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A comprehensive approach to health assessment of fish population using head kidney derived macrophages (HKM) and macrophage aggregates (MA) from rohu (*Labeo rohita*) and tilapia (*Oreochromis* sp.): the potentially sensitive biomarkers of contaminant exposure and several environmental stressors

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Abstract

Macrophages are key cells in both the innate and the adaptive immune system. The head kidney in teleost fish is a primary hematopoietic organ and act like bone marrow. Among head kidney leukocytes (HKL) only macrophages show the unique property of adherence on slides. The aim of the present study was to investigate the head kidney macrophages (HKM) morphology and phagocytic behavior in teleosts. Tissues from head kidney were removed and mashed in (0·1) M phosphate buffer saline (pH 7·2) in presence of trypsin- EDTA and tryton X 100. From cell suspension, adherent macrophages were stained by Giemsa. Presence of lysosomes was indicated using neutral red staining. Activated charcoal particles in normal saline (0·9% NaCl) was used for phagocytosis study. Light microscopy observations showed numerous macrophages as free macrophages or in aggregates (MAs). Formation of pseudopodia, attachment of charcoal particle on macrophage surface, tendency of cell fusion was noticed. Some cell also exhibited small vacuoles in cytoplasm. Macrophage aggregates (MAs), morpho-functional alteration of macrophages can be used as bio indicator to several environmental stressors which should be of great value as a first-line indicator early warning system of environmental pollution.

Key words : Head Kidney Macrophages (HKM), Macrophage Aggregates (MA), Phagocytosis, Bio indicator, Biomarkers.

Environmental conditions are certainly not static, and human influence has greatly accelerated the rate of environmentally deleterious changes, for example by continuously loading water systems with chemical xenobiotics. Particularly in the 20th century, countless thousands of organic trace pollutants have been produced, and in part released into the environment[10]. Fishes come into contact with multiple contaminants that are dissolved in the water or incorporated in their food chain, and so fishes are not only prone to endure negative toxicant-related health effects but also to bioaccumulate pollutants; fishes may therefore be used as bioindicators of environmental contamination[5,6].

There is growing awareness that a range of environmental chemicals target the immune system of fish. Macrophages play a significant role in defense mechanisms. Some previous studies showed Melano-Macrophage Centres (MMCs) or macrophage aggregates, increased in size in conditions of environmental stress and pollution. Numerous studies have shown an increase in number of macrophage aggregates (MAs), size or pigment content in fish collected at contaminated sites[7,11,12]. For this reason, MAs and morpho-functional alteration of macrophages have been suggested as potentially sensitive biomarkers of contaminant exposure and fish health[7,11,12].

Studies showed cultured head kidney macrophages (HKM) of rohu, *Labeo rohita* demonstrated the property of phagocytosis as evidenced by engulfment of yeast cells. Bacterial lipopolysaccharide (20 microg/ml) resulted in functional activation of macrophages as seen by enhanced lysosomal enzyme activity[3].

Researchers showed *in vitro* assays using piscine immune cells might be suitable to evaluate immunotoxic potentials of environmental chemicals to fish[9]. Previous reports showed the utility of splenic macrophage aggregates (MAs) as an indicator of fish exposure to degraded environments was evaluated as part of the Environmental Protection Agency's Environmental Monitoring and Assessment Program–Estuaries (EMAP-E). Densities of MAs that exceeded 40/mm^2 correlated with exposure to either hypoxic conditions or sediment contamination[7].

Studies also showed the ultrastructure and morphometrics of pigmented macrophages (PMs) were assessed in the spleen of European sea bass experimentally dosed with Cd and Hg. PMs occurred as solitary cells as well as structured aggregations, defined as macrophage aggregates (MAs)[8].

Alive fish samples of tilapia (*Oreochromis* sp.) and rohu (*Labeo rohita*) were collected from local markets and dissected in lab. Tissues from head kidney were removed using the forceps and mashed through the cell strainer into the petridish containing (0·1) M phosphate buffer saline (pH 7·2) in presence of trypsin- EDTA and triton X 100. Cell suspension was smeared directly on sterilized glass slides and incubated at 37 °C in a humid chamber for 3 hours. After incubation the nonadherent cells were removed by washing with PBS. The adherent macrophages were fixed by methanol and stained by Giemsa. Activated charcoal particles in normal saline (0·9% NaCl) was used for phagocytosis study. Functional activation of macrophages was seen by neutral red for lysosomal enzyme activity.

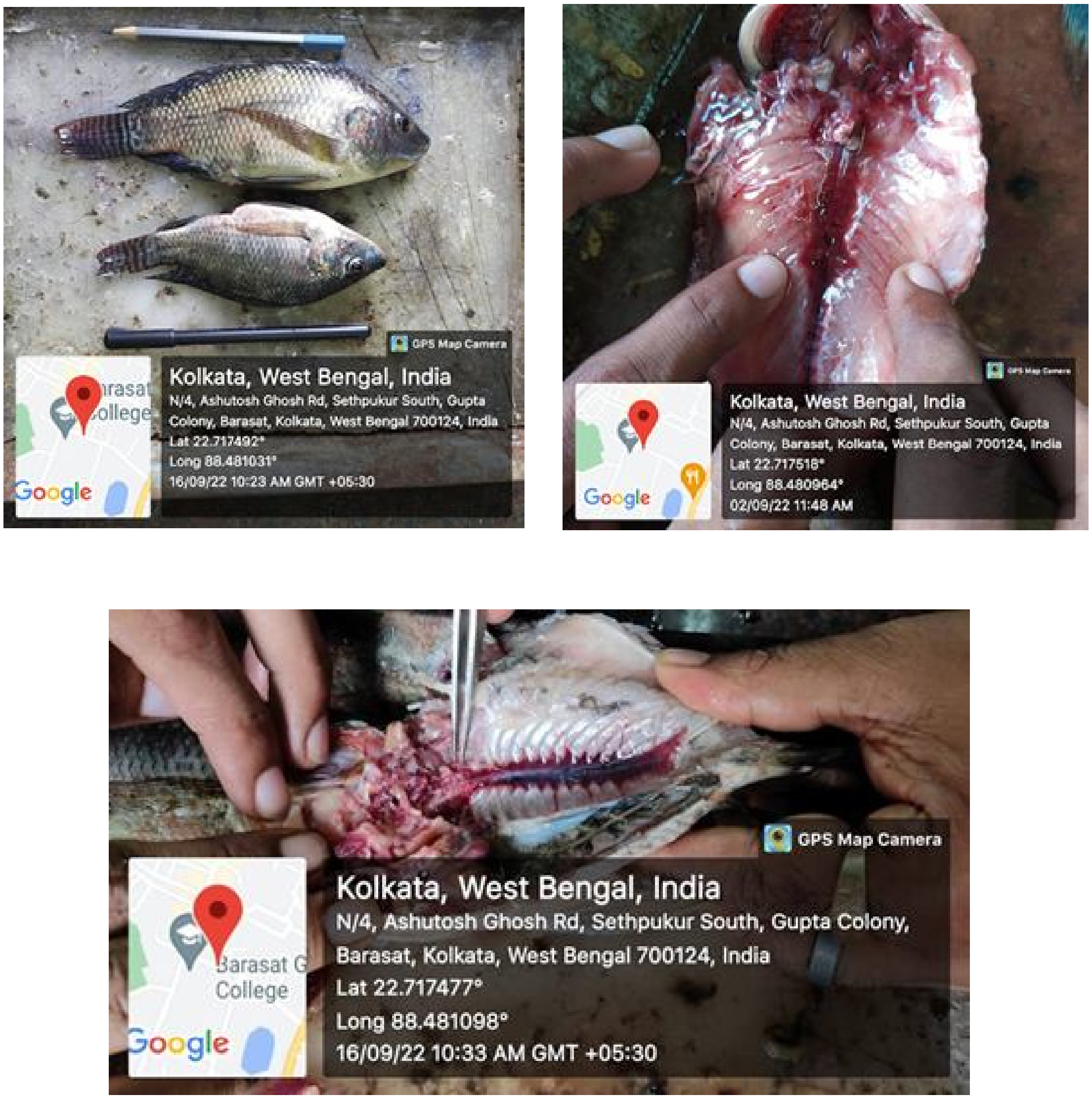


Fig. 1. Isolation of tissues from head kidney in the laboratory

Morpho-functional study:

Result showed numerous macrophages as free (Fig. 2) or in aggregates (MAs). Head kidney macrophages (HKM) showed the property of phagocytosis. Formation of pseudopodia, attachment of charcoal particle on macrophage surface, internalization of charcoal particles (Fig. 3), tendency of cell fusion was noticed. Some cell cytoplasm also exhibited vacuoles.

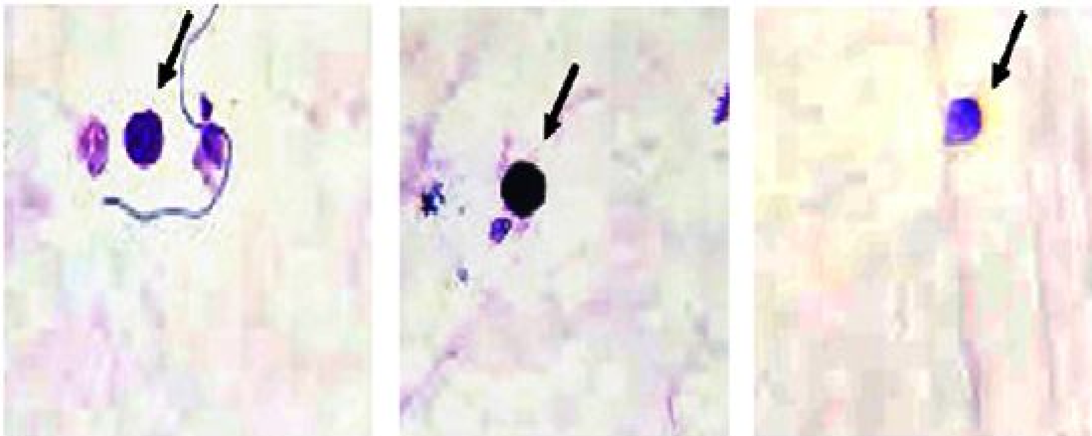


Fig.2. Giemsa stained head kidney macrophages showing their morphology (x400)

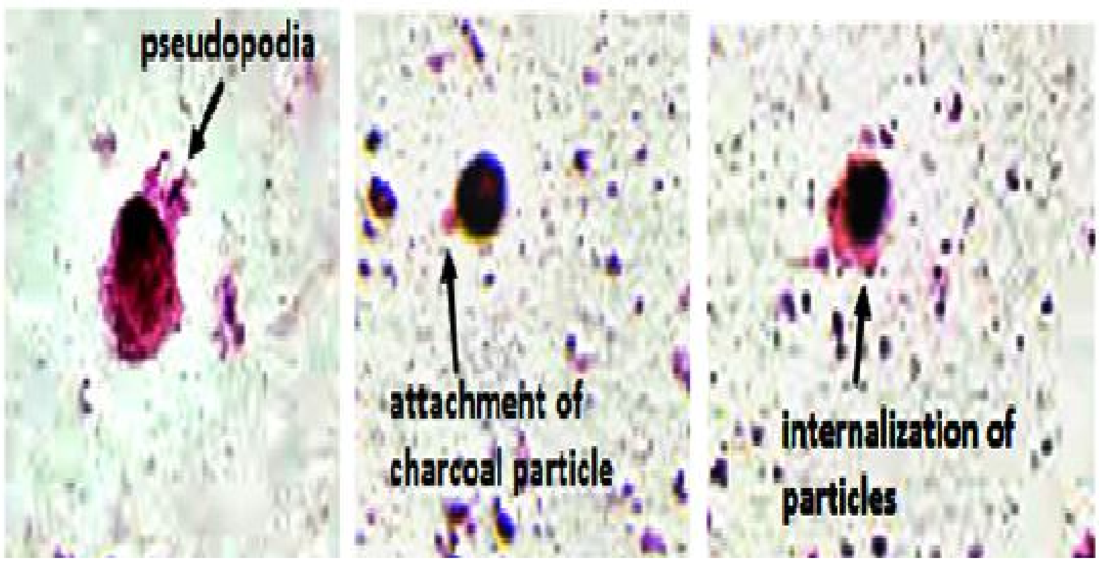


Fig. 3. Formation of pseudopodia, attachment of charcoal particle on macrophage surface, internalization of charcoal particles in HKM (x400)

Phagocytosis study :

Different stages of phagocytosis of charcoal particles by HKM on glass slides were determined (Fig. 4). Macrophages showed different stages of phagocytosis like attachment of charcoal particle on macrophage surface and tip of pseudopodia, formation of food cup and cell-cell fusion (Fig. 4).

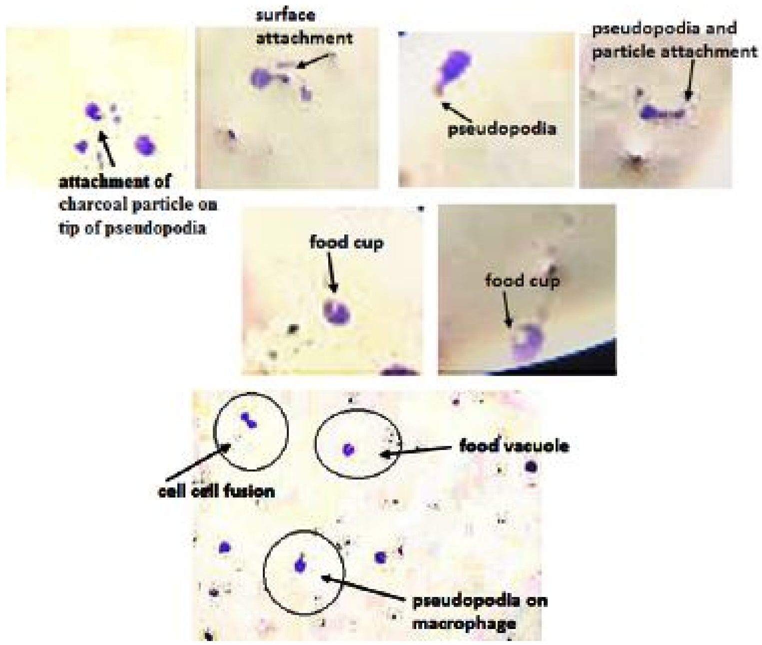


Fig. 4. Different stages of phagocytosis in HKM observed like formation of pseudopodia, formation of food cup and cell-cell fusion indicated by arrow (x400)

Neutral Red positive responses :

Functional activation of macrophages was seen by neutral red for lysosomal enzyme activity (Fig. 5).

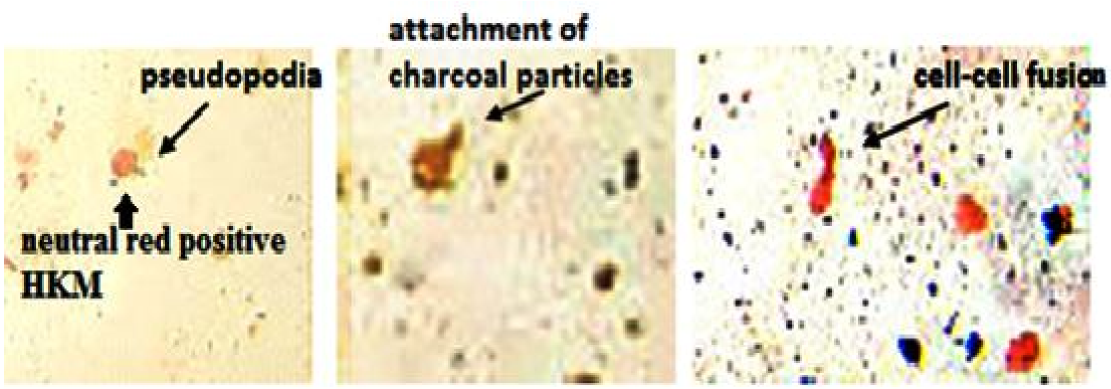
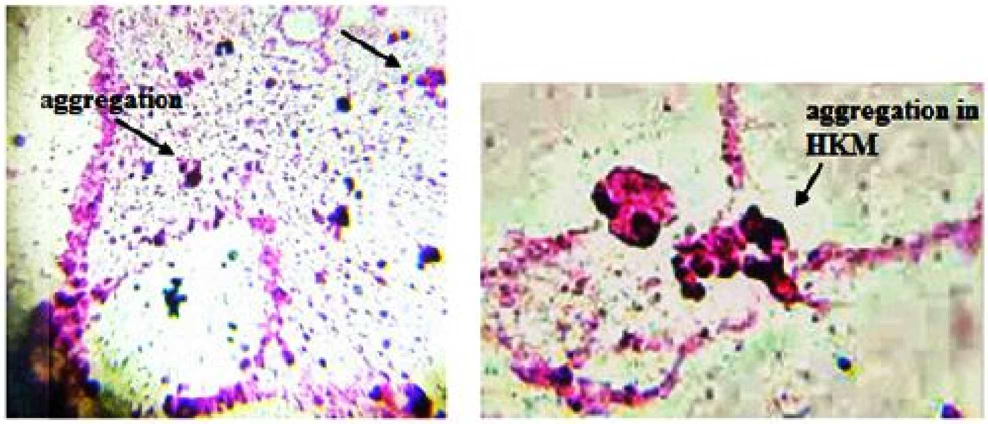


Fig. 5. Different stages of phagocytosis in neutral red positive HKM observed like formation of pseudopodia, attachment of charcoal particle and cell-cell fusion indicated by arrow (x400)

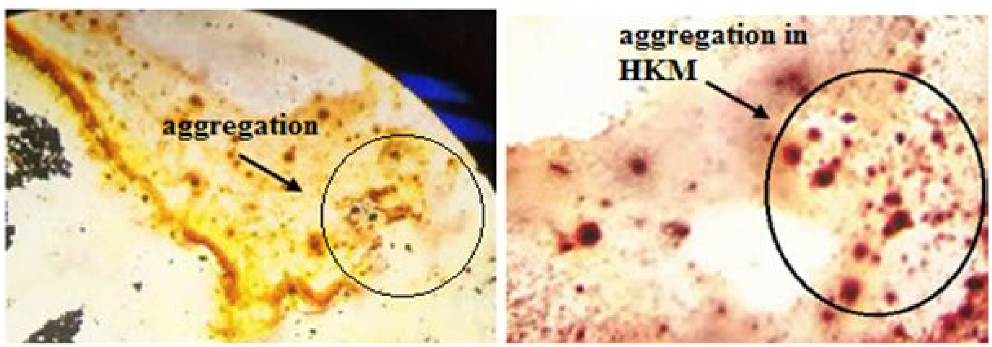
Cell aggregation :

Different stages of aggregation in macrophages (MA) were noticed (Fig. 6).



(A) (B)

Fig. 6. (A) Giemsa stained stages of aggregation in HKM in rohu (x100) and (B) Giemsa stained stages of aggregation in HKM in tilapia (x400)



(C) (D)

Fig. 6. (C) Neutral Red stained stages of aggregation in HKM in rohu (x100) and (D) Neutral Red stained stages of aggregation in HKM in tilapia (x400)

Present result showed numerous macrophages as free (Fig. 2) or in aggregates (MAs) (Fig. 6). Macrophage aggregates (MAs) are focal accumulations of macrophages found in the spleen, head kidney, and sometimes liver of teleost fishes. These structures are easily visualized in histologic sections through the presence of different pigments: hemosiderin, melanin, and ceroid/lipofuscin. Many factors are known to affect the accumulation and/or proliferation of these structures, including age[4], nutritional status[2], and infectious diseases[1].

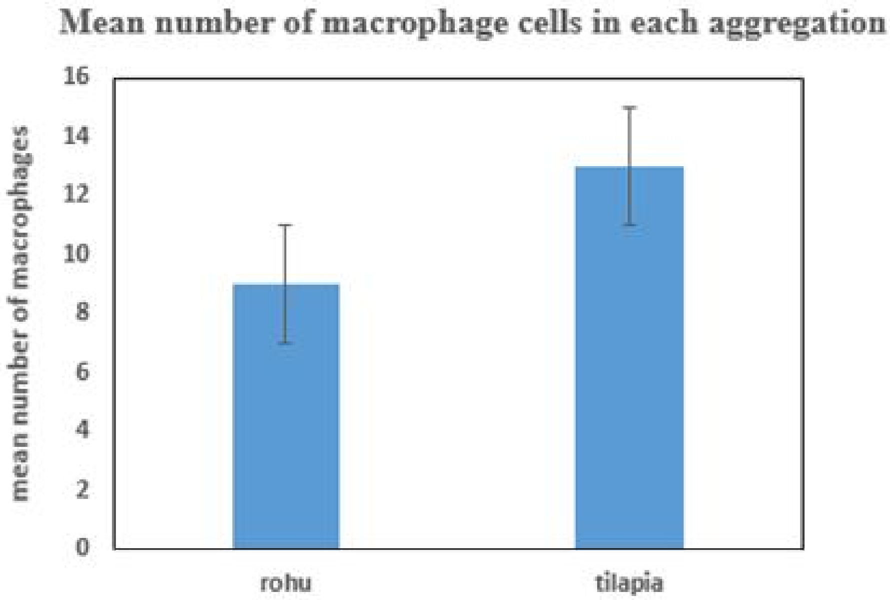


Fig. 7. Graph of average number of macrophage cells in each aggregation

Changes in various MA parameters (*e.g.,* number, size, percent area occupied) in relation to environmental contamination have been reported by several investigators[11,12]. MAs are known to change in number, size, and pigment content related to fish health and environmental degradation as biomarkers[7,11]. In the present result, average number of macrophage cells in each aggregation of tilapia was higher than rohu fish (Fig. 7).

In present result, Head kidney Macrophages (HKM) showed the property of phagocytosis. Formation of pseudopodia, attachment of charcoal particle on macrophage surface, internalization of charcoal particles and tendency of cell fusion was noticed (Fig. 3, 4).

Morpho-functional alteration of macrophages like phagocytosis can be used as bio indicator to several environmental stressors which should be of great value as a first-line indicator early warning system of environmental pollution. These are used to establish early-warning signals, biomarkers, to evaluate or predict the impact of chemicals in ecosystem and health monitoring system[12].

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