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# Morpho-functional variations of hemocytes in edible mud crab (*Scylla* sp.) under exposure of toxic metals

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**Abstract**

Benthic crustaceans such as crabs are suitable as bio indicators. The toxicity of contaminants can be examined in the cells by the use of animal models as biomonitoring or bio indicating tools. Metals in industrial wastewater sometime contain immuno-suppressors. Edible live mud crabs (*Scylla* sp.) were exposed to toxic metals like Sodium Arsenite, Mercury(II) Chloride ($HgCl_2$), Lead Nitrate and Zinc Sulphate in glass aquaria. Result showed that mean phagocytic index in lead (Pb) and arsenic (As) treated group was significantly reduced. Mean mortality index was significantly increased in arsenic and lead treated group. Control hemocytes showed neutral red stained lysosomal compartments. Hg and Pb treated cells showed neutral red positive response in their cytoplasm indicating damage of lysosomal membrane leading to the release of hydrolases in the cytoplasm. Increased roundness of the immune cells with decreased number of pseudopods in hemocytes exposed to mercury was noted. The results of present study corroborated the earlier studies. The present study provides a protocol to find out the effect of pollution on ecosystem using crab as a bio indicator species.

**Key words :** Hemocytes, Pollution, Bio Indicator, Toxic Metals.

Heavy metals are typical environmental pollutants due to their potential effects on human health and ecosystem[8]. Benthic crustaceans such as crabs are suitable as bioindicators of water pollution as they show a high sensitivity to environmental stressors[2]. The crustacean hemocytes play different functions such as phagocytosis, encapsulation and cell-cell communication. Classification of the hemocyte types in decapod crustaceans is based mainly on the presence of granules[7].

The crustacean haemocytes are generally hyaline cells (HC), small granular cells (SGC) and large granular cells (LGC)[7]. The toxicity of contaminants can be investigated

in the biological tissues and cells by the use of animal models as biomonitoring or bio indicating tools[13] which give information about the effects of xenobiotics on cellular level[1]. Metals in industrial wastewater sometime contain immuno-suppressors[18] that can influence defensive system[12]. Previous researches showed the effects of these chemicals on birds and mammals. However, not many investigations have been carried out on the effects of xenobiotics on invertebrate organisms[9]. The present study provides a protocol to find out the effect of pollution on ecosystem using crab as a bio indicator species.

Edible live mud crabs (*Scylla* sp.) were purchased from local fish markets. To investigate the immunotoxicity and ultrastructural changes of hemocytes, specimens of mud crabs were exposed to sodium arsenite ($NaAsO_2$) in water at 0.025 mg/L (group 1)[6], mercury (II) chloride ($HgCl_2$) in water at 0.30 mg/L (group 2)[19], lead nitrate in water medium at 50 mg/L (group 3)[5] and zinc sulphate at 0.43 mg/L in glass aquaria (group 4)[14]. Control specimens were untreated by any toxic metals (Each group, n=6).

The effect of toxicants was determined in edible mud crab (*Scylla* sp.) for 48 hrs and 72 hrs of span. Hemolymph of crabs was collected from the base of one of the second walking legs and hemolymph was smeared on glass slides, fixed by methanol and stained by Giemsa, Leishmans Eosin Methylene blue solution and neutral red. The phagocytosis of hemocytes were determined by activated charcoal particles[7]. The phagocytic index was calculated. Cells were treated with 50 µl of 0.25 % trypan blue dye solution for 5 minutes and mortality index calculated. Cells were stained with NBT.

Result showed that granular and semi-granular haemocytes were phagocytic. Binding of charcoal particles, pseudopodial growth formation were noted (Fig. 1). But significant numbers of hemocytes in treated group were not able to phagocytose the charcoal particles. Mean phagocytic index in arsenic (As) and lead (Pb) treated group was significantly reduced as the number of pyknotic cells and necrotic cells were increased (Fig. 2, 3).

Increased mean percentage of NBT positive cells in arsenic (As) treated group in 72 hrs was noted (Fig. 4, 5). The percentage of Trypan Blue (TB) positive cells represented a mortality index. Mean mortality index was significantly increased in arsenic and lead treated group (Fig. 6).

Neutral red stained cytoplasm of treated hemocytes indicated the damage of lysosomal membrane leading to the release of hydrolases in the cytoplasm indicating an alteration of the integrity of the lysosomal membrane. Control hemocytes showed neutral red stained lysosomal compartments. Treated cells showed neutral red positive response in their cytoplasm (Fig. 7, 8 and 9).

Average number of pyknotic cells was increased in treated group (p value was 0.0003) (Fig. 10). Mean number of cells in aggregates and mean number of total aggregates after treatment of toxic metals on glass slides was significantly reduced (Fig. 11 and 12).

Increased roundness of the immune cells with decreased number of pseudopods in hemocytes exposed to mercury was noted (Fig. 13).

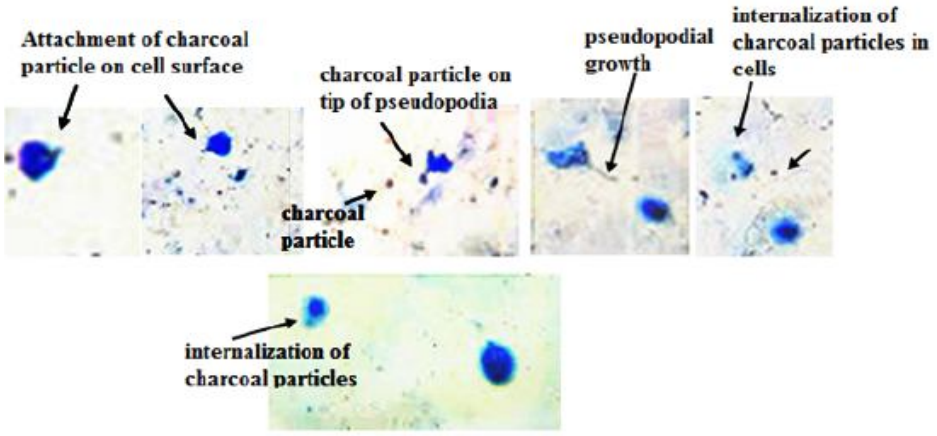
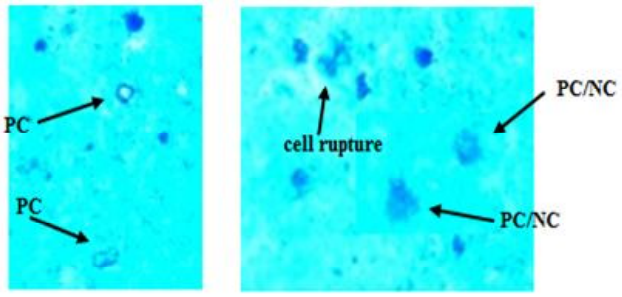



Fig. 1. Granulocytes (semigranular, granular cells) in control crabs evaluated by light microscopy. Leishmans Eosin Methylene blue stained hemocytes showed different stages of phagocytosis (x400)




**LEM stained pyknotic cells in Pb treated group in 72 hrs (x 400)**

Fig. 2. Leishmans Eosin Methylene blue solution stained pyknotic cells in Pb treated group in 72 hrs (x 400). PC=Pyknotic cells, NC=Necrotic cells (indicated by arrow)

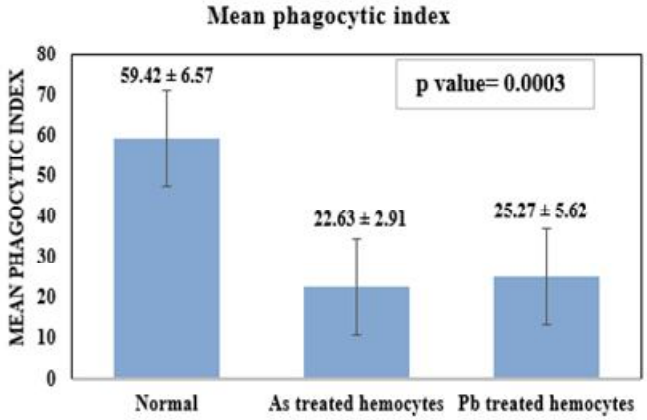



Fig. 3. Mean phagocytic index in Pb and As treated group was significantly reduced

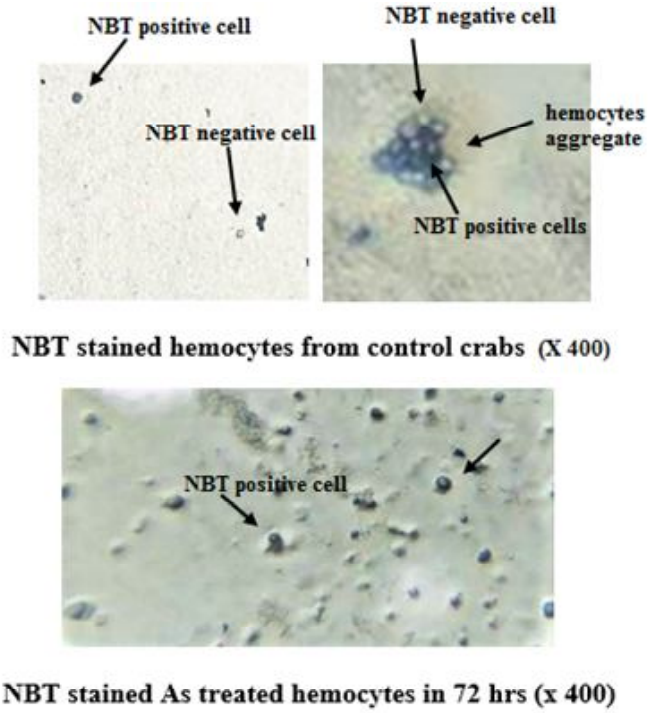



Fig. 4. Field of hemocytes with presence of NBT (Nitro Blue Tetrazolium) positive cells in control and arsenic (As) treated group (control group showed more aggregation)

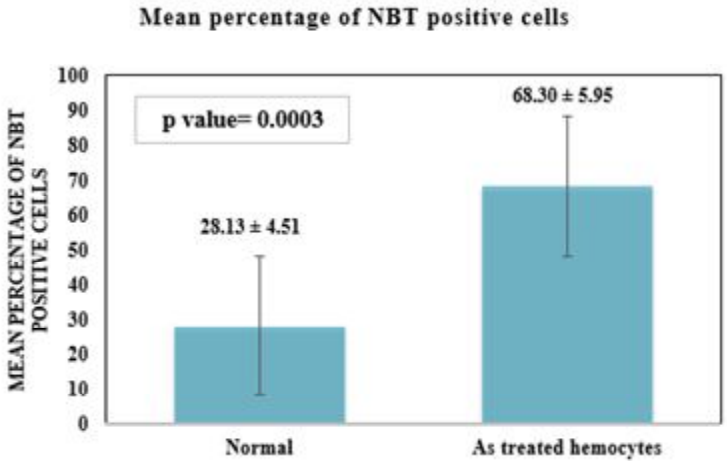



Fig. 5. Increased mean percentage of NBT positive cells in arsenic (As) treated group in 72 hrs

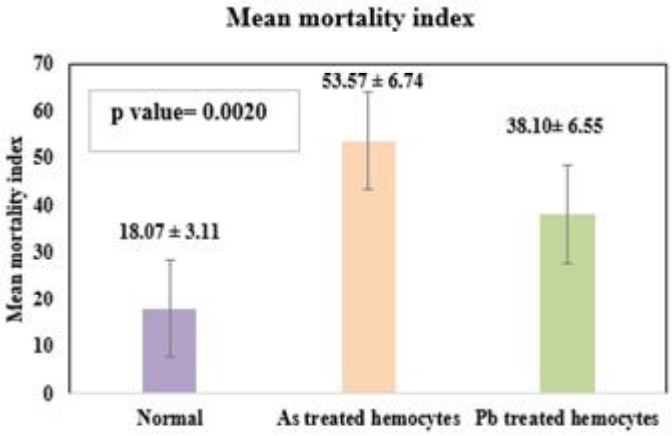



Fig. 6. Significant number of treated cells showed Trypan Blue (TB) positive response. Mean mortality index was significantly increased in arsenic and lead treated group

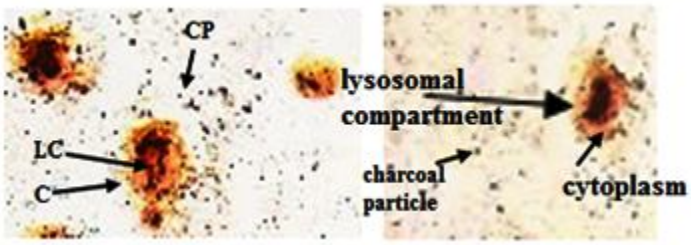



Fig. 7. Presence of lysosomal compartments and cytoplasm can easily be distinguished (x400) in control cells after staining by neutral red. LC= Lysosomal Compartments, C= Cytoplasm, CP= Charcoal Particles

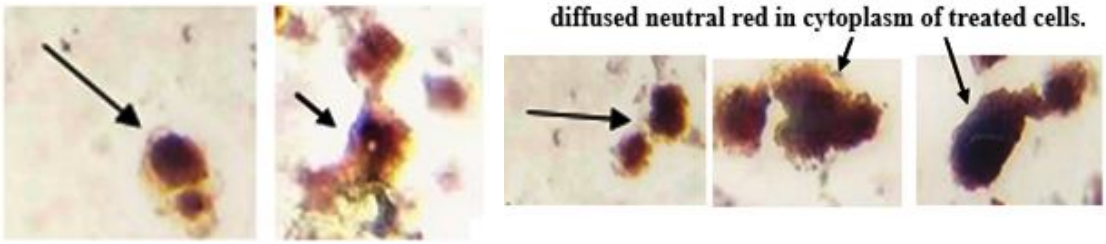



Fig. 8 (A) Fig. 8 (B)

Fig. 8. Progressive changes in Neutral Red positive cells during Hg treatment (x 400)

(A) Neutral Red (NR) positive response in Hg treated condition in 48 hrs,
(B) Neutral Red (NR) positive response in Hg treated condition in 72 hrs. Arrows indicates the diffused neutral red in cytoplasm of treated cells

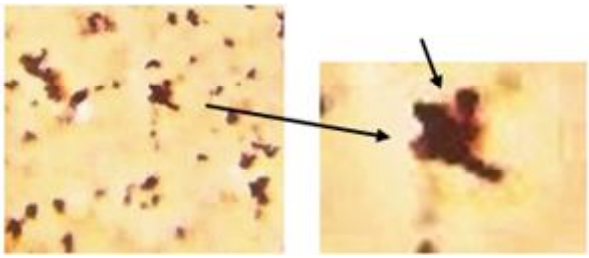


Fig. 9. Neutral Red positive cells during Pb treatment in 72 hrs (x 400). Treated cells showed neutral red positive response in their cytoplasm

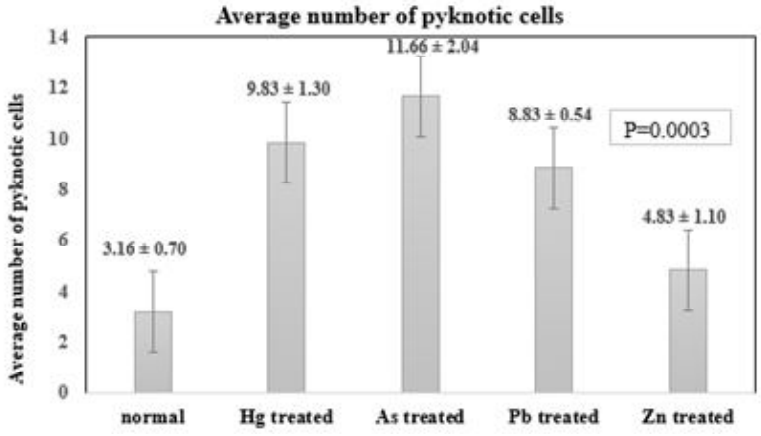



Fig. 10. Mean number of pyknotic cells after treatment of toxic metals. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

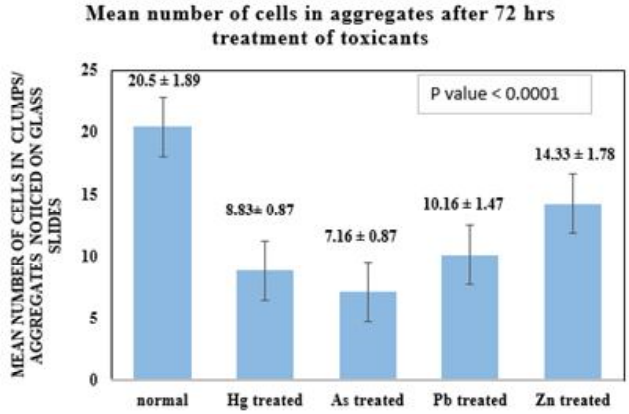



Fig. 11. Mean number of cells in aggregates or clumps after treatment of toxic metals noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

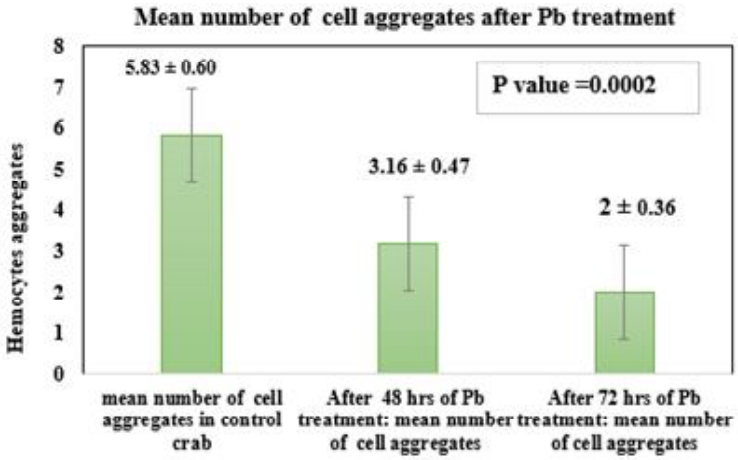



Fig.12 (A)

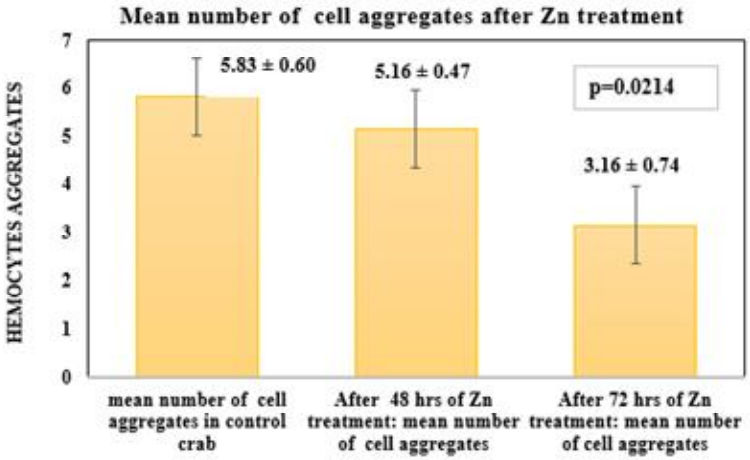



Fig. 12 (B)

Fig. 12. Mean number of cell aggregates after Pb and Zn treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

In an environment, high concentration of heavy metals destabilizes ecosystems[11]. Zheng *et al*.,[20] found that the enrichment level of the essential trace elements Fe, Zn, and Cu in the hepato pancreas of fish was higher than that in other tissues[20]. Du *et al*.,[4] showed the ecological risk of heavy metals to crustaceans was greater than that to fish[4]. Bian *et al*.,[3] showed that in aquatic animals, benthic oligochaetes were more sensitive to heavy metal pollution, followed by leeches, gastropods and insects[3]. The enrichment of heavy metals by aquatic animals was related to age, geographical distribution, seasons, and the form

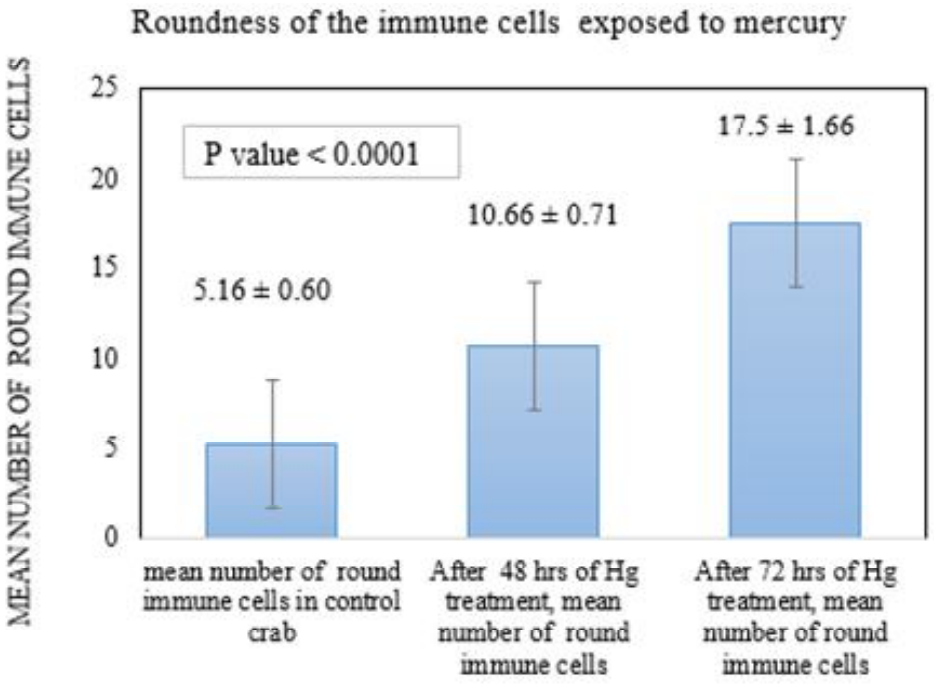



Fig. 13. Mean number of round immune cells (hemocytes) after treatment of mercury. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

of heavy metals[3].

Ramya *et al.*,[14] found histological alterations in gills in mud crab, *Scylla serrata* after exposures to 15% sublethal concentration of heavy metal zinc chloride[14]. Usharani[17] and Shagnas[15] showed similar type of observations in *Scylla serrata* exposed to toxic metals (zinc, lead and mercury).

Present result revealed the production of ROS (reactive oxygen species) in hemocytes indicating their phagocytic nature. NBT reacted with $O_2$- (superoxides) to form a dark blue colour (Fig.4). Toxic metal-induced cytotoxic events in the experiments may be due to excess ROS generation (Fig. 4).

Parisi *et al.*,[12] showed roundness effect of hemocytes by organic mercury. Changes in morphology of hemocytes of *Mytilus galloprovincialis* was noted due to the exposure to methylmercury. After xenobiotic exposure of methylmercury at sublethal concentration, the cells undergo contraction and rounding[12]. Mottin *et al.*,[10] stated increased mortality of haemocytes was associated with a change of cell shape, which becomes rounded, and reduction of phagocytic capacity[10].

Increased roundness of the immune cells with decreased number of pseudopods in hemocytes exposed to mercury was noted in our study (Fig. 13).

Zhao *et al*.,[19] showed $Hg_2$+ disrupted the histostructures of the hepatopancreas of juvenile Chinese mitten crabs. Moreover, as the $Hg_2$+ concentration increased, the survival rate of the crabs decreased, worst at 56.57% in 0.30 mg/L [19].

Silveira de Melo *et al*.,[16] stated Hg deregulated many crucial biological functions of freshwater amphipod *Gammarus pulex* exposed to two environmentally relevant concentrations (50 and 500 ng/L) at two temperature regime fluctuations (16$^{o}$C and 20 $^{o}$C) [16].

Guria and Ali.,[7] showed arsenic and mercury both inhibited the degree of hemocyte aggregation and assemblage of edible mud crab (*Scylla* sp.) that may affect "encapsulation response" [7].
The results of present study corroborated the earlier studies.

The present study provides a protocol to find out the effect of pollution on ecosystem using crab as a bio indicator species.

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