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Histopathological and Cellular Alterations in Digestive Glands/Tubules and Foot of *Pila* sp. to Speculate Environmental Pollution

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Abstract

Snail species are commonly used as bioindicators. Detailed information of the ecotoxic responses of a species is helpful for its conservation as well as environmental protection. Environmental pollutants like herbicides, pesticides, metals, others chemicals and genotoxic agents impose severe impacts on invertebrate cells. The freshwater snail *P. globosa* is very susceptible to organophosphates and various chemicals used as insecticides, pesticides, herbicides and pollutants. Live freshwater molluscs *Pila* sp. was manually collected from selected areas of Nadia district of West Bengal. The assessed parameters were cell morphology and histology of foot, digestive gland and intestine of *Pila* sp. The average number of pyknotic/necrotic cells of foot, digestive gland from the specimens collected from the sites associated with human effluents and agricultural fields was increased significantly. The histopathological alterations of digestive gland/tubules, intestine examined were disintegration of basement membrane, loss of epithelial cell lining in the samples collected from sites associated with human effluents and agricultural fields. The histopathological lesions in digestive glands/tubules, foot may result in the disturbances of overall metabolism and several related physiological processes.

Keywords

Bioindicators, Pollutants, Histopathological lesions

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INTRODUCTION

Biological markers are sensitive biological techniques for assessing stress induced by pollution (Marigomez *et al.*, 2013; Radwan *et al.*, 2024). They can be evaluated at several biological levels like molecules, cells and individuals which follow exposure to different types of pollutants (Vasseur and Cossu-Leguille, 2003; Gomot de Vaufleury and Pihan, 2000). Snail species are commonly used as bioindicators. Based on Gomot de Vaufleury, (2000) land snails exhibit remarkable tolerance to environmental pollution as because they can provide ecotoxicological effects due to environmental challenges.

Detailed information of the ecotoxic responses of a species are helpful for its conservation as well as environmental protection (Paital and Chainy, 2012). Recent researches showed much attention to find out cellular and physiological responses in invertebrates under the fluctuation of environmental factors and stress (Stankovic and Jovic, 2012; Sousa *et al*., 2014). Environmental pollutants like herbicides, pesticides, metals, others chemicals and genotoxic agents impose severe impacts on invertebrate cells (Karimi *et al*., 2017; Sigurnjak *et al*., 2021; Panda *et al*., 2022). Therefore, snails can be an ideal model of biomarker in freshwater as well as grassland ecosystems (Panda *et al*., 2021; Stankovic and Jovic, 2012; Sousa *et al*., 2014; Karimi *et al*., 2017; Sigurnjak *et al*., 2021; Panda *et al*., 2022).

The freshwater snail *P. globosa* is very susceptible to organophosphates such as phorate, formothion and trichlorfon and carbamates such as aldicarb, herbicides such as butachlor (Singh and Agarwal,1981; Sivaramakrishna *et al*., 1991; David *et al*., 2003). Various chemicals used as insecticides, pesticides, herbicides and pollutants can affect the non-target species like *P. globosa*.

P. globosa can be consumed as a food source, as it has a high source of protein and is cheaper than other animal protein sources (Nargis *et al*., 2011). So, it may negatively impact on human health (Panda *et al*., 2022).

MATERIALS AND METHODS

Live freshwater molluscs *Pila* sp. was manually collected from the selected areas of Nadia district of West Bengal like Morali River (Ballabhpur and near Morali bridge, Silinda-Darappur - Dudhpukur Rd, Hariankha, Nadia) and different sites of Raghunathpur, Nakashipara. Some collection sites were not associated with human habitats, human effluents and agricultural fields (site A). Some areas (site B) were associated with human effluents and agricultural fields (Guria, 2025).

Tissues of the foot, digestive gland/tubules and intestine 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 (Guria, 2025). Cell viability test was done by trypan blue assay. Cell counting was performed by hemocytometer. Investigated tissues were fixed in Bouin's fluid. Histological sections were made and stained with haematoxylin and eosin. Statistical analysis was done by GraphPad InStat.

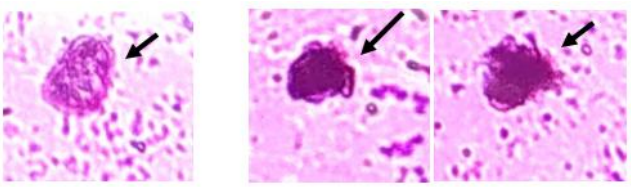
RESULTS

Giemsa-stained cells from digestive gland/tubules, intestine and foot in *Pila* sp. collected from the sites associated with human effluents and agricultural fields (site B) were necrotic indicating rupture of cell membrane (**Fig.1 and 5**). The histopathological alterations of digestive gland/tubules, intestine examined were disintegration of basement membrane, loss of epithelial cell lining, disruption of tubules, disappearance of cellular integrity and occurrence of cell debris in luminal space in the samples collected from site B when compared with site A. Digestive and secretory cells detached from basement membrane and from each other and adhesiveness of cells in digestive glands of *Pila* sp. was reduced in specimens of site B (**Fig. 2, 3 and 4**).

H-E-stained histological section of foot muscle of *Pila* sp. collected from site B showed disrupted connective tissue and necrotic columnar epithelia (**Fig.6**).

The mean number of Trypan Blue (TB) positive dead cells from digestive gland/tubules and foot was significantly increased in the specimens (*Pila* sp.) collected from the sites B (**Fig. 7, 8 and 9**).

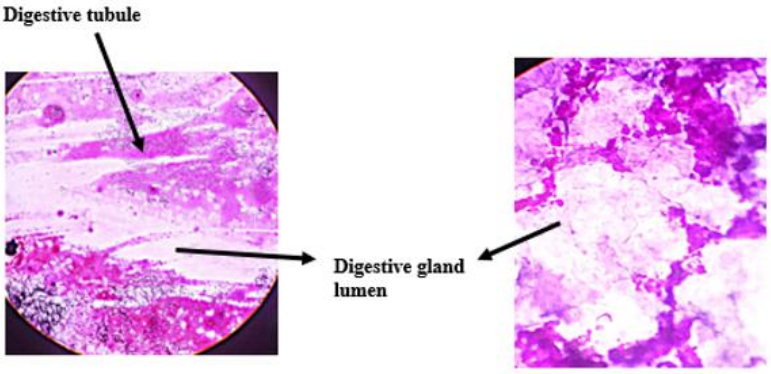
Increased pyknosis of cells was noted site B (**Fig. 10**). Mean number of digestive cells showing membrane rupture and mean percentage of digestive cells showing membrane blebbing were significantly increased in the specimens (*Pila* sp.) collected from the sites B when compared with sites A (**Fig. 11 and 12**).



1A 1B

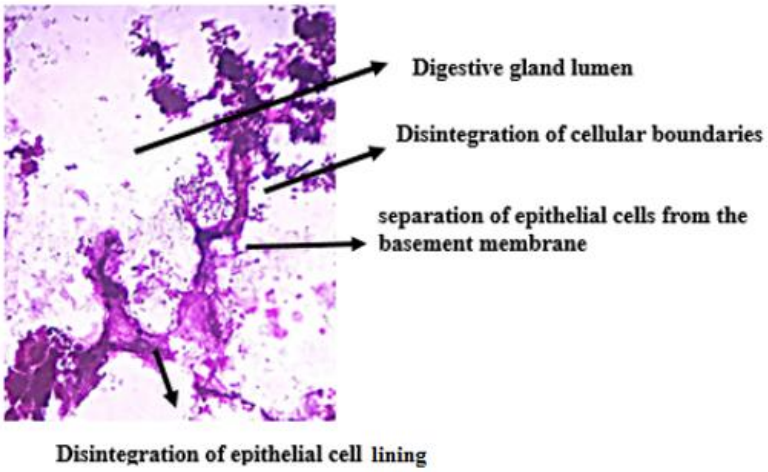
Fig. 1A: Giemsa-stained cells (indicated by arrow) from digestive glands in *Pila* sp. collected from the sites not associated with human effluents, human habitats and agriculture lands (site A).

Fig. 1B: Giemsa-stained necrotic cells (indicated by arrow) from digestive glands in *Pila* sp. collected from the sites associated with human effluents and agricultural fields showing progressive changes of cell membrane (site B).



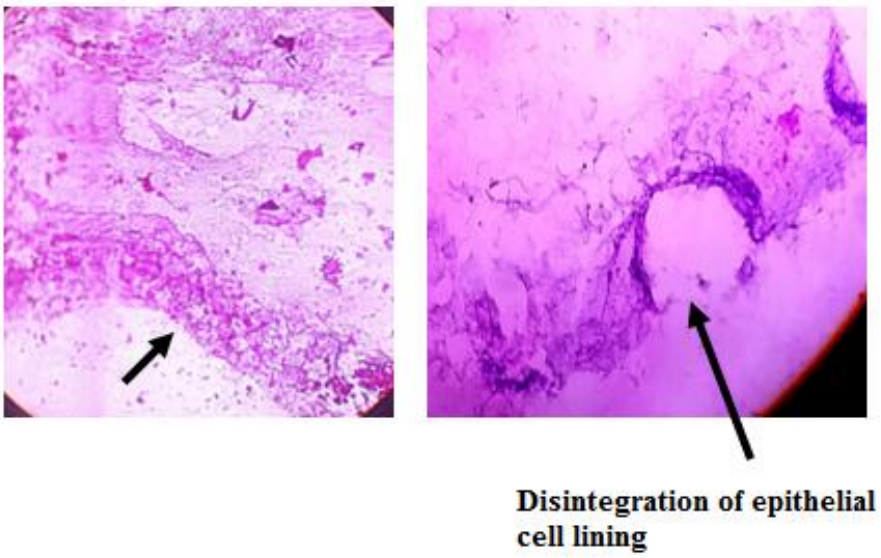
2A

Fig. 2A: H-E-stained histological sections of digestive glands /tubules of *Pila* sp. collected from site A.



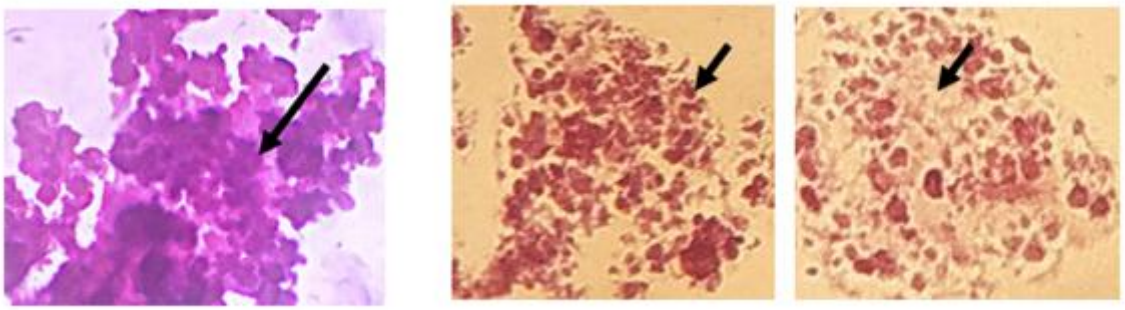
2B

Fig. 2B: H-E-stained histological sections of digestive glands/tubules of *Pila* sp. collected from site B.



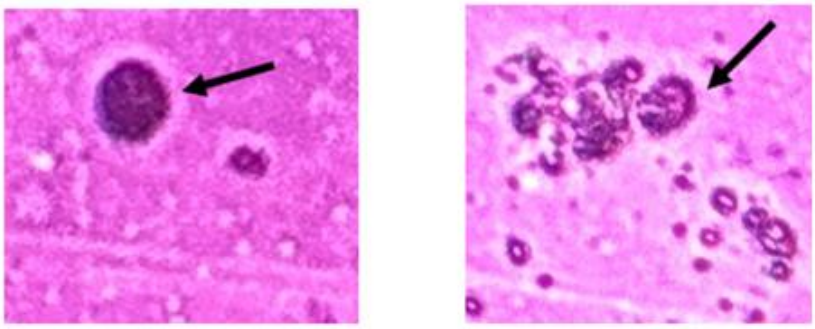
3A 3B

Fig. 3A: H-E-stained histological section of intestine of *Pila* sp. collected from site A.
Fig. 3B: H-E-stained histological section of intestine of *Pila* sp. collected from site B.



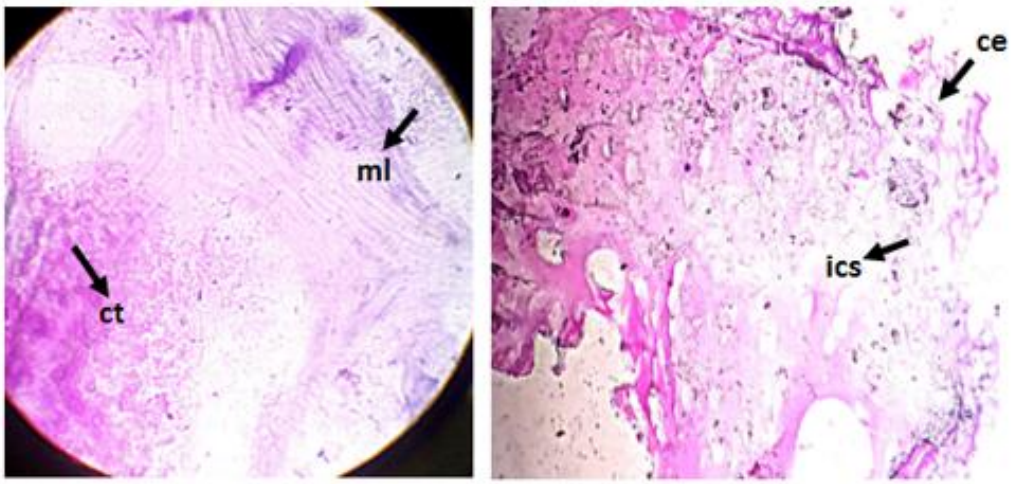
4A 4B

Fig. 4A: H-E-stained histological section showing compact adhesiveness of cells in digestive glands of *Pila* sp. collected from site A.
Fig. 4B: H-E-stained histological sections showing gradual loss of adhesiveness of cells in digestive glands of *Pila* sp. collected from site B.



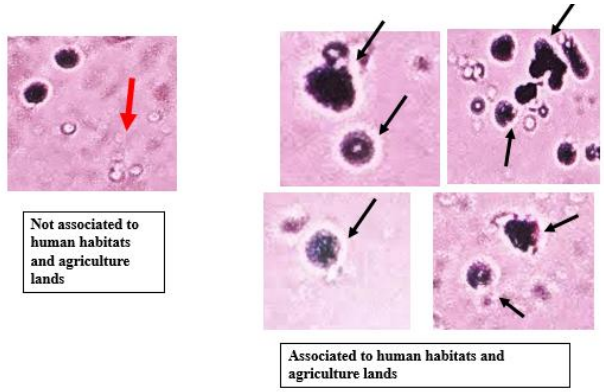
5A 5B

Fig. 5A: Giemsa-stained cells (indicated by arrow) from foot in *Pila* sp. collected from the sites not associated with human effluents, human habitats and agriculture lands (site A).
Fig. 5B: Giemsa-stained necrotic cells (indicated by arrow) from foot in *Pila* sp. collected from the sites associated with human effluents and agricultural fields (site B).



6A 6B

Fig. 6A: H-E-stained histological section of foot muscle of *Pila* sp. collected from site A.
Fig. 6B: H-E-stained histological section of foot muscle of *Pila* sp. collected from site B.
ct, connective tissue; ics, intercellular spaces; ml, muscle layer; ce, columnar epithelia



7A 7B

Fig. 7A: Trypan Blue (TB) positive foot cells in *Pila* sp. collected from site A.
Fig. 7B: Trypan Blue (TB) positive foot cells in *Pila* sp. collected from site B.
Trypan Blue (TB) negative cells (indicated by red arrow)
Trypan Blue (TB) positive cells represented dead cells (indicated by black arrow)

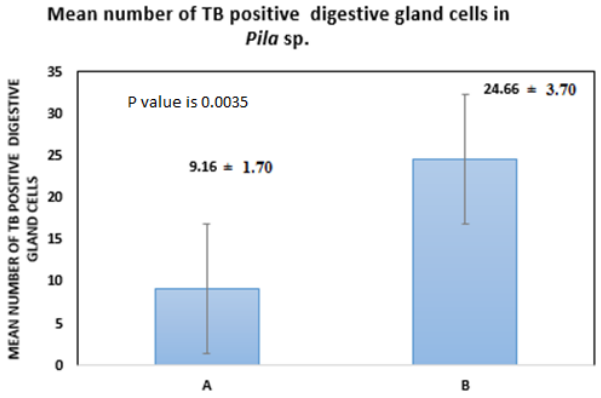


Fig. 8: Mean number of TB positive digestive gland cells in *Pila* sp.
A=cells from the specimens from the sites not associated with human effluents and agricultural fields,
B=cells from the specimens from the sites associated with human effluents and agricultural fields
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

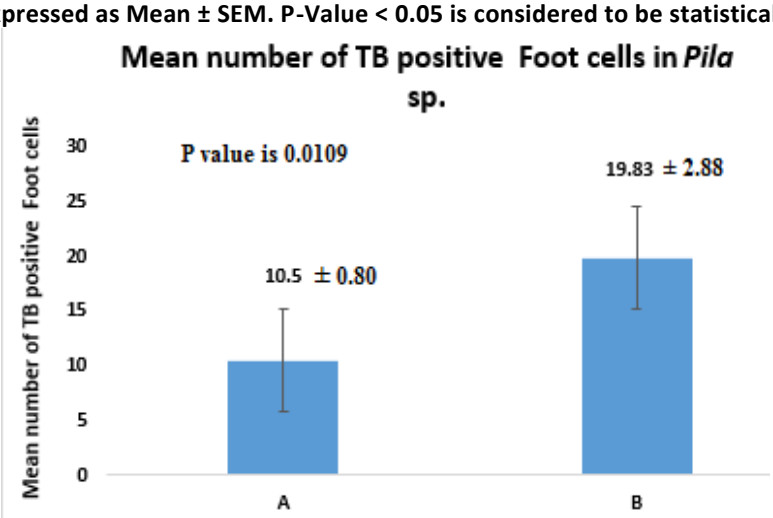


Fig. 9: Mean number of TB positive foot cells in *Pila* sp.
A=cells from the specimens from the sites not associated with human effluents and agricultural fields,
B=cells from the specimens from the sites associated with human effluents and agricultural fields
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

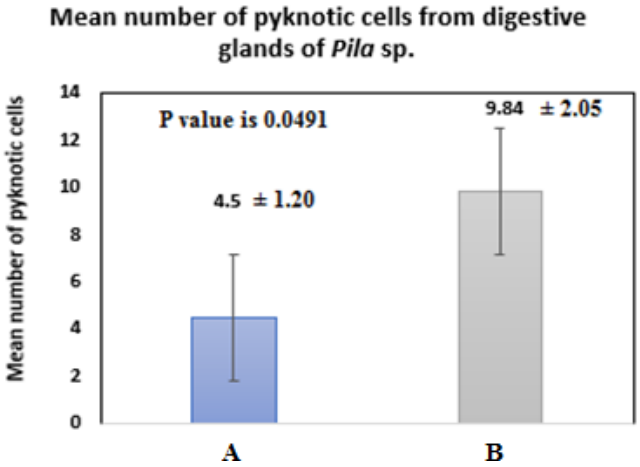


Fig. 10: Mean number of pyknotic cells from digestive gland in *Pila* sp.
A=cells from the specimens from the sites not associated with human effluents and agricultural fields,
B=cells from the specimens from the sites associated with human effluents and agricultural fields
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

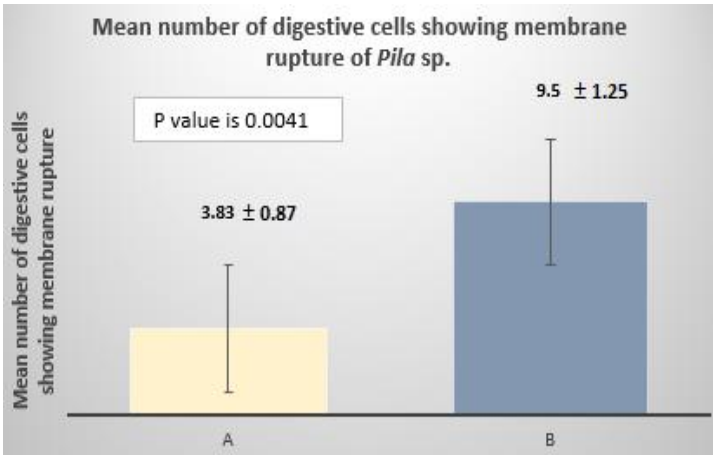


Fig. 11: Mean number of digestive cells showing membrane rupture in *Pila* sp.
A=cells from the specimens from the sites not associated with human effluents and agricultural fields,
B=cells from the specimens from the sites associated with human effluents and agricultural fields
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

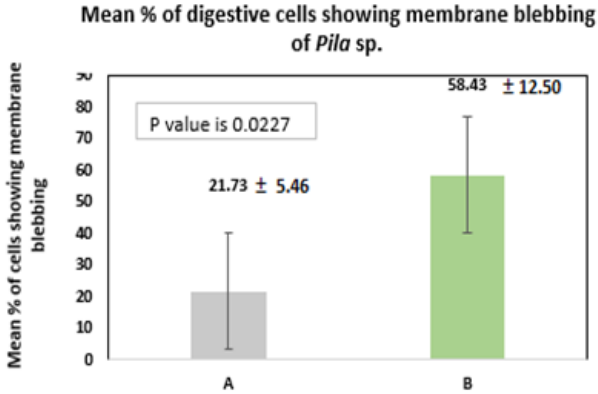


Fig. 12: Mean percentage of digestive cells showing membrane blebbing in *Pila* sp.
A=cells from the specimens from the sites not associated with human effluents and agricultural fields,
B=cells from the specimens from the sites associated with human effluents and agricultural fields
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

DISCUSSION AND CONCLUSION

This work is the extended work of Guria, (2025). Collection sites B were associated with human habitats and human effluents and agricultural fields may pollute the environment of aquatic bodies through anthropogenic activities rather than collection sites A which were not associated with human habitats and human effluents and agricultural fields.

Guria, (2025) showed cells from hepatopancreas in molluscs collected from the sites associated with human effluents and agricultural fields were necrotic. The average number of pyknotic cells from hepatopancreas from the specimens collected from the sites associated with human effluents and agricultural fields was increased significantly (Guria, 2025). Water pollution occurs because of synthetic pesticides which are commonly used in agricultural practices and industrial organic chemicals which are harmful in terrestrial and aquatic environments (Demi and Sicchia, 2021).

Pesticides are vital to modern agriculture but their improper use has resulted in a significant environmental challenge (Pathak *et al*., 2022). Previous researches showed snails subjected to 0.02 µg/mL indoxacarb displayed histo-architectural damages, alteration of digestive cells and excretory cells with numerous vacuoles. On the other hand, the group of snails exposed to 0.2 µg/ml indoxacarb exhibited rupture of digestive cells, increased number of vacuoles and the presence of excretory cells with clumps (Radwan *et al*., 2024).

Plant fertilizers are used to enhance the growth of the different parts of the plant. Several studies showed beside the role of fertilizers in the growth of the plant it has a molluscidal effect. Sheir, (2015) found that Caselio (plant fertilizer) caused damage of tissue in the digestive tubules showing necrosis of cells in the freshwater snail, *Lanistes carinatus*. El-Deeb *et al*., (2015) exposed *Biomphalaria alexandrina* snails to the fertilizers with high phosphorus and high nitrogen content and showed histological alterations in the digestive glands. Triebskorn, (1989) studied the effect of metaldehyde molluscicides on the digestive tract of *Deroceras reticulatum* and found mucus deficiency in the digestive tract. Hamed *et al*., (2007) found severe vacuolization in digestive cells of *Eobania vermiculata* treated with molluscicidal carbamates, methiocarb and methomyl. The results showed degenerative changes of epithelia.

Triebskorn and Künast, (1990) reported that carbamate molluscicides caused damages of basal parts of the epithelial cells, the underlying connective and muscle tissues in the digestive gland of the land snail, *Monacha obstructa* treated with methomyl.

Abd-El Azeem & Sheir, (2018) showed the effect of exposure to 200 µl/l Caselio, plant metabolism regulator (PMR) in slugs` (*Deroceras reticulatum*) skin and foot showed cellular degeneration in the epithelial layer indicating detachment of connective tissue from the foot muscles and formation of gaps between muscle fibers. Exposure to 500 µl/l of PMR caused appearance several vacuoles in the muscle layer. The effect of 800 µl/l of PMR showed complete necrosis of the columnar epithelia (Abd-El Azeem & Sheir, 2018).

Present results showed cells from digestive gland/tubules and foot in *Pila* sp. collected from site B were necrotic. The histopathological alterations included disintegration of basement membrane, loss of epithelial cell lining. The mean number of Trypan Blue (TB) positive dead cells from digestive gland/tubules and foot was significantly increased in the specimens (*Pila* sp.) collected from the sites B. The histopathological lesions in digestive glands/tubules may result in the disturbances of overall metabolism and several related physiological processes.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT

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REFERENCES

Abd-El Azeem HH and Sheir SK. (2018). Impacts of the plant fertilizer, Caselio on the slug, *Deroceras reticulatum* (Gastropoda, Stylommatophora): laboratory studies. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 9(1): 386-396.

David M, Mushigeri SB, and Prashanth M S. (2003). Nickel induced changes on some aspects of protein metabolism in the tissues of *Pila globosa*. *J. Environ. Biol*, 24 (1): 69–75.

Demi SM and Sicchia SR. (2021). Agrochemicals Use Practices and Health Challenges of Smallholder Farmers in Ghana. *Environmental Health Insights*, 15: 1–11.

El- Deeb F, Assem MM, Hasheesh W, Tantawy A and EL-Sayed S. (2015). *Adv Environ Biol*, 9 (21): 19-21.

Gomot de Vaufleury A. (2000). Standardized growth toxicity testing (Cu, Zn, Pb, and

pentachlorophenol) with *Helix aspersa*. *Ecotoxicol Environ Saf*, 46: 41–50.

Gomot de Vaufleury A and Pihan F. (2000). Growing snails used as sentinels to evaluate terrestrial environment contamination by trace elements. *Chemosphere*, 40: 275–284.

Guria S. (2025). Study of Cells of Hepatopancreas of *Bellamya bengalensis* and *Pila* sp. Collected from some areas of Nadia District of West Bengal. *International Journal of Life Sciences,* 14 (4): 151-155.

Hamed SS, Abdelmeguid NE, Essawy AE, Radwan MA and Hegazy AE. (2007). Histological and ultrastructure changes induced by two carbamate molluscicides on the digestive gland of *Eobania vermiculata*. *J. Biol. Sci*, 7: 1017- 37.

Karimi B, Maron PA, Chemidlin-Prevost BN, Bernard N, Gilbert D, and Ranjard L. (2017). Microbial diversity and ecological networks as indicators of environmental quality. *Environ. Chem. Lett*, 15: 265–281. doi:10.1007/s10311-017-0614-6

Marigomez IA, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville MP. (2013). Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "mussel watch. *Ecotoxicology*, 22: 486–505.

Nargis A, Talukder D, Parmanik SHA and Hasan MR. (2011). Nutritional value and physico-chemical characteristics of apple snail *Pila globosa* (swainson) and *Lymnaea luteola* lamark. *Bangladesh J. Sci. Ind. Res*, 46 (4): 539–542.

Paital B and Chainy GB. (2012). Biology and conservation of the genus *Scylla* in India subcontinent. *J. Environ. Biol*, 33 (5): 871–879.

Panda F, Pati SG, Bal A, Das K, Samanta L and Paital B. (2021). Control of invasive apple snails and their use as pollutant ecotoxic indicators: A review. *Environ. Chem. Lett*, 19: 4627–4653. doi:10.1007/s10311-021- 01305-9

Panda F, Pati SG, Das K, Samanta L, Sahoo D K & Paital B. (2022). Biochemical and molecular responses of the freshwater snail *Pila* sp. to environmental pollutants, abiotic, and biotic stressors. *Frontiers in Environmental Science*, 10: 1033049.

Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S, Mohapatra A, Pandey V, Rana N, Cunill JM. (2022). Current status of pesticide effects on environment, human health and its eco-friendly management as bioremediation: a comprehensive review. *Front Microbiol*, 13: 962619.

Radwan MA, Gad AF, Abd El-Aziz AM, El-Gendy KS. (2024). Does commercial indoxacarb pose ecotoxicological consequences? Employing a multi-marker approach in the model species *Theba pisana*. *Environ Sci Pollut Res Int*, 31(22): 31911-31924.

Sheir SK. (2015). The role of Caselio (plant fertilizer) exposure on digestive gland histology and heavy metals accumulation in the freshwater snail, *Lanistes carinatus*. *J Biosci App Res*, 1(5): 223-233. DOI: 10.21608/jbaar.2015.106031

Singh O and Agarwal R A. (1981). Toxicity of certain pesticides to two economic species of snails in northern India. *J. Econ. Entomol*, 74 (5): 568–571. doi:10.1093/jee/74.5.568

Sigurnjak M, Cvetnić M, Miloloža M, Kučić Grgić D, Markić M, Kušić H., Bolanča T, Rogošić M and Ukić Š. (2021). Modeling the toxicity of pollutants mixtures for risk assessment: A review. *Environ. Chem. Lett*, 19: 1629–1655. doi:10.1007/s10311-020-01107-5

Sivaramakrishna B, Radhakrishnaiah, K and Suresh A. (1991). Assessment of mercury toxicity by the changes in oxygen consumption and ion levels in the freshwater snail, *Pila globosa*, and the mussel, *Lamellidens marginalis*. *Bull. Environ. Contam. Toxicol*, 46(6): 913–920. doi:10.1007/BF01689738

Sousa ACA, Pastorinho MR, Takahashi S and Shinsuke T. (2014). History on organotin compounds, from snails to humans. *Environ. Chem. Let*, 12: 117–137. doi:10.1007/s10311-013-0449-8

Stankovic S and Jovic M. (2012). Health risks of heavy metals in the mediterranean mussels as seafood. *Environ. Chem. Lett*, 10: 119–130. doi:10. 1007/s10311-011-0343-1

Triebskorn R. (1989). Ultrastructural changes in the digestive tract of *Deroceras reticulatum* (Muller) induced by a carbamate molluscicide and by metaldehyde. *Malacologia*, 31(1): 141-156.

Triebskorn R and Künast C. (1990). Ultrastructural changes in the digestive system of *Deroceras reticulatum* (Mollusca: Gastropoda) induced by lethal and sublethal concentrations of the carbamate molluscicide Cloethocarb. *Malacologia*, 32: 89-106.

Vasseur P and Cossu-Leguille C. (2003). Biomarkers and community indices as complementary tools for environmental safety. *Environ Int*, 28: 711–717.