MS.ID-IJPBS-6080
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2025) 15 (2): 21-29
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Effect Of Metal Pollutants on Hemocytes of Freshwater Molluscs, *Bellamya bengalensis*: The Reliable Markers of Aquatic Pollution

Srikanta Guria[1]*, Monoj Das[2], Anindita Chakraborty[2] and Tithi Dutta[2#]

[1]Post Graduate Department of Zoology, Krishnagar Government College, Krishnagar, West Bengal, 741101

[2]Post Graduate Department of Zoology, Barasat Govt. College, Barasat, Kolkata-700124, West Bengal

#All are equal contributors

Received: 15 Feb 2025/Accepted: 16 Mar 2025/Published online: 01 Apr 2025
***Corresponding Author Email:** guriasrikanta@gmail.com

Abstract

Hemocytes are useful tool for assessing the toxicity of heavy metals on freshwater gastropods. Freshwater molluscs *Bellamya bengalensis* were exposed to sodium arsenite ($NaAsO_2$), mercury (II) chloride ($HgCl_2$), lead nitrate, zinc sulphate and copper sulphate ($CuSO_4.5H_2O$) for the different exposure periods as 24hrs, 48hrs, 72hrs and 96hrs. Control specimens were untreated by any toxic metals. Hemolymph was collected and hemocytes were stained by Giemsa, Leishmans Eosin Methylene blue solution, neutral red and trypan blue. Results showed that heavy metal toxicity affected the morphology and function of chief immune effector cells (hemocytes) in freshwater molluscs and also inhibited the degree of hemocyte aggregation, clot formation which may affect "encapsulation response", microaggregation and wound healing. Cells showed neutral red (NR) positive reaction in cytoplasm indicating damage of lysosomal membrane due to metal toxicity. Mortality index was also significantly increased in 96hrs of treated group. This supports the idea that hemocyte analysis is a reliable tool to obtain information about pollution which may act as markers of aquatic biomonitoring approach.

Keywords

Hemocytes, Aggregation, Freshwater gastropods, Environmental pollution

*****

INTRODUCTION

A global concern about the role of heavy metals in aquaculture has been raised due to health risks for consumers (Lakra and Nagpure, 2009). The ecotoxicological assessment is essential to identify potential environmental risk. Hemocytes are useful tool for assessing the toxicity of heavy metals, and pesticides on aquatic invertebrates like freshwater gastropods. Researchers showed that aquatic animals like benthic oligochaetes, gastropods, insects, crustaceans, fishes are more sensitive to ecological risk of heavy metal pollution (Ishrat, 2020). Hemocyte analysis of freshwater molluscs *Bellamya bengalensis* could be used as effective biomarkers for evaluation of environmental pollution.

MATERIALS AND METHODS

The snails *B. bengalensis* were collected in the early morning from some selective ponds during November- December 2024. A total 80 *B. bengalensis* were collected by handpicking. They were then transported to laboratory. They were acclimatized in glass aquaria for 3 days to laboratory regimes before experimentation. Freshwater molluscs *Bellamya bengalensis* were exposed to sodium arsenite

($NaAsO_2$) in water at 0.025 mg/L (group 1), mercury (II) chloride ($HgCl_2$) in water at 0.30 mg/L (group 2), lead nitrate in water medium at 50 mg/L (group 3), zinc sulphate at 0.43 mg/L in glass aquaria (group 4) and copper sulphate ($CuSO_4.5H_2O$) at 0.56 ppm (group 5) for the different exposure periods as 24hrs, 48hrs, 72hrs and 96hrs. Control specimens were untreated by any toxic metals (Each group, n=6) (after Guria, 2020; Guria, 2023; Guria and Ali, 2024; Kamble and Kamble, 2012).

Hemolymph was collected from *B. bengalensis* and smeared on glass slides and stained by Giemsa, Leishmans Eosin Methylene blue solution, neutral red and observed under light microscope. Cell viability test was done by trypan blue assay. Cell counting was performed by hemocytometer.

RESULT

A clotting formation on glass slides by granulocytes was found in control molluscs (Fig. 1). Hemocytes fused together to form clots or cell clumps. Small clumps of cells then aggregated to form larger masses. The aggregation of molluscs by hemocytes in vitro can be categorized into weak aggregation and cohesive aggregation (Fig. 1). Significant number of cells became pyknotic after treatment (Fig. 2, 4, 6 and 12).

As, Hg, Pb, Zn, Cu inhibited the degree of hemocyte aggregation, clot formation. Mean number of compact aggregates was found to be less after 24 hrs, 48hrs, 72hrs and 96hrs of metal treatment. Mean number of cells in each clump/ aggregate was also decreased after 48hrs,72hrs and 96hrs of metal treatment which may affect "encapsulation response", microaggregation and wound healing (Fig. 8, 9, 10 and 11).

As and Hg treated cells showed neutral red (NR) positive response in their cytoplasm indicating damage of lysosomal membrane leading to the release of hydrolases in the cytoplasm (Fig. 3).

The cytosol of granular hemocytes were rich in carbohydrates (PAS positive) in control animals. But the mean percentage of Periodic acid-Schiff (PAS) positive cells became less in treated animals after 72hrs and 96hrs of treatment of Hg (Fig. 7).

The percentage of Trypan Blue (TB) positive cells represented a mortality index which was significantly increased in 96hrs of treated group (P value < 0.0001) (Fig. 5 and 13).

Increased roundness of the immune cells with decreased number of pseudopods in hemocytes after Hg treatment was noted (Fig. 14)

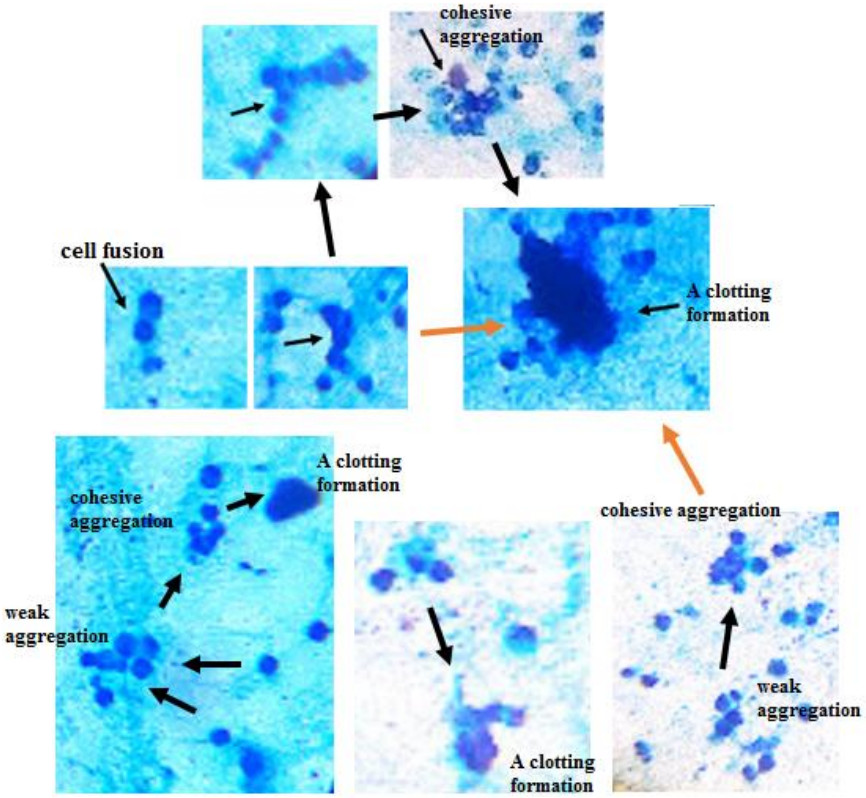


Fig. 1. Control hemocytes showed different stages of clotting formation (x400). A clotting formation from beginning (cell fusion, weak aggregation) to complete process (cohesive aggregation, clotting formation) to show a central point surrounding with numerous granulocytes.

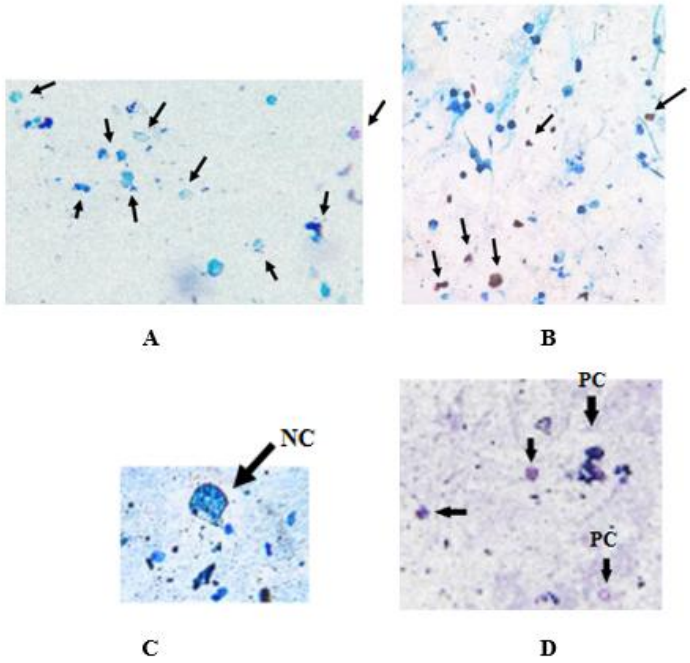


Fig. 2. Leishmans Eosin Methylene blue solution stained pyknotic cells/ necrotic cells in arsenic (As) treated group in 24 hrs (A), 48hrs condition (B), and 72hrs condition (C), and 96hrs condition (D) (x 400) (indicated by arrow) PC=Pyknotic cells, NC=Necrotic cells

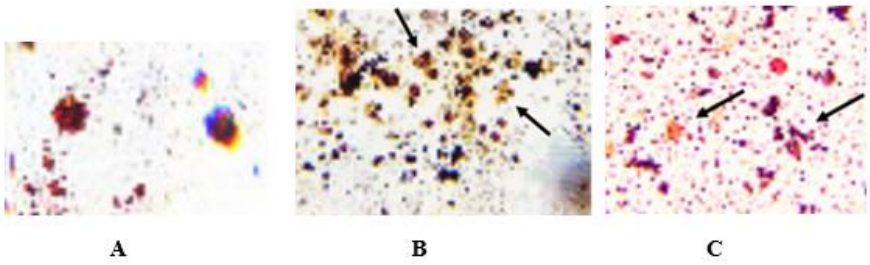


Fig. 3. Neutral Red stained control Bellamya hemocytes (A), Neutral Red stained Bellamya hemocytes in arsenic treated group in 48hrs condition (B), and in Hg treated group in 72hrs condition (C) and cell rupture indicated by arrow (x 400) PC=Pyknotic cells, NC=Necrotic cells

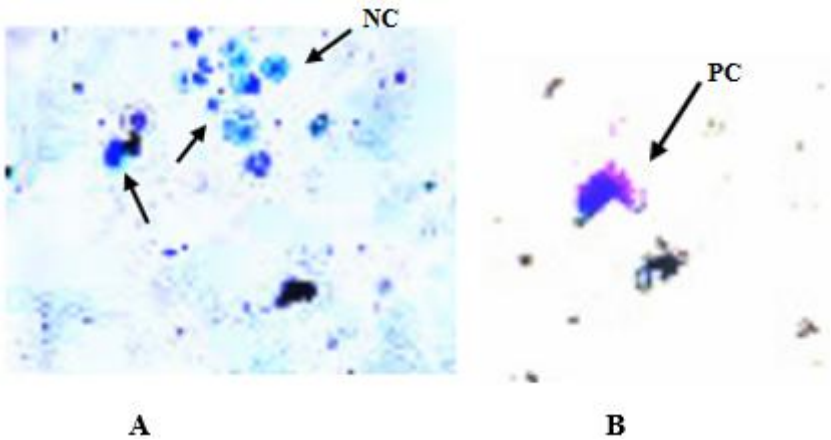
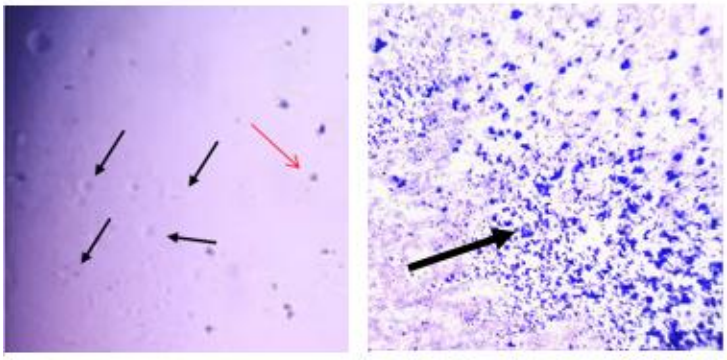
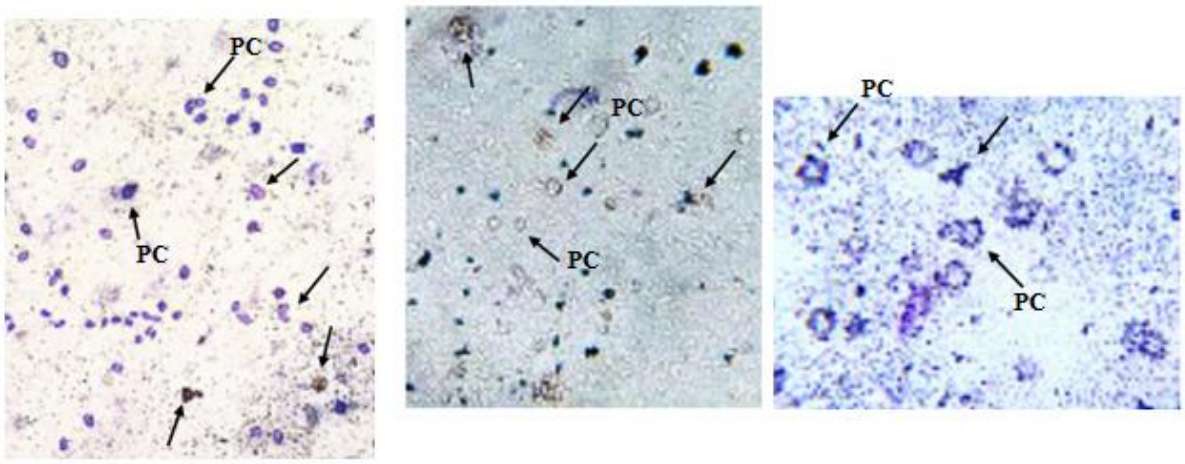


Fig. 4. Leishmans Eosin Methylene blue solution stained pyknotic cells/ necrotic cells in mercury (Hg) treated group in 48 hrs (A) and Giemsa stained pyknotic cells in Hg treated group in 72hrs condition (B) (indicated by arrow) PC=Pyknotic cells, NC=Necrotic cells



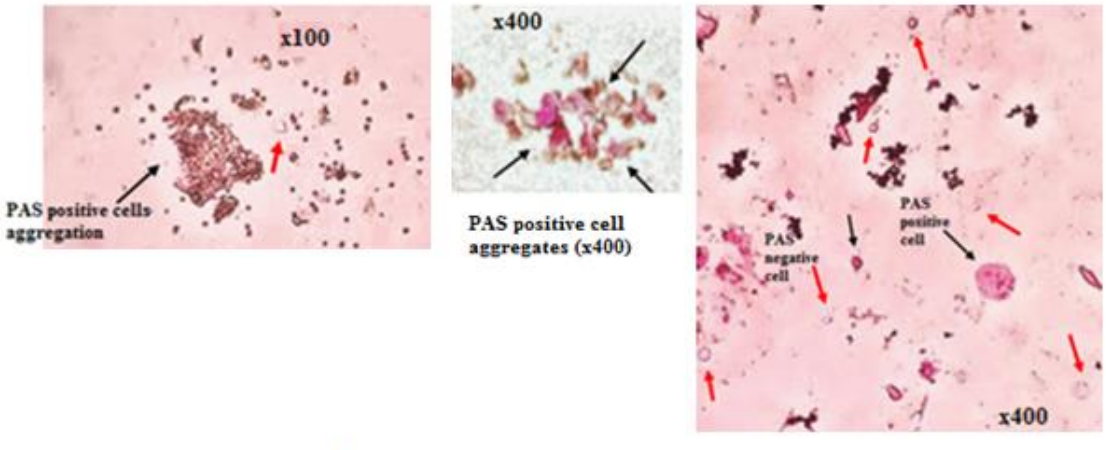
A B

Fig. 5. Trypan blue stained cells in control group (indicated by red arrow) (A) and Trypan blue stained cells in copper sulphate treated group in 72hrs condition (B) (indicated by black arrow)



A B C

Fig. 6. Leishmans Eosin Methylene blue solution stained pyknotic cells/ necrotic cells in ZnSo4 treated group in 48 hrs (A), 72 hrs condition (B), and 96 hrs condition (C) (x 400) (indicated by arrow)
PC=Pyknotic cells



A B

Fig. 7. A=SGH (small granular hemocytes) and LGH (large granular hemocytes) (periodic acid Schiff (PAS)-positive) in control animals, B= PAS positive cells after 72hrs of Hg treatment PAS positive cells- indicated by black arrows, PAS negative cells-indicated by red arrows.

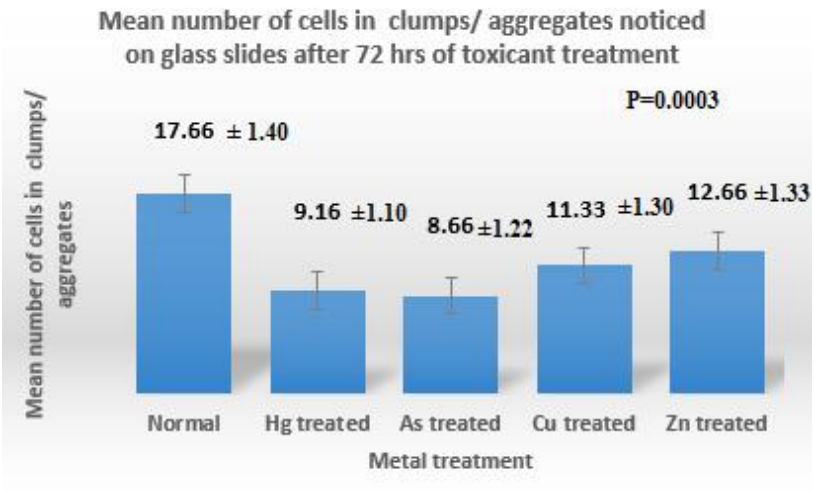


Fig.8. Mean number of cells within aggregates after metal treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value =0.0003).

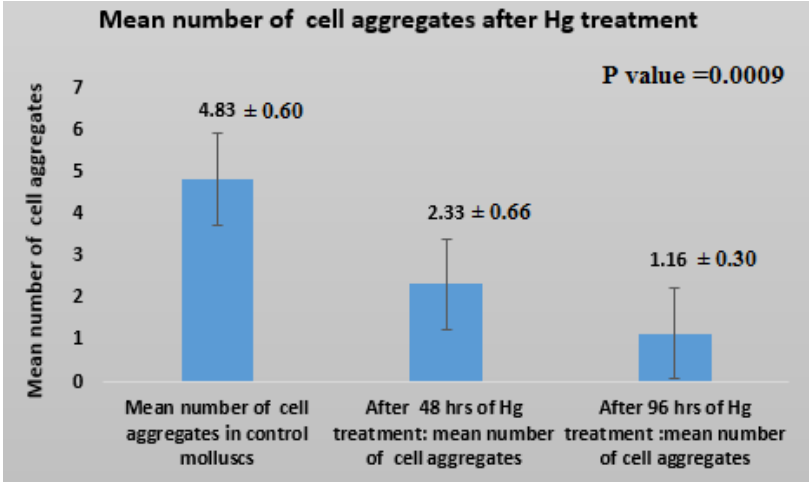


Fig.9. Mean number of cell aggregates after Hg treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value =0.0009).

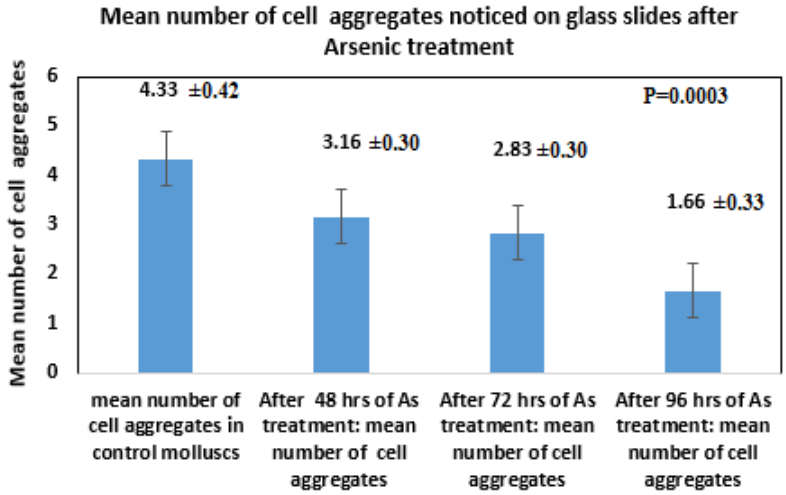


Fig. 10. Mean number of cell aggregates after As treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value =0.0003).

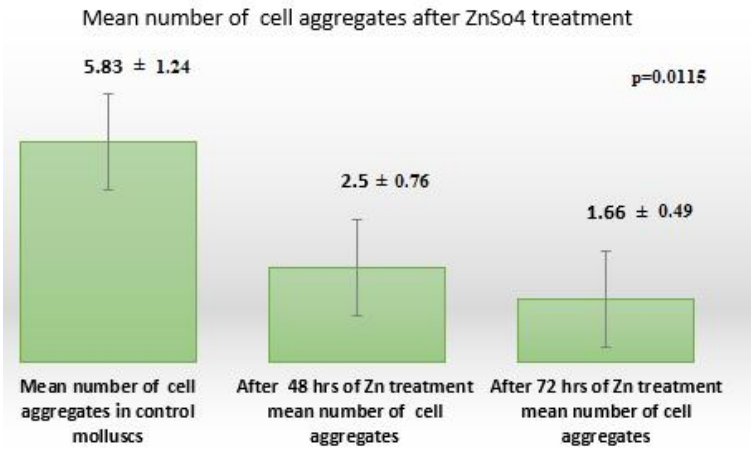


Fig.11. Mean number of cell aggregates after Zn treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value =0.0115).

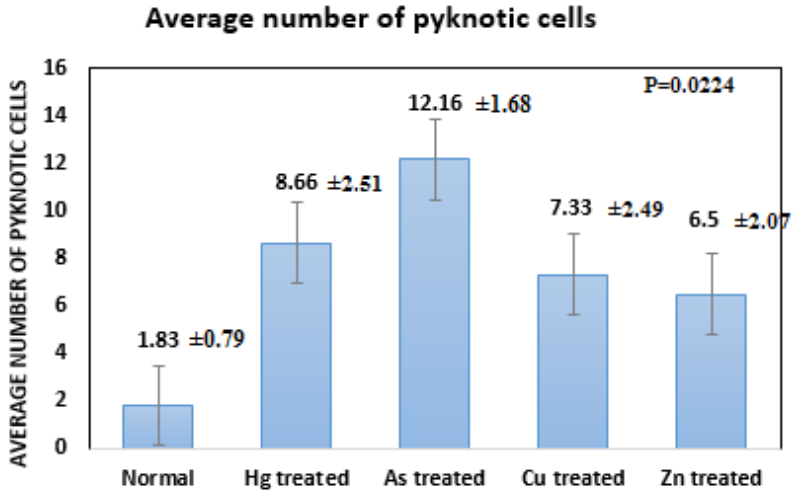


Fig. 12. Mean number of pyknotic cells on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value= 0.0224).

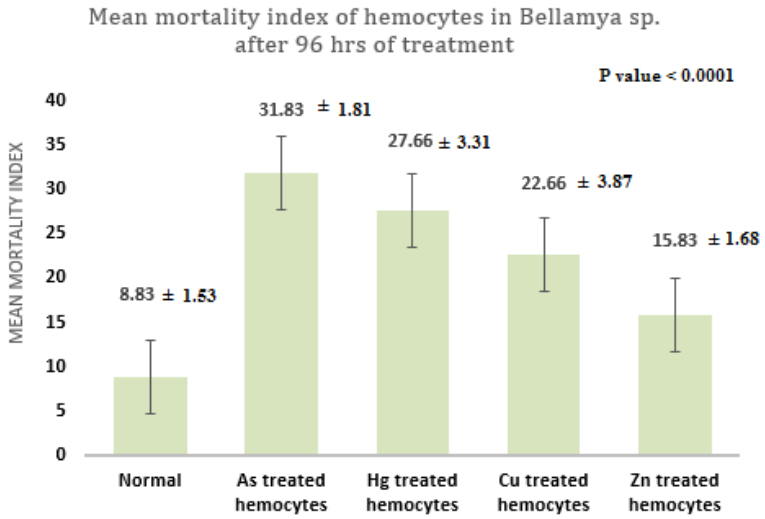


Fig. 13. Mean mortality index after metal treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value <0.0001).

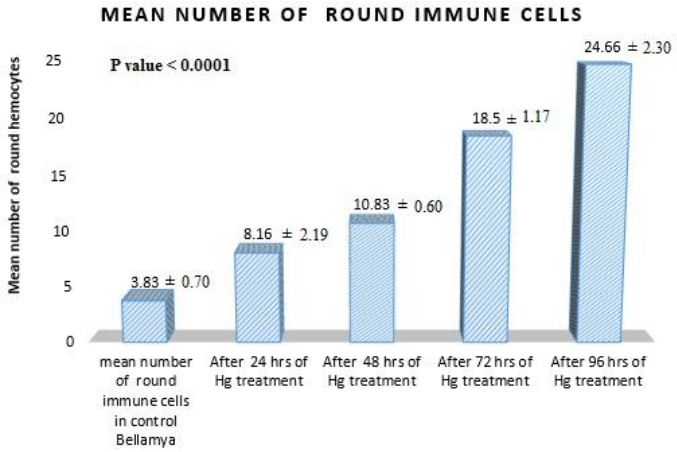


Fig.14. Mean number of round immune cells after Hg treatment on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant (ANOVA, P value< 0.0001).

DISCUSSION AND CONCLUSION

The defense mechanisms of molluscs involve hemocytes performing cellular reactions like phagocytosis, nodule formation, encapsulation. Encapsulation is caused by infiltration and aggregation of hemocytes at the site of infection. Pollutants can affect biological cycles of aquatic organisms (Khayatzadeh and Abbasi, 2010).

Copper sulphate is used as algaecide or herbicide for the control of phytoplankton or weeds in aquatic bodies (Watson and Yanong, 2002). In copper treated group of *Filopaludina bengalensis*, increased mucus secretion (%) and decreased foot protrusion was noted (Ghosh *et al*., 2021). Guria (2020) revealed cellular death of hemocytes of *Pila* sp. after treatment with arsenic. Guria (2023) indicated cytoplasmic vacuolation and membrane blebbing of hemocytes in *Bellamya bengalensis*, after lead (Pb) exposure.

Many workers studied mercurial compounds toxicity to aquatic animals (Rehowldt *et al*., 1973). Eldon *et al*., (1980) studied the effect of low concentration of heavy metals on the bivalve, *Macoma balthica* and observed that mortality was high on in animals exposed to Hg and Cd. Methylmercury form of mercury is a concern because it is absorbed easily into the food chain. Necrosis, vacuolation and degeneration of hepatocytes were observed when *Channa punctatus* was exposed to mercuric chloride for 30 days (Sastry and Gupta, 1978; Rani and Ramamurthi, 1989). Rana and Sharma (1982) observed mercurial toxicity in the liver of a freshwater teleost, *Channa punctatus*. Greater accumulation of Hg in liver after a longer treatment and the respective change in enzyme activity showed a close response relationship. Mercury exposition on filter feeder molluscs induced structural changes and permeability in cell membrane, alteration in a number of enzymatic reactions and imbalance in the levels of vital inorganic cations (Sivaramakrishna and Radhakrishnaiah, 2000). Mussels showed a higher capacity for mercury (Hg) accumulation compared to oyster and clams (Briant *et al*., 2017). Previous papers reported exposure to Hg increased roundness of the immune cells due to less pseudopods in hemocytes of mussels and snails (Marchi *et al*., 2004; Leomanni *et al*., 2016). Also, sub-lethal methylmercury concentration induced immunosuppression in ascidian *Styela plicata*, (Cammarata *et al*., 2007). The effects of LC50/10 concentrations of mercuric chloride on histology of hepatopancreas have been documented by some researchers in *Bellamya bengalensis* (Mahajan., 2023). The histopathological alterations included damaged epithelial cells and disruption of hepatic tubules at 0.109 ppm $HgCl_2$ for 7 days.

Parisi *et al*., (2021) showed methyl mercury (CH3HgCl) promoted the alteration of hemocyte viability, modification of the morphological features and cellular spreading of the mussel, *Mytilus galloprovincialis*.

Pagano *et al*., 2017 showed the effects of two heavy metals (CdCl2 and ZnCl2) on digestive glands and haemolymph of *Mytilus galloprovincialis* which caused damage to haemocytes and digestive cells, mainly after 7 days of exposure.

Belal and Gad, 2023 showed that zinc oxide nanoparticles (ZnO-NPs) at concentrations of 50 and 100 µg/ml have significantly decreased the total hemocyte count (THC) and different hemocyte count (DHC) except the number of oenocytes as they were significantly increased in 5th larval instar of *Bombyx mori*. It caused morphological deformities like loss of pseudopods and destroyed cell membranes, nuclear degeneration, cytoplasmic lysis, and appearance of cytoplasmic vacuoles.

Present results showed that heavy metal toxicity has affected the morphology and function of chief

immune effector cells (hemocytes) in freshwater molluscs and also inhibited the degree of hemocyte aggregation, clot formation which may affect "encapsulation response", microaggregation and wound healing. Cells showed neutral red (NR) positive reaction in cytoplasm indicating damage of lysosomal membrane due to metal toxicity. Mortality index was also significantly increased in 96hrs of treated group. These results corroborated the previous researches.

This supports the idea that hemocyte analysis is a reliable tool to obtain information about pollution. Therefore, comprehensive research on aquatic bodies is necessary to identify the bioindicators which are essential to address the environmental challenges.

CONFLICT OF INTERESTS

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

ACKNOWLEDGEMENT

We thank Head, Post Graduate Department of Zoology and Principal, Barasat Govt. College for necessary support.

REFERENCES

1. Belal R and Gad A. (2023). Zinc oxide nanoparticles induce oxidative stress, genotoxicity, and apoptosis in the hemocytes of Bombyx mori larvae. *Sci Rep*. 13(1): 3520. doi: 10.1038/s41598-023-30444-y.
2. Briant N, Chouvelon T, Martinez L, Brach-Papa C, Chiffoleau JF, Savoye N, Sonke J and Knoery J. (2017). Spatial and temporal distribution of mercury and methylmercury in bivalves from the French coastline. *Mar Pollut Bull*. 114(2):1096-1102. doi: 10.1016/j.marpolbul.2016.10.018.
3. Cammarata M, Parisi MG, Benenati G, Arizza V, Cillari T, Piazzese D, Gianguzza A, Vazzana M, Vizzini A and Parrinello N. (2007). In vitro effects of methylmercury on ascidian (*Styela plicata*) immunocyte responses. *Appl Organomet Chem*. 21:1022–1028. https ://doi. org/10.1002/aoc.1335.
4. Eldon J, Marketta P and Rolf K. (1980). Effect of low concentration of heavy metal on the bivalve *Macoma blthica*, *Ann.Zool. Fenn*. 17(4): 233-242.
5. Ghosh P, Dutta M and Panigrahi AK. (2021). Behavioral biomarker responses of *Filopaludina bengalensis* to acute copper toxicity. *Curr World Environ*. 16(1): 227-235. DOI: 10.12944/CWE.16.1.23.
6. Guria S and Ali R. (2024). Morpho-functional variations of hemocytes in edible mud crab (*Scylla* sp.) under exposure of toxic metals. *Indian J. Applied and Pure Bio*. 39(3): 1693-1702.
7. Guria S. (2023). Effect of lead (Pb) toxicity on hemocytes of freshwater molluscs, *Bellamya bengalensis* with emphasis to cell aggregation. *Indian J. Applied & Pure Bio*. 38(3): 1434-1440.
8. Guria S. (2020). Threats of arsenic on morphological shift of hemocytes of molluscs: a hint towards arsenic-induced cell death. *Rom. J. Biol. – Zool.* 65(1–2): 99–106.
9. Ishrat B, Lone FA, Bhat RA, Shafat AM, Zubair A and Shakeel A. (2020). Concerns and threats of contamination of aquatic ecosystems: Hakeem. K, Qadri. H, Bhat. R, biotechnology and bioremediation; Springer. *Chem*; c 2020.
10. Kamble NA and Kamble SB. (2012). Reactivity of digestive mucins in freshwater snail *Bellamya bengalensis* against copper sulphate induction. *Asian Journal of Biological and Life Sciences*.1(3): 208-212.
11. Khayatzadeh J and Abbasi E. (2010). The Effects of Heavy Metals on Aquatic Animals. The 1st International Applied Geological Congress, Department of Geology, Islamic Azad University – Mashad Branch, Iran. 1: 26-28.
12. Lakra WS and Nagpure NS. (2009). Geno toxicological studies in fishes; A review. *Indian Journal of Animal Science*. 79(1): 93-97.
13. Leomanni A, Schettino T, Calisi A and Lionetto MG. (2016). Mercury induced haemocyte alterations in the terrestrial snail *Cantareus apertus* as novel biomarker. *Comp Biochem Physiol C Toxicol Pharmacol*. 183–184:20–27. https ://doi.org/10.1016/j. cbpc.2016.01.004.
14. Mahajan P R. (2023). Natural Cure of Histoarchitecture in Hepatopancreas of Freshwater Gastropod Snail, *Bellamya Bengalensis* (L.) After Heavy Metal Stress. *World Journal of Pharmaceutical and Life Sciences*. 9 (11). 113-116.
15. Marchi B, Burlando B, Moore MN and Viarengo A. (2004). Mercury- and copper-induced lysosomal membrane destabilisation depends on [Ca2+]i dependent phospholipase A2 activation. *Aquat Toxicol*. 66:197–204. https ://doi.org/10.1016/j.aquat ox.2003.09.003.
16. Pagano M, Porcino C, Briglia M, Fiorino E, Vazzana M, Silvestro S and Faggio C. (2017). The Influence of Exposure of Cadmium Chloride and Zinc Chloride on Haemolymph and Digestive Gland Cells from *Mytilus galloprovincialis*. *International Journal of Environmental Research*. 11(2): 207–216. DOI: 10.1007/s41742-017-0020-8.
17. Parisi MG, Pirrera J, La Corte C, Dara M, Parrinello D, Cammarata M. (2021). Effects of organic mercury on *Mytilus galloprovincialis* hemocyte function and morphology. *J Comp Physiol B*. 191(1):143-158. doi: 10.1007/s00360-020-01306-0.
18. Rana SV and Sharma R. (1982). Mercurial toxicity in the liver of a freshwater teleost, *Channa punctatus*. *Toxical Lett. (Amst)*. 11(1-2): 7-10. doi: 10.1016/0378-4274(82)90097-2.
19. Rani AU and Ramamurthi R. (1989). Histopathological alterations in the liver of freshwater teleost *Tilapia mossambica* in response to cadmium toxicity. *Ecotoxicol. Environ. Saf.* 17(2): 221-226. doi: 10.1016/0147-6513(89)90041-9.

20. Rehwoldt R, Lasko L, Shaw C and Wirhowski E. (1973). The acute toxicity of some heavy metals ions towards benthic organisms. *Bull Environ Contam Toxicol*. (10): 291-294.
doi: 10.1007/BF01684818.
21. Sastry KV and Gupta PK. (1978). Effect of mercuric chloride on the digestive system of *Channa punctatus*, A histopathological study. *Environ. Res*. (16): 270-278.
22. Siva ramakrishna B and Radha krishnaiah K. (2000). Chapter 14 Mercury induced alterations in the energetics of hepatopancreas of two freshwater molluscs, *Pila globosa* and *Lamellidens marginalis*. *Trace Met Environ*. 4: 389–409.
https ://doi.org/10.1016/S0927 -5215(00)80017–X.
23. Watson CE and Yanong R. (2002). Use of copper in freshwater aquaculture and farm ponds. *Fact Sheet*. FA-13, 10: 246-249.
https://doi.org/10.32473/edis-fa008-1989.