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Study of Cellular Markers in Fresh Water Mollusc, *Bellamya bengalensis*

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ARTICLE DETAILS

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ABSTRACT

Molluscs are invertebrates with the capacity to act as bio-indicators of water pollution. Bioaccumulation of pollutants occurs in different organ such as gills, digestive glands, mantle, foot but the digestive gland in mollusc is considered as a target organ for detoxification. The using of multiple biomarkers (biochemical, molecular, cellular and histological) are considered valuable tool in determination of effects of pollutants. Contamination of aquatic ecosystem by pesticides, metals, organic compounds has been posing an ecotoxicological threat. The objective of the present study was to elucidate the role of cellular biomarkers identification in pollution detection. In the present work we have emphasized on hemocytes, foot cells and digestive gland cells and histological analysis. Freshwater mollusc *Bellamya bengalensis* were manually collected from different aquatic bodies of Nadia district of West Bengal. Some aquatic bodies were not associated with human habitats, human effluents and agricultural fields (sites A). Some aquatic bodies were associated with human habitats, human effluents and agricultural fields (sites B). Cell isolation, histological sections and staining were performed. Paraptosis and necrosis like features of cells were noted in site B group as compared to site A group of snails. Collection sites B may pollute the environment and affect aquatic mollusc due to anthropogenic activities. The present results corroborate with the findings in various gastropod snails by many scientists. Hemocyte function, cell death/apoptosis of foot cells, digestive glands cells have been claimed as markers of toxicity of different chemical compounds. Monitoring the health of aquatic ecosystems through molluscan cellular marker has been gaining special attention in recent researches.

1. Introduction

The molluscan immune system depends on circulating hemocytes which are migrating throughout the tissues in response to foreign toxic particles including pathogens. Hemocytes are involved with phagocytosis and encapsulation (Carballal *et al.*, 1997). Phagocytosis is reported as a biomarker of aquatic pollution (Oliver and Fisher, 1999). These cells are the target of many environmental contaminants. Guria, (2023) reported, in the freshwater mollusc, *Bellamya bengalensis*, after the lead (Pb) treatment, both paraptosis and apoptosis like features were noticed in the hemocytes. Pb inhibited the degree of hemocyte aggregation as poorly organized loose clump/ aggregates (Guria, 2023).

Guria *et al.*, (2025) stated when *Bellamya bengalensis* were exposed to sodium arsenite, mercury (II) chloride, lead nitrate, zinc sulphate and copper sulphate for the different exposure periods, the morphology and function of hemocytes were altered. Cells showed neutral red (NR) positive reaction in cytoplasm due to metal toxicity (Guria *et al.*, 2025). Guria, (2018) determined the toxic effect of lead on the cytomorphology of hemocytes of *Lamellidens marginalis* to evaluate its potential as a bio monitor for detecting a heavy-metal polluted environment. Treated cells showed vacuolation like features. The histological techniques are the promising area of research as it gives the information of the effects of the xenobiotics on tissues. Digestive gland is important route of entry for toxicants. Hepatopancreas is central metabolic organ

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involved in detoxification (Hamed *et al*., 2007; Guria, 2025). Accordingly, hepatopancreas is target organs in excretion of xenobiotics and highly recommended for toxicological studies (Otldil and ayaz, 2020). Structural changes within hepatopancreas, changes of the epithelium of digestive and basophilic cells are the markers of pollution (Lobo, 2019; Guria, 2025). The objective of the present study was to elucidate the role of cellular biomarkers and histological biomarkers identification in pollution detection.

2. Materials and Methods

2.1 Study area:
The study areas for specimen collection were different aquatic bodies of Nadia district of West Bengal.

2.2 Sampling method: Freshwater molluscs *Bellamya bengalensis* were manually collected from different aquatic bodies of Nadia district of West Bengal like Ballabhpur, Silinda-Darappur, Hariankha and Raghunathpur of Nakashipara areas. Some aquatic bodies were not associated with human habitats, human effluents and agricultural fields (sites A). Some aquatic bodies were associated with human habitats, human effluents and agricultural fields (sites B) (Guria, 2025).

*2.3 Cell isolation, histological sections and staining:*Hemolymph was collected from *B. bengalensis*. The syringe with a needle was inserted in foot, through the operculum and drops of hemolymph were aspirated and smeared on glass slides and hemocytes were stained by Giemsa, Leishmans Eosin Methylene blue solution and observed under light microscope (Guria *et al*., 2025; Guria, 2023). Tissues of digestive glands and foot were removed and mashed through the cell strainer into the petridish in presence of trypsin- EDTA. Cell suspension was smeared on glass slides, fixed by methanol and stained by Giemsa. Trypan blue assay showed cells that have taken up the dye were dead. Cell counting was performed by hemocytometer. Histological sections of digestive glands were made and stained with haematoxylin and eosin (double staining). Statistical analysis done by GraphPad InStat (Guria, 2025).

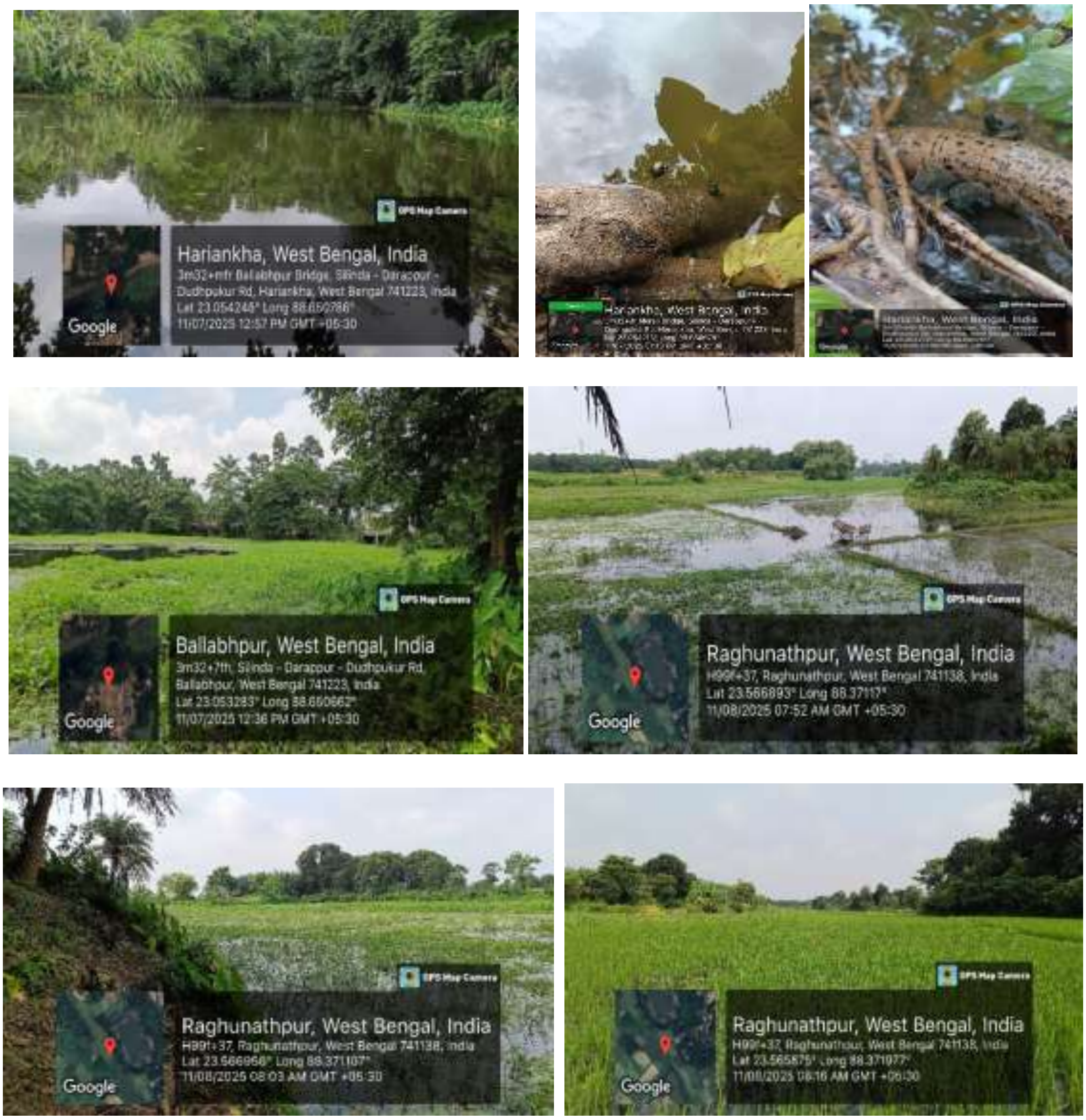
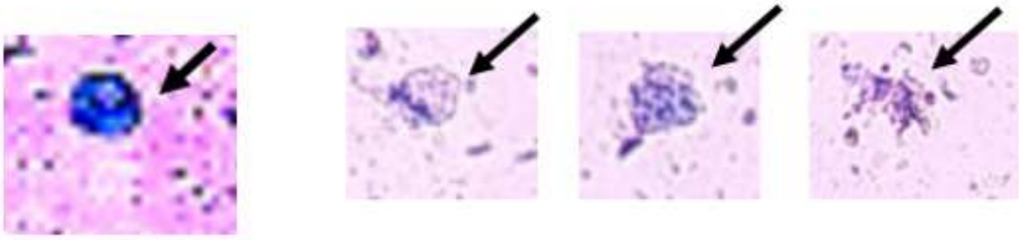


Fig 1: Specimen collection sites

3. Results

Vacuolization of cells (paraptosis like features), degeneration of nucleus, rupture of membrane (necrosis like features) were noted in the hemocytes, foot cells and digestive glands cells of site B group as compared to site A group of snails. Mean number of pyknotic hemocytes, foot cells and digestive glands cells of *Bellamya bengalensis* were increased in site B. The histopathological alterations were damage of epithelial cell lining of tubules. Loss of cellular boundaries was noticed and luminal space was filled by cellular infiltration in site B group.



2A 2B

Fig 2A: Giemsa stained hemocytes (indicated by arrow) from sites A.
Fig 2B: Giemsa stained necrotic cells (indicated by arrow) from sites B. Progressive Changes of hemocyte morphology in *Bellamya bengalensis* at sites B

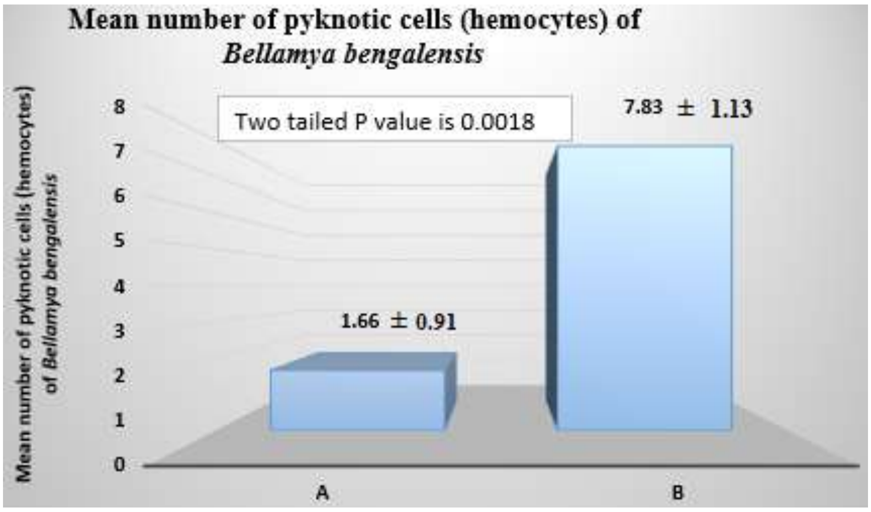
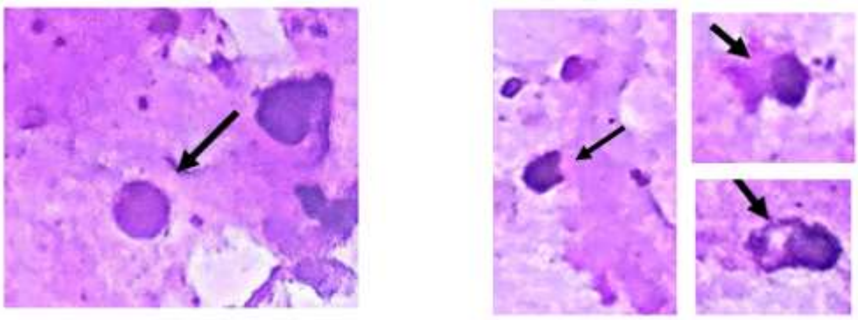


Fig 3: Mean number of pyknotic hemocytes of *Bellamya bengalensis*
A=cells from collection sites A
B= cells from collection sites B
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant



4A 4B

Fig 4A: Giemsa stained cells (indicated by arrow) from digestive glands in *Bellamya bengalensis* collected from sites A.
Fig 4B: Giemsa stained necrotic cells (indicated by arrow) from digestive glands in *Bellamya bengalensis* collected from the sites B.

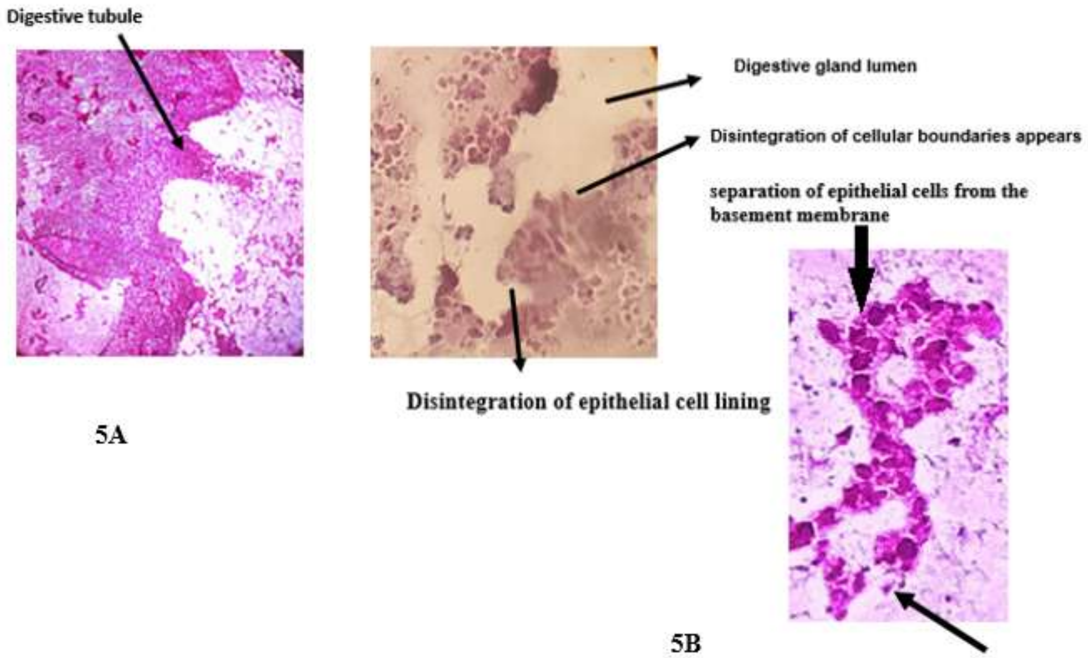


Fig 5A: H-E stained histology of digestive glands of *Bellamya bengalensis* collected from sites A**.**
Fig 5B: H-E stained histology of digestive glands of *Bellamya bengalensis*collected from sites B.

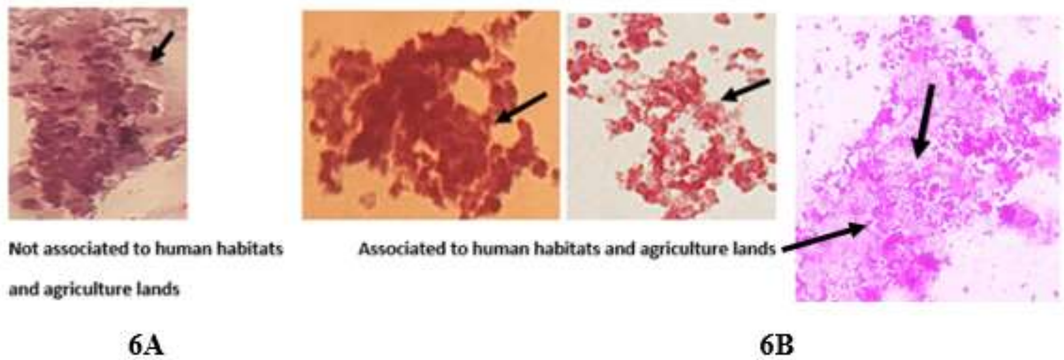


Fig 6A: H-E stained compact adhesiveness of cells of digestive glands of *Bellamya bengalensis* collected from sites A.
Fig 6B: H-E stained gradual loss of adhesiveness of cells of digestive glands of *Bellamya bengalensis* collected from sites B.

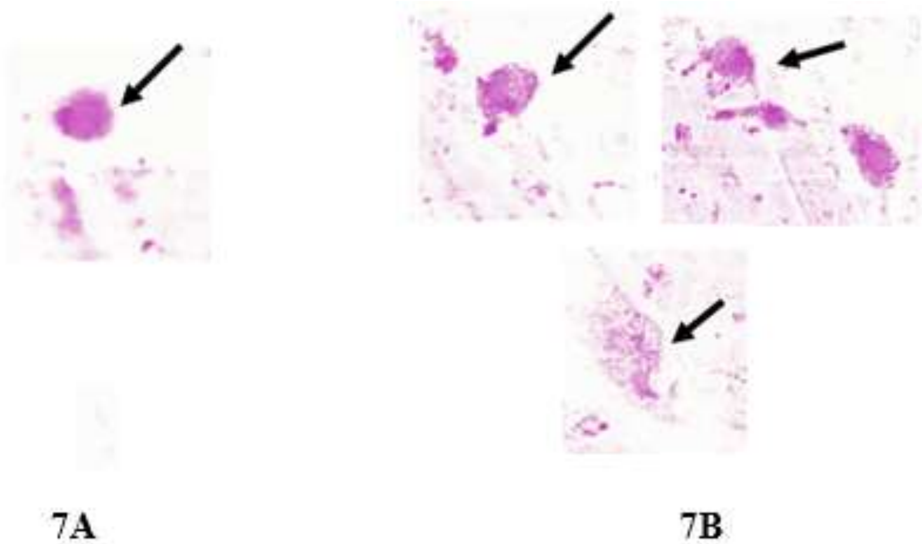


Fig 7A: Giemsa stained foot cells in *Bellamya bengalensis* collected from sites A
Fig 7B: Giemsa stained foot cells in *Bellamya bengalensis* collected from sites B (cell vacuolation found)

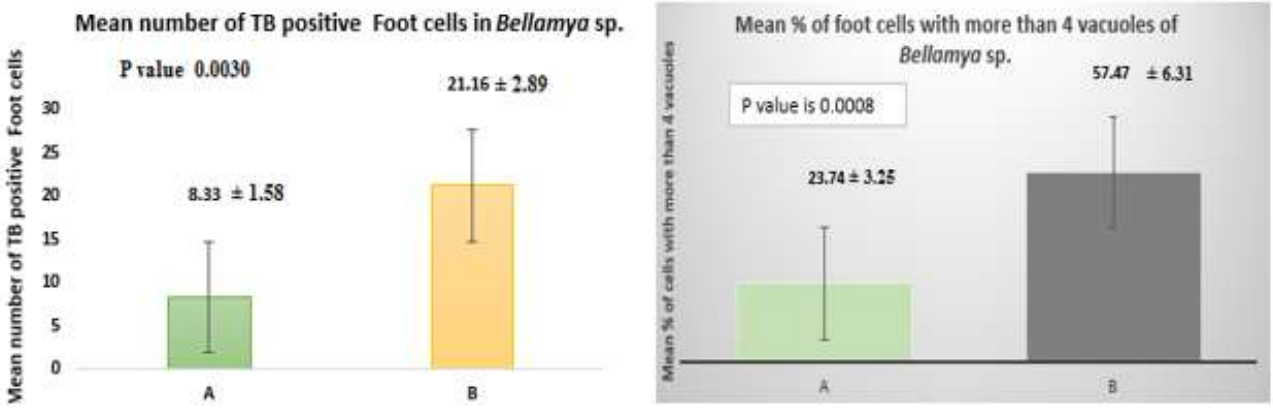


Fig 8: A=cells from collection sites A; B= cells from collection sites B
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

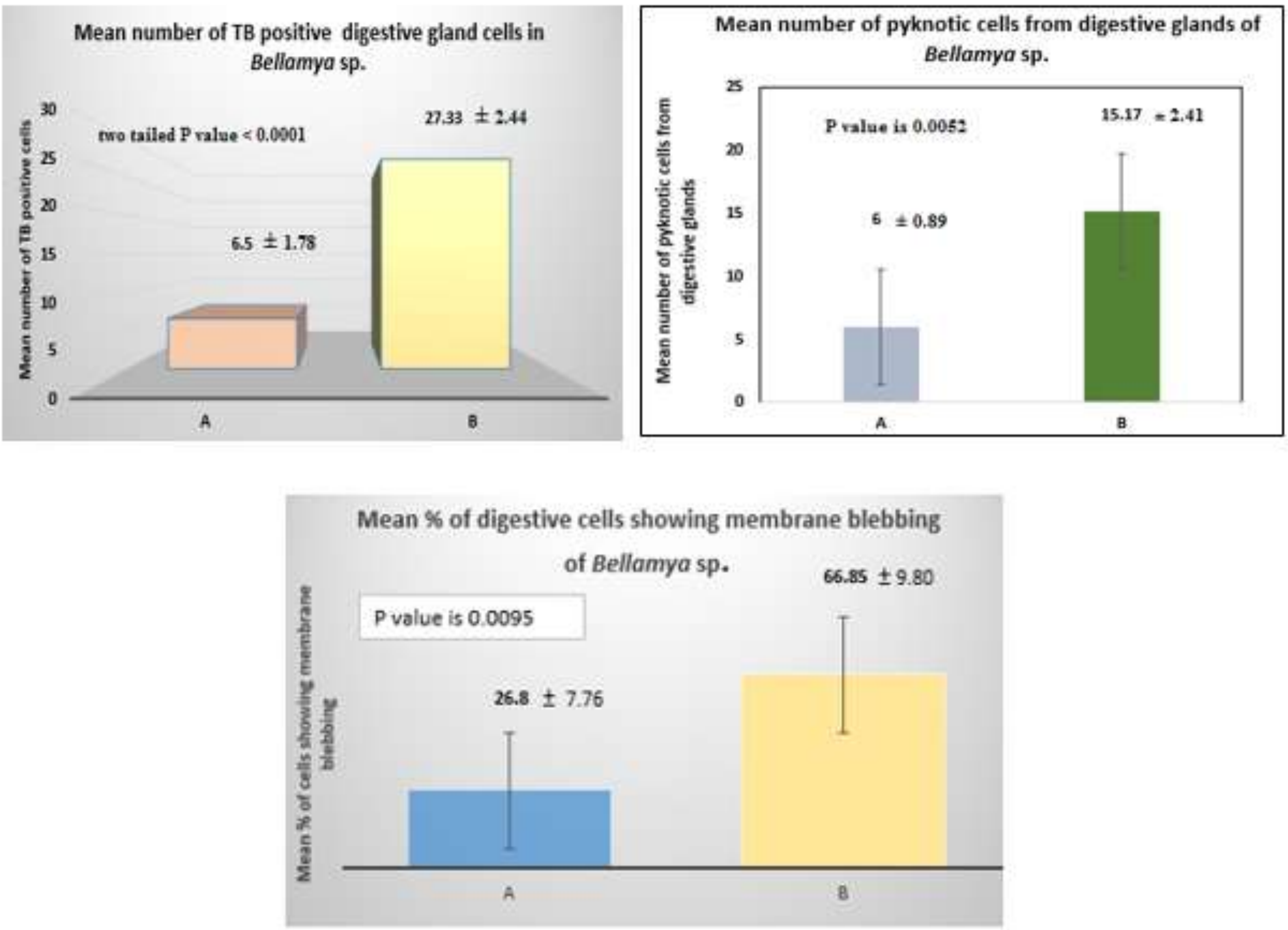


Fig 9: A=cells from collection sites A; B= cells from collection sites B
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

4. Discussion

The present study is the extended work of Guria, (2025). In the present work we have emphasized on hemocytes, foot cells and digestive gland cells and histological analysis. Guria, (2025) showed cells from hepatopancreas in mollusc collected from the sites associated with human habitats, human effluents and agricultural fields were necrotic. Collection sites B that were associated with human habitats, human effluents and agricultural fields may pollute the environment and affect aquatic mollusc due to anthropogenic activities rather than other collection sites (sites A) which were not associated with human habitats, human effluents and agricultural fields. Many workers have reported the degenerative changes in tissues of the animals in response to pollution by various toxicants (Shaikh *et al*., 2010; Andhale *et al*., 2011). Victor *et al*., (1990) observed histopathological changes in the hepatopancreas of *P. hydrodromous* in response to cythion organophosphate insecticide resulting damages of tubular epithelium, and atrophy. These degenerative changes result in the impairment of physico-metabolic processes of mussels. Gravato *et al*., (2005) reported breakage of DNA strands in cells of hepatopancreas of mussels exposed to resin acids. The normal histological structure of digestive gland of *B. bengalensis* shows large number of acini. Each acinus consists of digestive absorptive cells and excretory cells lined with epithelium.

An empty space called lumen is present in the centre of acinus. Kumari *et al*., (2011) revealed, digestive gland showed histopathological changes due to the stress caused by specific detergent in *Bellamya bengalensis* like vacuolization of epithelial cells, inflammation of tubules and presence cell debris in lumen. Histopathological alterations such as gathering of haemocytes in areas between the tubules have also been reported in the digestive gland of snail, *B. dissimilis* exposed to endosulfan, methyl parathion (Jonnalagadda and Rao, 1996). Heng and Rusin (2004) reported the cellular alterations in the digestive gland like cellular hypertrophy in snail, *Turritella* sp. due to exposure of zinc and copper. Kamble and Potdar (2010) reported the histopathological changes after the exposure of *Bellamya bengalensis* to lead acetate up to 96 hrs were swelling and rupturing of digestive absorptive cells and atrophy in the muscular layer (Kamble and Potdar, 2010).

5. Conclusion

The present results corroborate with the findings in various gastropod snails by many researchers. Hemocyte function, cell death/apoptosis of foot cells, digestive glands cells have been claimed as markers of aquatic pollution which may help in monitoring the health of aquatic ecosystems.

6. Acknowledgement

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7. Conflicts of Interest

The authors declare no conflicts of interest.

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