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Aquatic Contamination: Histopathology Of Female Gametocytes in *Barytelphusa cunicularis*

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Abstract:
Reproduction has vital role to maintain the biodiversity and ecobalance in nature. In present investigation major physico-chemical parameters like chloride, sodium, COD, TS, TDS and SS has induces histopathology of oocytes in crab *Barytelphusa cunicularis*. In which, ovary showed disturbed epithelial layer with degenerated oocytes. Vacuolization was appeared at the periphery of the oocyte cells due to high concentration of COD. In conclusion, physico-chemical parameters caused histopathology on developing oocytes under stressful conditions.

Keywords: *Barytelphusa cunicularis,* histopathology, ovary, pollutants, water quality.

1.0 Introduction:
Water pollution by industrial effluent has become a major problem due to their extreme toxicity to human health and too biological ecosystems (Katsuro *et al.*, 2004). The healthy aquatic ecosystem depends on the biological diversity and Physico-chemical characteristics (Venkatesharaju *et al.,* 2010). The pollution of rivers and streams with chemical contaminants became one of the critical environmental problems. Natural water resources as rivers, lakes and streams have enough factors responsible for growth of various organisms in the aquatic body (Dhasarathan *et al*., 2006). Environmental pollution found to be undesirable side effect of industrialization and an important aspect of environmental degradation (Jothinarendiran, 2012). Pesticides have different modes of action to aquatic inhabitants. As a result release of pollutants from industrial areas, and agricultural runoff into the environment severely mixes into water bodies (Tyagi, 2000). Chemical persistence are facing spatial or temporal alarming high levels of xenobiotic chemicals (Brack *et al.* 2002; Diez *et al.,* 2002). Some of these chemicals are nonbiodegradable and remain dangerous for a long time. Effluents are discharged into environment with enhanced concentration of nutrient, sediment and toxic substances can cause a serious negative impact on the quality and life forms of the receiving water body (Forenshell, 2001; Miller and Siemmens 2003; Schulz and Howe, 2003). The direct discharge of industrial effluents and runoff comprising versatile chemicals exert their toxic effect on the aquatic organisms (Mathivanan, 2004). Industrial, sewage, municipal wastes are continuously added to water bodies leading to changed physiochemical property making them unfit for use of livestock and other organisms (Dwivedi and Pandey, 2002). Aquatic ecosystems are more sensitive to the release of industrial wastewater (Pálaez-Cid et al. 2013).

As crustaceans were considered as healthy food `known by their high quality protein and less contents of fat. Reproduction is a physiological process and biological need of animals for the continuity of generation which is known to dominate all other physiological processes. Reproduction serves to replace population losses due to death and migration (warren, 1971). Long term exposure to the pollutant can causes remarkable damage to the tissues of regenerative cells restricting the development of eggs, hatching of the eggs and newly hatched young ones are also affected by the exposure to the pollutants and ultimately reduces their long term exposure more pollutants gets accumulated in the tissue of the animal and thus it becomes unfit for aquatic life. Histopathological observations give an early indication of pollution hazard, and provide useful data on nature and degree of damage to cells and concerning tissues. It is a common tool for determining the deleterious effects of toxic substances in animals.

Histopathological effect of pollutants on various tissues of aquatic animals was studied by many workers. Stress exerted by exposure of freshwater crabs *paratelphusa (Barytelphusa) jacquemontii* to insecticide endosulphon drained into waterbodies had altered activity of enzyme constituents, which indicated significant influence of toxic nature of this insecticide to crab as an important species of aquatic ecosystem. (Patil *et al* 2014). Literature revealed that less work has attended to effects of water quality on histological changes in the gametogenic cells specifically in female so, present work aimed to investigate the effect of water contaminant on ovarian cells of freshwater crab, *Barytelphusa cunicularis.* Obtained results were interpreted with reproductive capacity of organism in the study region.

2.0 Material and Methods:

2.1 Study area:

Gadhinglaj is a tahsil from district Kolhapur, state Maharashtra. Geographically has latitude 16°13´26"N and longitude 74°26´9" E. River Hiranyakeshi flows through two states of India (Maharashtra and Karnataka). Local inhabitants use water for their daily needs along with agriculture and industrial processes. For present study, three sites were selected as, (Figure 1) site I Mahagoan river (N 16°9'54" E 74°19'58") considered as normal. Site II Nilgi river (N 16° 14´ 16" E 74° 25´ 41"), the local inhabitants depend on these for their daily needs like drinking, cloth washing, cattle washing and agriculture use etc. and site III Harali river(N 16°9'54" E 74°19'58") situated near the sugar industry. The molasses from this industry directly poured into Harali river. At site II and III agricultural and industrial waste was added continuously (Fig.1).

2.2 Parameters under study:

For the physico-chemical assessment, the water samples were collected for the year February 2012 to January 2013 from sites from where animals were collected. Water samples were brought to the laboratory by plastic containers for analysis of physico-chemical parameters. Physico-chemical parameters were analyzed by applying standard physico-chemical methods. (APHA, 1985,). Heavy metal analysis was carried out by using Atomic Absorption Spectrophotometer (AAS) (Kemito company- 201).

2.3 Animal collection:

The freshwater crab *barytelphusa cunicularis* were collected from site I, II and III. Healthy crabs having equal size (carapace width 13.1- 13.5 cm and body weight 215-217 g) were used for experiment.

2.4 Histology:

To study Histopathological lesions in the ovary the female crab were dissected immediately and ovary were quickly removed and fixed in aqueous bouins fluid. After fixation for 24hrs. the tissue was passed through alcohol grades for dehydration and cleared in xylene. The tissue was embedded in paraffin wax (MP. 58-60 °C) and serial sections were cut at 4-5 micrometer and stained in Delafield's Hematoxylene and eosin.

3.0 Results and Discussion:

3.1 Physico-chemical parameters of water:

3.1.1 Chloride:

The most important source of chloride in natural water is discharge of sewage. The minimum value of chloride was (11.49 mg/l) at site I whereas maximum (52.87 mg/l) at site II and site III it was 63.16 mg/l (Table 1, 2 and 3). Sreenivasan, (1965) documented, chloride concentration between 4-10 ppm indicating purity of water. Chloride content was reported as an indication of pollution if present in higher concentration. Royal Commission suggested that, water having 30 mg /l of chloride was reported to be fairly clean. Sources of chloride pollution in water include fertilizers, sewage, effluents from drainage, salts and human as well as animal wastes. High chloride content cause high blood pressure in people (Subin *et. al*., 2011). Chloride of water found toxic to most plants so it should be checked for irrigation water. The tolerance limit for surface water used for irrigation was 600 mg /l (Fadtare *et. al,* 2007).

3.1.2 Sodium:

The maximum sodium analyzed at site II (42.25mg/l) and 34mg/l at site III while minimum at site I (7.25mg/l) (Table 1, 2 and 3). Maximum sewage discharge was found at site II leading to its pollution as compared to others. The industrial discharge and sewage disposal has increased the content of sodium, when present in high concentration which limits the biological diversity (Adoni *et al.,* 1985).

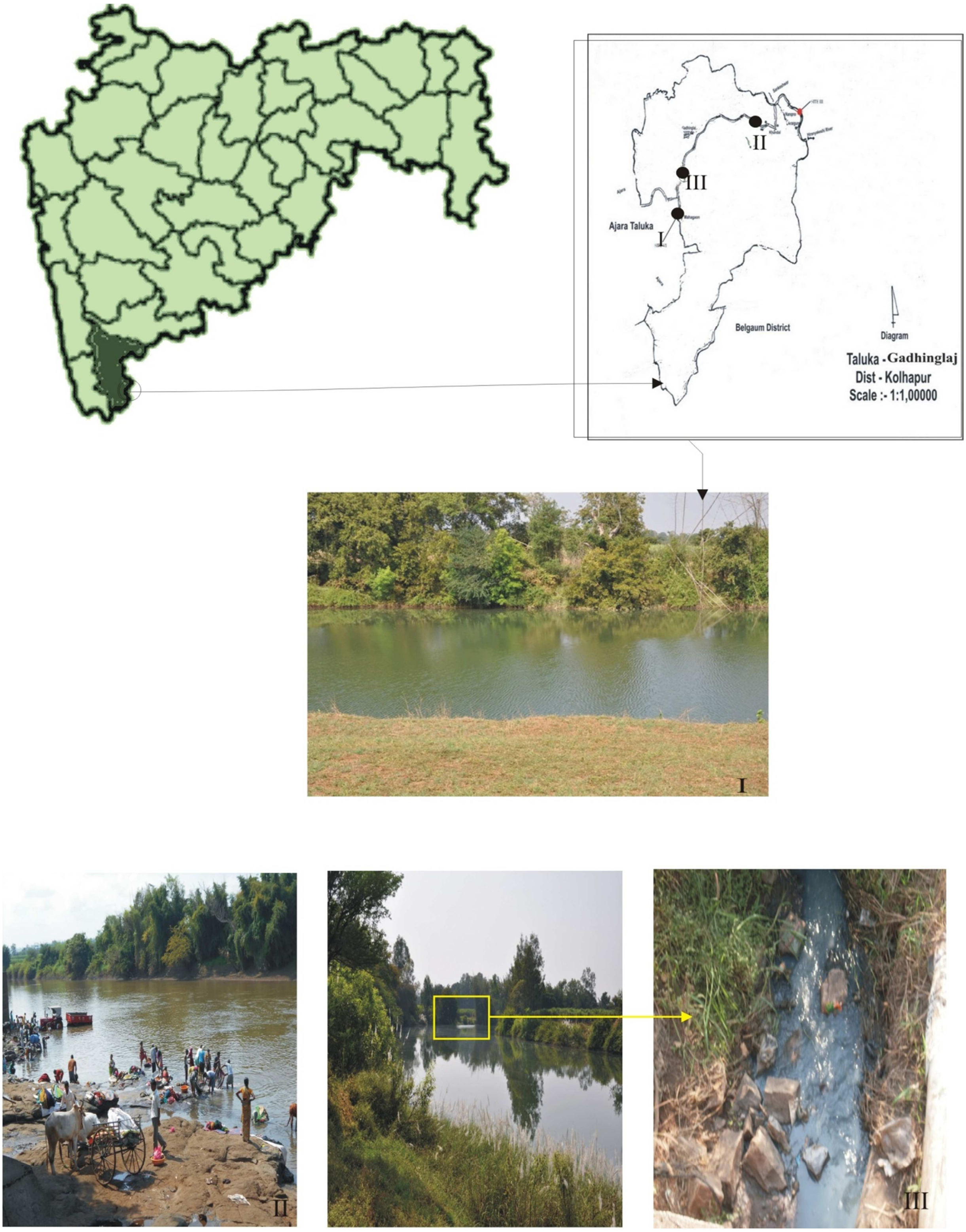

Fig. 1: Sampling sites of study area, Site I: Mahagoan river, II: Nilgi river, III: Harali river

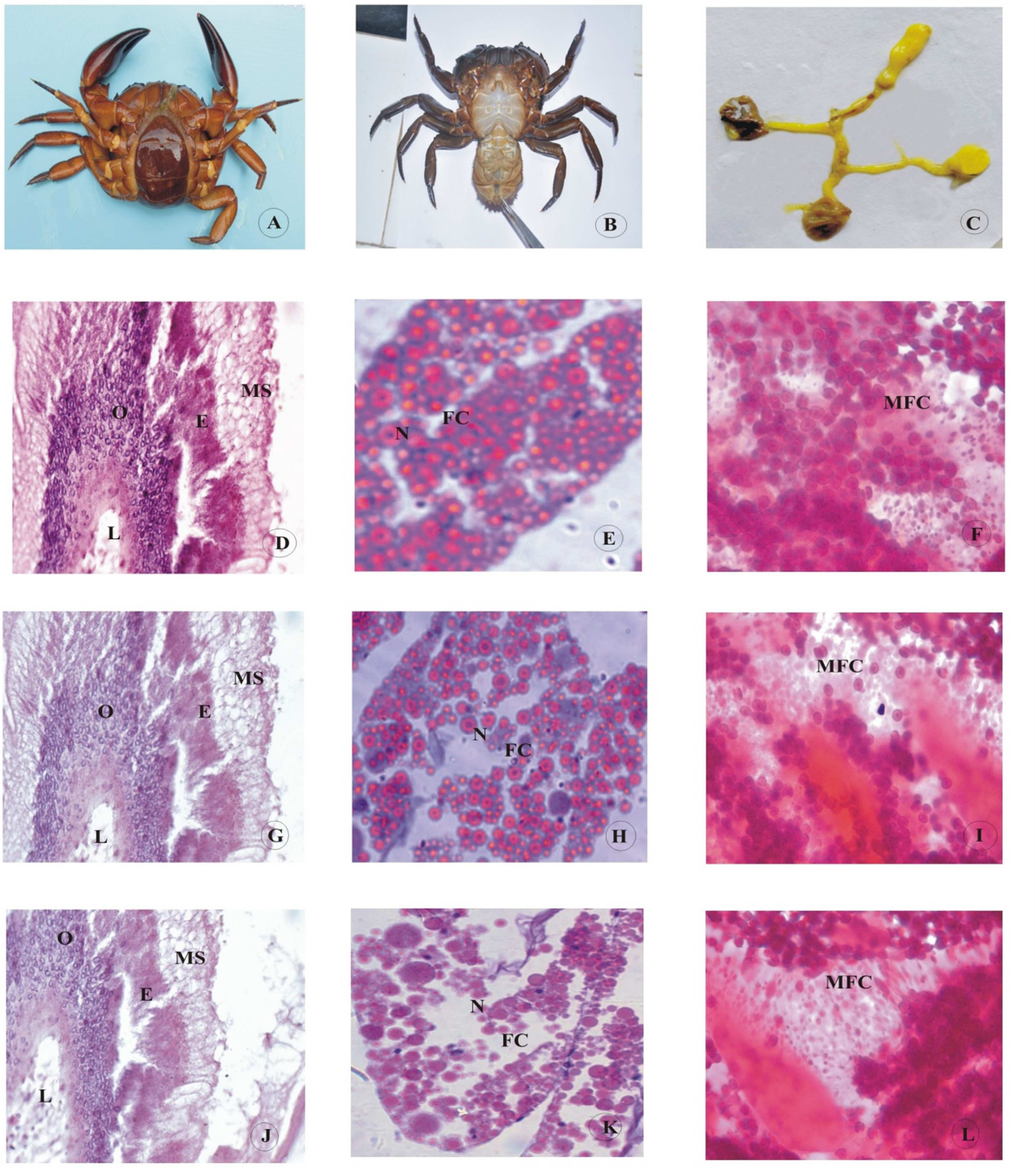

Fig. 2: A: Freshwater crab, **B**: External openings of crab ovary, C: Female reproductive system of crab.
1) D, E, F: Histology of crab ovary from site I 2) G, H, I : Histology of crab ovary from site II 3) J, K, L: Histology of crab ovary from site III

Abbrevations: MS: multilayered sheath, E: enlarged spermathical epithelium, O: ovarian follicles, L: lumen, N: nucleus, FC: follicular cells, MFC: matured follicular cells.

Table 1: Seasonal variation in physico-chemical parameters from site- I

Parameter	Season		
	Summer	Rainy	Winter
Chloride	21.3±8.829	11.492±1.894	19.00±0.849
Sodium	8±3.162	7.25±2.753	5±0.816
COD	5.625±4.269	2.75±0.957	9±10
TS	63±10.893	60±19.866	76.5±18.645
TDS	58±13.165	52.5±13.279	68±12.754
SS	5±3.464	7.5±7.852	8.5±6.608
Potassium	1.33±0.577	1.25±0.5	1.5±0.577
Iron	0.291±0.213	0.667±0.653	0.02±0

Table 2: Seasonal variation in physico-chemical parameters from site- II

Parameter	Seasons		
	Summer	Rainy	Winter
Chloride	51.83±35.951	19.0275±2.699	52.87±17.741
Sodium	42.25±19.362	28±14.165	19.75±2.872
COD	25.5±5.196	8±6.164	92.5±62.55
TS	229±149.75	84±51.562	175.5±111.40
TDS	192.25±99.633	75.75±51.771	157.5±115.243
SS	36.75±58.351	8.25±0.5	11.25±4.991
Potassium	3.75±2.872	3.33±0.577	4.25±2.629
Iron	0.885±0.876	1.313±0.604	0.228±0.07566

Table 3: Seasonal variation in physico-chemical parameters from site- III

Parameter	Seasons		
	Summer	Rainy	Winter
Chloride	58.75±1.369	38.21±20.051	63.16±7.062
Sodium	34±17.435	28.75±9.358	28±7.0710
COD	834±748.16	249.25±66.269	495.5±514.049
TS	209.75±122.31	369±194.357	570.75±69.615
TDS	196.5±121.179	343.25±194.93	541±47.483
SS	13.25±12.841	24.5±22.590	30.25±28.663
Potassium	3.5±2.645	4.25±4.0311	10.75±3.403
Iron	0.859±0.961	1.64±1.659	3.531±2.169

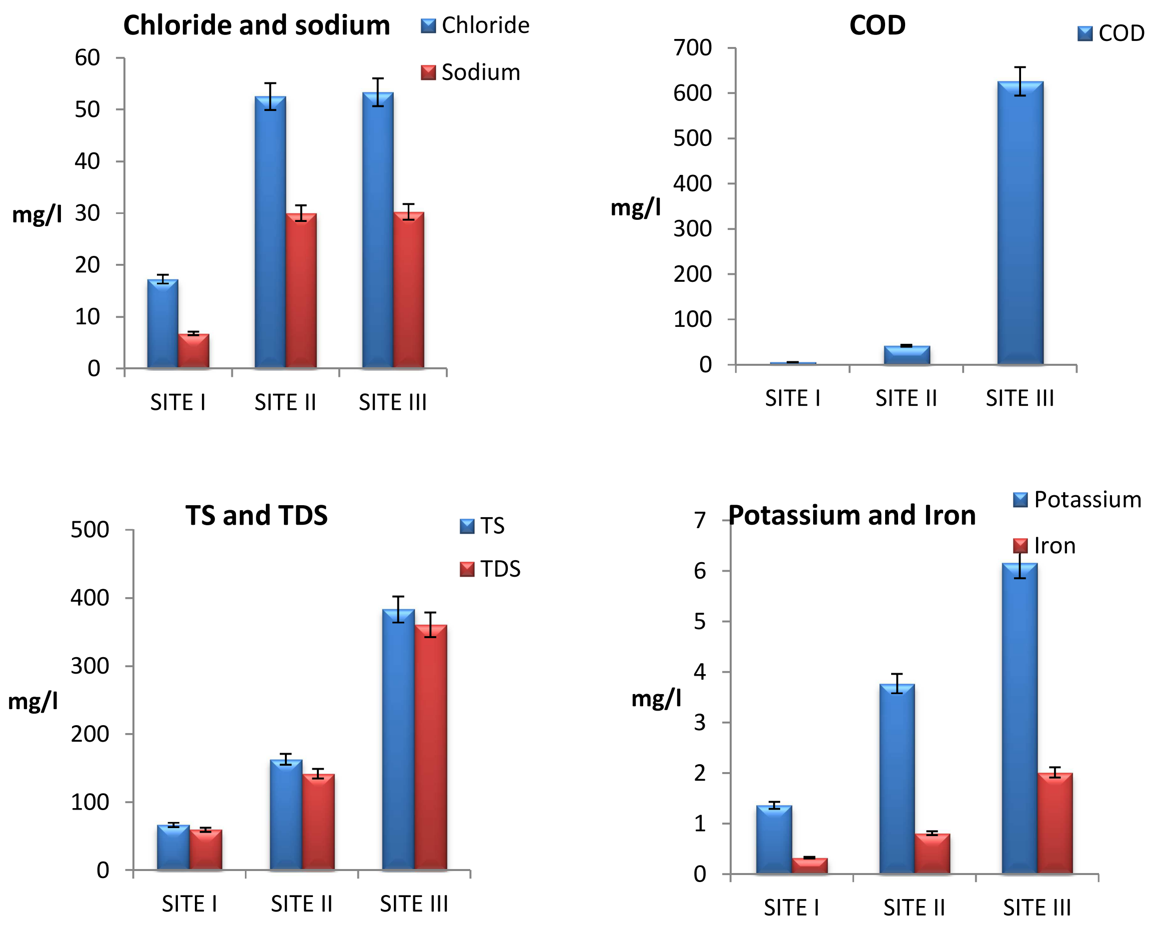

Fig. 3 Graphical representation of Physico-chemical parameters of study area

3.1.3 Chemical Oxygen Demand:

COD found reliable parameter for analysis of water pollution. Maximum COD found at site III (834 mg/l) and at site II (92.5 mg/l), while minimum at site I (2.75mg/l). Chemical pollution in aquatic ecosystems, found to be major environmental concern. Pollution decreases the water quality, subsequently affects organisms inhabiting in the system. Chemical oxygen demand (COD) found measure of the oxygen equivalent of the organic matter of water which was susceptible to oxidation by a strong chemical oxidant. Thus, COD found to be, reliable parameter for judging the extent of pollution in water (Amirkolaie, 2008). The COD of water increases with increasing concentration of organic matter (Boyd, 1981). In our investigation COD concentration was comparatively higher at site 2 and 3 due addition of chemical and municipal waste as being discharged. Thus we found that, industrial waste has affect over the hatching, failure of trout eggs, and effect over diurnal and seasonal behavior, changed migration capacity. Overall resulted to decrease the tolerance of species. It has been observed that, because of pollution of chemicals, domestic wastes, metal pollution cause change in the species diversity.

3.1.4 Total solids:

The total solid content was maximum at site II and III (229 mg/l and 570mg/l), while minimum at site I (60 mg/l) (Table 1, 2 and 3).

3.1.5 Total dissolved solids:

In the present investigation the concentration of total dissolved solid found high at site II and III (192mg/l and 541mg/l) which has decreased potability and reduced utility of water for drinking,

irrigation and industrial purposes. Minimum range of TDS was seen at site I (52.5 mg/l) (Table 1, 2 and 3).
The animal pollution and human interference also contributed to enrichment of dissolved solids (Krishna ram *et al.,* 2007). The total concentration of dissolved solids in a water body found useful parameter in describing the chemical density and productivity of the water (Jhingran, 1982). High content of dissolved solids has elevated density of water, influencing osmoregulation and reducing gas solubility utility of water for drinking, irrigation and industries (Manivasakam, 2003).

3.1.6 Suspended solids:
The suspended solid content was maximum at site I and II (36.75 mg/l and 30.25mg/l) while minimum at site I (5 mg/l) (Table 1, 2 and 3).
According to Jain *et al.,* (2003), the higher concentration of total suspended solids was found due to insoluble organic matter in sewage. The disposal of sewage and industrial effluent has contributed suspended matter to the rivers. WHO, (1984) recorded that, 30-80 % human diseases were occurred due to impurities of water. Our study revealed that, total concentration of dissolved solids in a waterbodies was high at site II and III as compare to site I. Results Indicated enrichment of suspended solids at site II and III found unfit for drinking, irrigation and also for industrial purpose.

3.1.7 Potassium:
The high concentration of potassium was noted at site 2 (4.25 mg/l) and at site 3 (30.25 mg/l) while minimum at site I (1.5mg/l) (Table 1 and 2). Potassium found vital role in the metabolism of freshwater organisms (Krishna ram *et al.,* 2007). Cell membrane continually pumps the potassium and sodium, which requires the expenditure of large amount of energy (Goldman and Horne, 1983). We found higher concentration of potassium recorded in rainy season at site II and lower concentration in winter season at site I.

3.1.8 Iron:
Iron content of water was high at site III (3.531 mg/l) while minimum (0.02 mg/l) at site I (Table 1 and 3). The iron concentration high at site III, it might be due to mixing of sugar industrial effluent. Iron concentration up to 0.1 mg. L^{-1} is acceptable while 1 mg. L^{-1} or more iron in fresh water could be harmful for life (Trivedi and Gurdeep,1992). concentration of iron in present study at site III was 3.531 mg. L^{-1} which does not falls in safe limits recommended by Trivedi and Gurdeep (1992). Iron was found vital element of life and natural component of soil and its concentration can be influenced by industrialization. We observed the higher concentration of iron was at site III because of mixing of industrial waste in it.

3.2 Anatomical features of the female reproductive system of, *Barytelphusa cunicularis*:
The female reproductive system found bilaterally symmetrical. An H-shaped ovary consisting of a pair of long ovarian sacs connected by a narrow bridge tube was located in the cephalothorax on the dorsal side of the stomach. A short oviduct with a seminal receptacle was connected with the posterior end of each ovarian sac, and has genital pore opens on the sternum of the sixth thoracic segment. Anatomically ovary showed following stages,

1. Immature (stage I) ovary with thin, white to pale yellow in color.
2. Maturing (stage II) ovary dark yellow in colour.
3. Vitellogenic-I (stage III) ovary having deep or dark yellow in colour. The ovarian lobes were large and extend upto the abdominal regions.
4. vitellogenic-II (stage IV) ovary with fully matured and appeared dark yellow colour.
5. Spent restorative stage (stage V) ovary has aged oocytes almost of the same size as that of the vitellogenic oocytes.

Vacuoles were appeared in ova. Vacuolization extended slowly into the interior of ova and has the last stage whole ooplasm which was vacuolated. Oviduct and spermatheca were joined together to ovary (Figure 1-C). The color of ovary also showed variation during the course of development and maturity.

3.3 Histological alterations in ovary from site I:
The histological structure of ovary of crab, *Barytelphusa cunicularis at* site I showed that, entire ovary was enclosed by a thin capsule of fibrous connective tissue and associated with multilayred sheath. Fibrous connective tissue has separated lobes of mature ovaries. There were two types of cells in the ovarian lobes, the developing oocytes and the follicle cells (oogonia and oocytes). The oocytes found covered with follicles cells. The ovarian follicles were filled with different types of maturing oocytes. The thin membrane oocyte has large rounded nucleus. The mature stage was characterized by ovary with well-developed lobes and fully matured eggs. Thus histologically developing stages of ovarian follicles were observed

with huge number of maturing oocytes (Fig.2 D, E, F) at site I.

3.4 Histological alterations in ovary from site II and III:

The histological structure of ovary from site II and III showed thin capsule of multilayered sheath. Ovarian cells were destructed, outer thin spermathical epithelium and inner germinative epithelial layer were damaged. The oocyte covering thin membrane was also enlarged and damaged, with destructed follicle cells. Vacuolation and fragmentation in the follicular cells were observed. Follicular membrane was damaged.Vacuolation in the follicular cells (Fig. 2 H, K) was prominent with less number of matured follicle cells. In the present investigation, the ovary of Barytelphusa cunicularis subjected to sugar industrial effluent showed marked degenerative changes.

3.5 Histopathology of ovary:

Shinde *et al* (2002), identified the rupturing of oocytes membrane in the oocytes, Vacuolization in the peripheral oocytes and disturbances in the supporting connective tissue after acute and chronic exposure of sugar industrial effluent in crab *Barytelphusa gurini.* Machale *et al* (1990) studied on histipathological changes in the ovary of freshwater crab *Barytelphusa guerini* exposed to copper sulphate and reported extensive damage to oocytes. Chourpagar and Kulkarni (2011), observed histological changes in the tissues of freshwater female crab, *Barytelphusa cunicularis* when exposed to heavy metal pesticides. The reproductive cycle of crustaceans has been widely studied, mainly of those species that have commercial value or ecological potential reported by Castiglioni and Negreiros-Franzoso, (2006). Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale *et al.,* 2011). Histological changes provides an early indication of pollution hazard, and also useful data on nature and degree of damage to cells and tissues (Shaikh *et al.,* 2010). In present study we observed the pathological conditions of ovary at site II and III the damages might be due to pollution stress. 5-hydroxytryptamine present in the central nervous system of crustaceans exerts its effect indirectly, by stimulating release of the ovary stimulating hormone (GSH) which in turn acts directly on the gonad. Several reports also exist on the effects of the neurotransmitter, 5-hydroxytryptamine on gonadal development of crustaceans (Nagur babu *et al*., 2013). In present study, Damage to ovary due to exposure of hard water pollutants might be decline neurotransmitter, 5-hydroxytryptamine.

Machale *et al.,* (1990) studied the effect of cuprous oxide on the ovary of *B. guerini* and noticed shrinkage in ooplasm, vacuolization on peripheral side, rupture of oocytes, structural damage, necrosis of nuclei and nucleoli with disintegration of oocytes. Sarojini *et al.,* (1990) observed the degeneration of oocytes, vacuolization and replacement of oogonia with fibrous tissue in the ovary of freshwater crab, *B. guerini* after exposure to zinc sulphate. Rao *et al.,* (1990) reported the shrinkage of ooplasm. Kharat, *et.al,* (2011) observed the gametogenic changes in ovary of freshwater prawn, *Macrobrachium kistensis* exposed to TBTCL. Histolopathological studies were useful in evaluating the pollution potential since trace amount of these chemicals producing considerable damages in various organs (Indira1989). In addition, analysis of histological changes in target organs provides a valuable tool in understanding the role of specific cell and organelles metabolism (Hinton *et al* 1992 and Rubio *et al* 1993) The histopathological changes in ovarian cells due to contaminated water showed progressive damage and degeneration. This was evident with the exposure of animal to water pollutants extent of tissue damage increases with the increase in water pollution. Damage to ovary due to exposure of hard water pollutants decline in reproductive activity and indirectly reduces the regenerative capacity in the population indices.

4.0 Conclusion:

Exceeded inorganic and inorganic compounds causes' heavy contamination. The aquatic organisms absorb the pollutants directly from water, leads to abnormal developmental growth. In the present investigation site I can be suitably used for irrigation with less hazardous effect. Water analysis from site II and III indicated that, it was contaminated due to organic content of sewage, agricultural wastes inducing fertilizers etc. Pollution in aquatic ecosystem, especially riverine systems has major environmental disturbance. Study revealed that, there was an adverse impact on physico-chemical parameter by the discharge of sewage and industrial effluent. Gonadal system of *Barytelphusa cunicularis* showed hypertrophy with structural damage, due to the direct effect of induction of parameter as chloride, sodium, COD, TS, TDS and SS on developing oocytes under stressful conditions leading to gametogenic abnormalities. Less reproduction may leads to alterations in eco balance and its diversity indices in natural condition.

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