mode of action on the vital organs is still not fully understood. The importance of study on the histological changes brought about under the stress of different toxicants in different organs and organ systems of crab has been documented (Patil *et al.*, 2006).

In the freshwater crabs it is observed that the external organs are affected due to the toxic chemicals causing loss of equilibrium, abnormal changes [jumping to avoid toxin, paralysis] and finally lead to death (Paul *et al.*, 2005; Joshi, 2006). This may be attributed to the significant damage to the internal organs. Heavy metal pesticides pollute aquatic ecosystem and find their way in the body of aquatic animals by means of ovary and spermatheca [Paul *et al.*, 2005].

Barytelphusa cunicularis possesses these criteria and is a sensitive indicator of heavy metal both at acute toxicity and at accumulation levels, indicating the possible use of this species in monitoring pollution. The contamination of aquatic resources with a wide range of pollutants has become a matter of concern over the past few decades and it affecting the aquatic animals specially crabs vary widely. The objective of the present study is impact assessment of mercury in the ovary and spermatheca with special reference to histological study of the freshwater crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentration of mercuric chloride for 5 and 10 days of exposure.

Material and Method

The freshwater crabs, *Barytelphusa cunicularis* were collected from Pimpalwadi site (Jaikwadi Dam Paithan) located at (19°29′6″N 75°22′12″E) Aurangabad District. They were acclimatized to laboratory conditions under normal day/night of 11 L : 13 D illumination at 27 ± 1º C for about one week in plastic troughs [18" diameter] containing sufficient tap water so that crabs are submerged. Before experimentation female crabs in intermoult stage [C_3 Diwan, 1973] of approximately equal carapace width (45 to 50 mm) and body weight (50 to 55 gm) were sorted into 3 groups (Control, $HgCl_2$ treated) with similar biomass (n = 6 for each group), each group being maintained in laboratory condition. The crabs were exposed to sublethal concentration of mercuric chloride 1/5 (0.208 ppm) for 5 and 10 days. After their respective exposure period, the ovary and spermatheca were dissected out from both the control and experimental were fixed in aqueous Bouins fluid. After fixation for 24 h the tissues were further processed to study histological details as per procedure of Bancroft and Stevens [1982]. In brief, the tissues were dehydrated through 30 % to 100 % different alcohol grades and cleared in xylene. Cold and hot impregnations were followed by embedding the tissue in paraffin wax [M.P. 58-60 ° C]. Serial sections were cut at 7 µm serial using rotary microtome. The sections of ovary and spermatheca were stained using Harris Haematoxylin and Eosin-Y as counter stain [Bancroft and Stevens, 1983]. Damage to the tissues of treated crabs is recorded by comparing the data obtained from control.

OBSERVATION AND RESULTS

Impact assessment of mercury was studied in the ovary and spermatheca of freshwater crab, *Barytelphusa cunicularis* (Westwood) exposed to sublethal concentrations of mercuric chloride for 5 and 10 days of exposure.

The experimental crabs showed many histological changes the ovary showed distortion of yolk granules and slight necrosis are observed in the oocytes. The outer thin epithelium and inner germinative epithelial layers are damaged. Oocytes covering thin membrane are also damaged and follicle cells are destructed. In addition oocytes show vacuolization; fragmentation and necrosis, and the nuclei are disintegrated [Fig. 1].

The spermatheca of experimental crab showed drastic changes in the cuticular, muscular and epithelial layers. The thickness of middle muscular and inner epithelial layer is enlarged. Damages in the spermathecal wall are clearly observed. Luminal content consisting of granular substances, sperm mass and spermathecal fluid substances is evenly distributed. Vacuolization is observed in the lumen of the spermatheca [Fig. 2].

DISCUSSION

The chemical exposures of organisms in polluted ecosystems and extent of occurrence or accumulation of trace metals by organisms in different tissues is dependent on the route of entry, that is, either from surrounding medium or in the form of food or chemical form of material available in the media [Ghosh and Kshirsagar, 1973; Phillips and Rainbow, 1994].

Season may influence body burdens of heavy metals. This seasonal variability may results from either internal biological cycle of the organism or from changes in the availability of the metals in the environment of the organism [Yilmaz and Yilmaz, 2007; Olowoyo, *et al.,* 2010].

Several different studies have been carried out on the determination of levels of heavy metals and their effects in aquatic organisms particularly in crab (Falusi and Olanipekun, 2007). The increase of the mercury in the water body results in the excess accumulation of mercury by the aquatic animals like crabs, fishes, bivalves which are consumed by the people as their food, thus, posing a human health risk;

elevated levels of mercury in the crabs can also have ecologically significant effect, such as affecting reproduction [Wiener, 1995; lliopoulou and Kotsanis, 2001].

The mode of action of heavy metals on biological systems is thought to be enzymes systems, although extra ordinary concentrations may result in direct tissue damage (Abubakar and Garba, 2006).

The nutritional implication is that consumers of these food materials may be exposed to heavy metal toxicity if bioaccumulation results due to regular consumption [WHO, 1972, Goyer, 1995, Ross and Morison 2002]. The levels are far beyond the tolerable level of 0.001 μg/g, [WHO, 1972].

Though these food materials are processed [heating, cooking] before consumption, the effect of processing could be minimal, since the heavy metals are non-degradable. Mercury toxicity can occur after microbial degradation of Hg to dimethyl mercury. Human exposure to dimethyl mercury occurs through consumption of contaminated aquatic foods. Hg affects the central nervous system and brain due to its ability to cross the blood brain barriers (Goyer, 1995).

The histological techniques are the promising area of research in aquatic toxicology as it gives the real picture of the effects imposed and the involvement of the xenobiotics in either disturbing or destroying the vital organs of living organisms. Many workers have reported the degenerative changes in selected tissues of the animals in response to pollution by various toxicants (Kale, 2002; Reddy, 2005; Samyappan, 2006; Wu, *et al.,* 2008; Shaikh, *et al.,* 2010; Andhale, *et al.,* 2011).

Swelling and vacuolization in the oocytes, degeneration of oolemma and loss of normal shape of oocytes, disorganized ooplasm, hypercromatic nuclei and fibrosis of ovarian wall rupturing of oocytes membranes in the oocytes and disturbances in the supporting connective tissues of *Barytelphusa cunicularis* in response to endosulfan and thimet toxicity (Jadhav, 2002).

Spermatheca in crab is paired sac-like organs, which present two zones: one dorsal, glandular zone of mesodermic origin and the other one the ventral zone, with chitinized walls, of ectodermic origin. They are usually referred to as ‘storage’ and ‘fertilization chamber’, respectively located just below the heart [Jonson, 1980; Lopez, *et al.,* 1999].

Suresh [2001] reported damage of ovarian wall, vacuolation and foaming in ooplasm in the ovary and in spermatheca disruption and disintegration of the wall layer and non-homogeneity in the distribution of the granular substances of the brackish water crab, *Uca annulipes* in response to cadmium and mercury exposure.

The present study revealed that the significant differences on ovary and spermatheca. The concentration rates of mercury in the tissues of freshwater crab vary significantly as a function of season and the pollution load of tissue.

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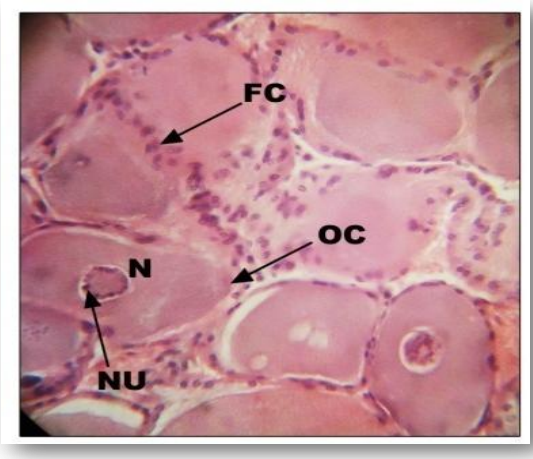
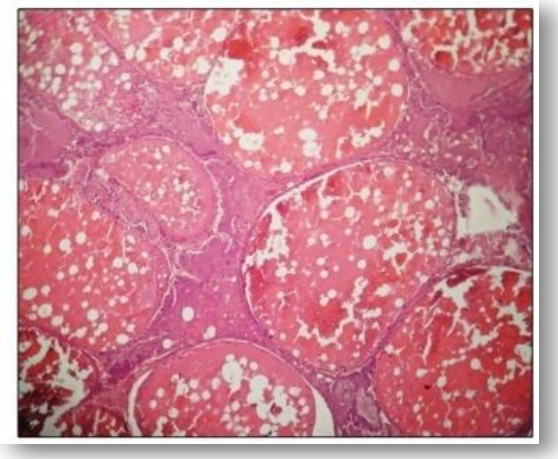
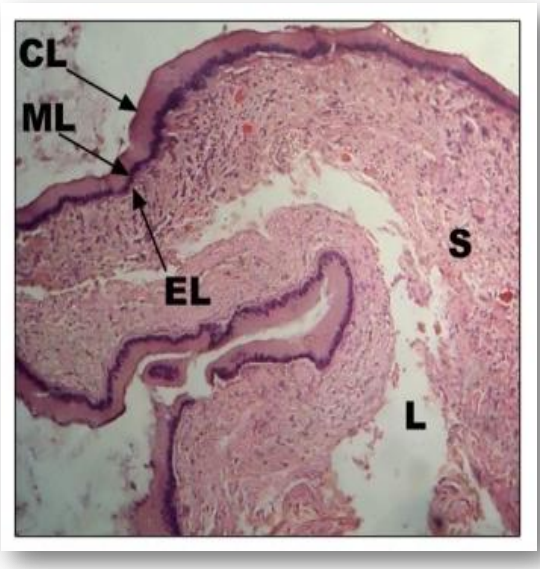


CONTROL CRAB [X 400]
Nucleus is distinct with nucleoli, oocytes emerge from the centre of each lobe,

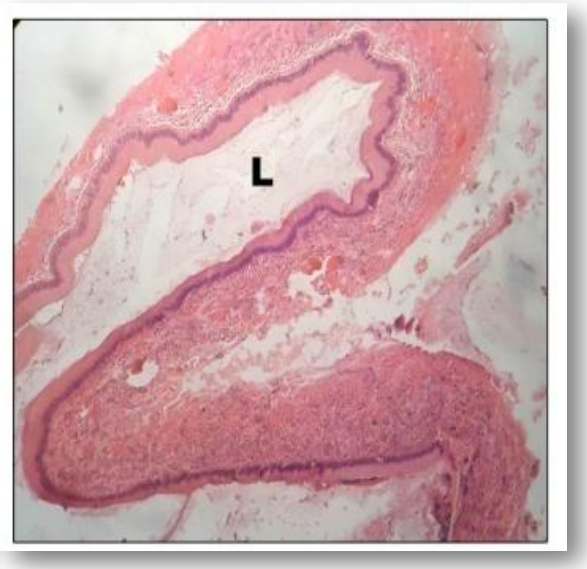


EXPERIMENTAL CRAB [X 400]
Distortation of yolk granules, necrosis are observed in the oocytes, outer and inner layers damaged, oocytes show vacuolization; fragmentation & necrosis, nuclei are disintegrated.

Fig. 2. T.S. OF OVARY OF CRAB, *BARYTELPHUSA CUNICULARIS* [WESTWOOD]
OC: OOCYTE, **FC**: FOLLICLE CELL, **N**: NUCLEUS AND **NU**: NUCLEOLUS. STAIN H & E.



CONTROL CRAB [X 400]
Layers are distinct, clearly visible and lumen filled with free sperm, sperm mass, spermatophores.



EXPERIMENTAL CRAB [X 400]
Thickness of middle muscular layer & inner epithelial layer, damage spermathecal wall, vacuolization is observed in the lumen, spermathecal fluid, is perm mass evenly distributed.

Fig. 3. T.S. OF SPERMATHECA OF CRAB, *BARYTELPHUSA CUNICULARIS* [WESTWOOD].
CL: CUTICULAR LAYER, **ML**: MUSCULAR LAYER, **EL**: EPITHELIAL LAYER, **L**: LUMEN ,**S**: FREE SPERM, SPERM MASS AND SPERMATOPHORE. STAIN H & E.