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Pollution-Induced Suppression of Phagocytic Activity in Freshwater Snails Collected from Selected Sites of Churni River, Nadia District, West Bengal

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ABSTRACT

Churni River is flowing through Nadia district in West Bengal, India. There was huge biological diversity in this river for fishes. But the water of the river has been polluted, creating loss of biodiversity since last few decades due to anthropogenic activities. Freshwater snails, *Bellamya bengalensis*, were manually collected from different selected sites of the Churni River in Nadia district. Some sites were not associated with human habitats, human effluents (site A). Some sites were associated with wastewater, domestic sewage, and other anthropogenic activities (site B). Hemocytes, the immune cells, were isolated, and phagocytic index was calculated.

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Degeneration of nucleus of cells and increased roundness of hemocytes with decreased number of pseudopods were noted in the hemocytes of site B group as compared to site A group of snails. Mean phagocytic index was significantly reduced in site B polluted group which may lead to alter the immune status of the animals, considering as biomarker of aquatic pollution.

Keywords: Churni River, Hemocytes, Phagocytosis, Pollution

1. INTRODUCTION

Churni is a river in the southern part of Nadia district of West Bengal, India. It has a length of approximately 56 kilometers. The river is a distributary of the Mathabhanga river. Churni is important because it provides livelihood opportunities for many fishermen.

But industrial effluents, sewage from adjacent area and sugar mill effluents are directly released into the river. The water is contaminated by agricultural wastes from adjacent crop fields and burning-ghat effluents [1, 2].

There was huge biological diversity in this river for tiger prawn, hilsa, and pabda, along with other aquatic animals like crab and snails [3,4]. But the water quality of the river has been affected, creating loss of biodiversity since last few decades due to imbalances of anthropogenic activities [4,5].

2. MATERIALS AND METHODS

Freshwater snails *Bellamya bengalensis* were manually collected from different selected sites along the banks of the Churni River in Nadia district, West Bengal. Some sites were not associated with human habitats, human effluents (site A). Some sites were associated with wastewater discharged from human habitats, discharge of domestic sewage, immersion of idols (site B) [6,7,8].



Collection site A (some sites of Shibnibash)



Collection site B (some sites of Krishnaganj)

Figure 1. Collection sites of specimens

Hemolymph was collected from *B. bengalensis*, and hemocytes were stained by Giemsa and neutral red. Cell counting was done by hemocytometer. Activated charcoal particles suspended in normal saline was injected into foot and the hemolymph was taken for phagocytosis study. The foot was gently penetrated with a needle, and hemolymph was easily collected using gentle intermittent suction [9]. The phagocytic index was calculated as per the following formula.

$$\text{Phagocytic index} = \frac{\text{Number of cells with phagocytosed charcoal particle}}{\text{Total number of cells counted}} \times 100$$

3. RESULTS

Different stages of phagocytosis of charcoal particles by hemocytes on glass slides were determined like formation of long pseudopodia, food cup, food vacuole (Fig. 2A). Degeneration of nucleus was noted in the hemocytes of site B group as compared to site A group of snails (Fig. 2B). Formation of long cellular process, attachment of charcoal particles on cell surface as well as tip of pseudopodia was noted in the Neutral Red stained cells collected from specimen of site A (Fig. 3). But significant numbers of hemocytes in site B group were not able to phagocytose the charcoal particles properly. Few hemocytes were completely laden by charcoal particles in site B group (Fig. 4). Increased roundness of hemocytes with decreased number of pseudopods was observed in site B group (Fig. 4 and 6). Mean phagocytic index was reduced in site B group (Fig. 5).

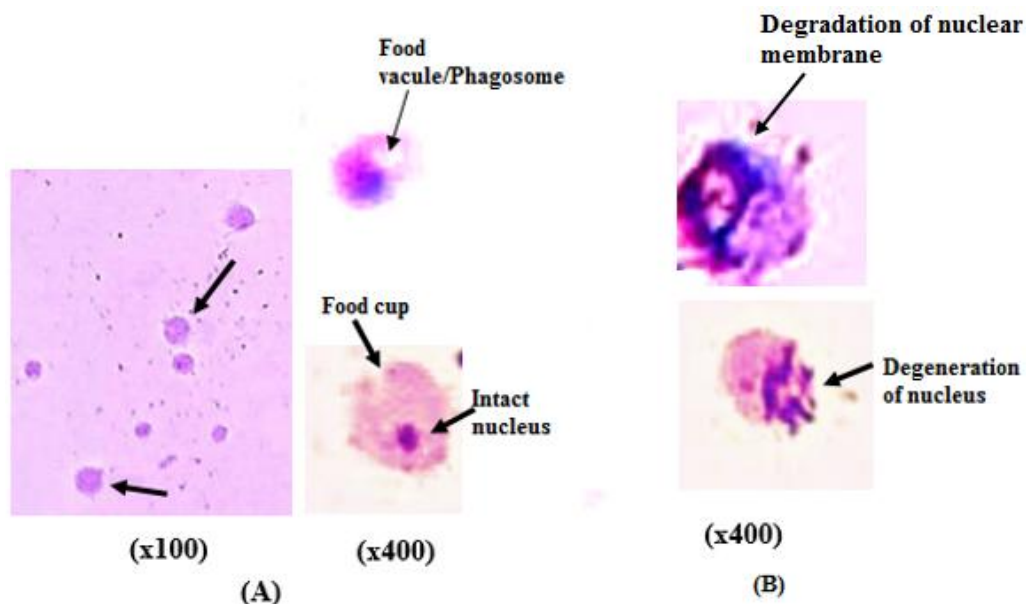


Figure 2. Microscopic images of hemocyte subpopulations stained with Giemsa.
(A) Hemocytes showing normal morphology of site A of Churni river of study area
(B) Necrotic hemocytes showing degeneration of nucleus of site B of Churni river of study area
 (indicated by arrow)

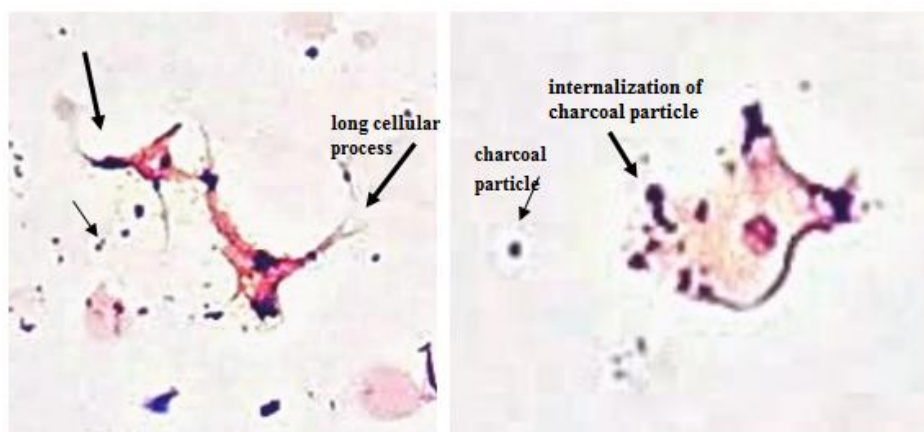


Figure 3. Formation of long pseudopodia, attachment of charcoal particle on cell surface and tip of pseudopodia in the Neutral Red stained cells collected from specimen of site A of Churni river (x400).

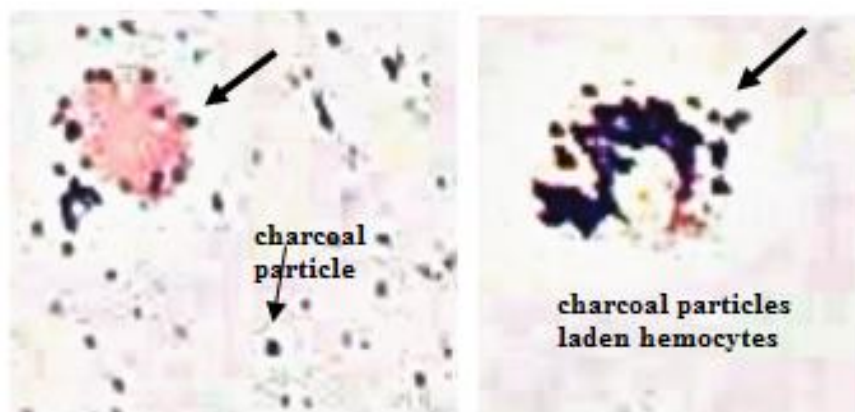


Figure 4. Charcoal particles laden rounded hemocytes stained by Neutral Red in site B group indicated by arrow (x400).

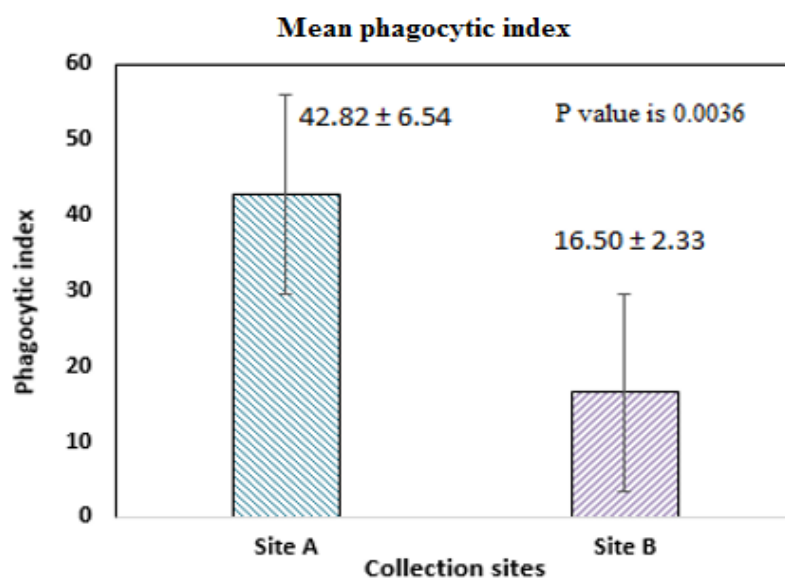


Figure 5. The mean phagocytic index of hemocytes from site B was reduced significantly when compared to site A . Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

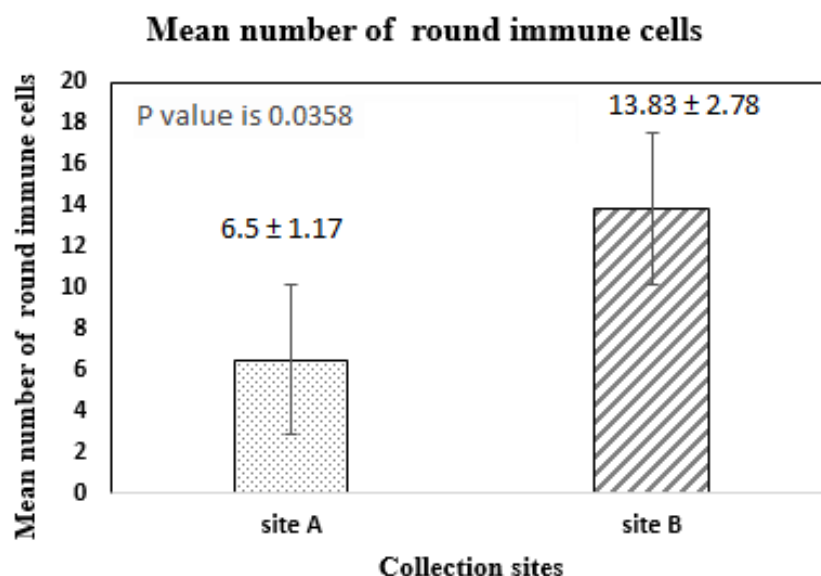


Figure 6. Mean number of round hemocytes with less or no pseudopodia in collection sites of Churni river. Values are expressed as Mean $\pm$ SEM. P-Value < 0.05 is considered to be statistically significant.

4. DISCUSSION AND CONCLUSION

Collection sites B of Churni River that were contaminated by anthropogenic activities may pollute the environment and affect aquatic snails rather than sites A, which were not associated with wastewater discharged from human habitats and anthropogenic activities.

Studies [9] determined the toxic effect of lead on hemocytes of *Lamellidens marginalis* for detecting a heavy-metal polluted environment. Lead treated cells showed apoptosis and paraptosis like features. According to Ray *et al.*, (2013) [10] in *L. marginalis*, round granulocytes act as principal immune effector cells showing strong phagocytic efficiency, and both the vertebrate macrophage and the invertebrate hemocyte phagocytic systems are homologous, related with recognition and engulfment of foreign material [11].

Present result reported the phagocytic response of hemocytes under the challenge of charcoal particles and showed Neutral Red positive response indicating the presence of lysosomal compartments. Phagocytosis is considered a classical innate immune response and is considered as a biomarker of pollution [12]. Mean phagocytic index was significantly reduced in site B polluted group, which may lead to imbalance of the immune status of the animals in the contaminated water.

Conflict of Interests

No conflict of interests declared.

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