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EFFECT OF LEAD NITRATE ON HAEMOCYTE MORPHOLOGY OF FRESHWATER PRAWN, *Macrobrachium dayanum* (CRUSTACEA-DECAPODA)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lead (Pb), a non-essential "Grey listed" heavy metal is a serious threat to aquatic flora and fauna. The haematological, nephrological, histopathological and neurological effects of lead are well known in vertebrates. Present study aims to investigate effect of lead on morphology of haemocytes, a important component of immune system of freshwater prawn, *Macrobrachium dayanum*. Prawns, collected at "Gulala-Ghat" from river Gomti, Lucknow, subjected to acute and sub-acute concentrations of lead nitrate (116.46 mg/l, 96h LC_{50}; 29.12 mg/l, 25% of 96h LC_{50}) showed morphological changes in haemocytes after 24, 48, 72 and 96h in acute exposure and after 10, 20 and 30 days in sub-acute exposure. The chief morphological changes like surface blebbing, vacuolization, cytoplasmic darkening, fragmentation of nuclear material, cone formation, nuclear pycnosis and breaking of plasma membrane were noticed after acute exposure (116.46 mg/litre). Black granular depositions, nuclear pycnosis and fragmentation of nuclear material; pseudopodial projections, blackening of the plasma membrane, achromatia were observed after sub-acute exposure (29.12 mg/litre). These changes were pronounced in prohaemocytes, granulocytes, plasmatocytes and spindle cells. The severity of morphological changes was dose- and duration-dependent. The present study found a positive correlation between heavy metal lead exposure and morphological changes in haemocyte morphology leading to severe immune dysfunction in freshwater prawn *M. dayanum*. This parameter can be used as bio-marker in health monitoring of prawns as well as environmental monitoring without sacrificing the animal.

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1. INTRODUCTION

To combat the nutritional requirement of increasing population, human beings started using different types of fertilizers, pesticides and insecticides to increase their crop yield and also developed various industries to fulfill their basic requirements of food, cloth and shelter. Industrial effluents and domestic sewage contain hazardous chemicals directly discharged into our riverine system and other freshwater bodies. Heavy metals also enter into our aquatic ecosystem by various natural activities, causing deleterious effects on aquatic flora and fauna [1]. Heavy metals cause serious threats because of their long half-life period, persistent accumulative and amplificative tendencies in the food chain, thereby increasing the problem many folds [2]. Nowadays, heavy metal contamination has become a major environmental issue [3, 4, 5].

Among heavy metals, lead (Pb) is an ubiquitous environmental contaminant belonging to the most toxic heavy metals released in the biosphere. Lead is considered a non specific poison affecting physiological systems and can cause brain damage, kidney damage, and gastrointestinal and reproductive disorders [6].

Toxic effects of lead and other heavy metals on blood cell morphology have been mostly investigated in fishes [7-11] and other invertebrate, gastropods [12,13] while crustaceans despite being an important member of aquatic food chain and having high economic and medicinal value have been documented less in reference to metal toxicity [14-19].

Freshwater Prawns belong to the family Palaemonidae of Decapod-Crustacea. These are economically important as well as potential animals for freshwater aquaculture and may serve as a good laboratory model for fundamental and toxicological research [20, 17, 21, 22, 19, 23].

Therefore present work has been taken into account to evaluate the toxic effects of lead as lead nitrate on the haemocyte morphology of freshwater prawn, *Macrobrachium dayanum* (Crustacea-Decapoda), a good bio-indicator of the freshwater aquatic ecosystem.

2. MATERIALS AND METHODS

The experimental animal freshwater prawns, *M. dayanum* (Henderson) were collected from river Gomti, Lucknow (U. P.), with the help of local fishermen and brought to the laboratory (N 26º 5' 59" E- 80º 56' 17") in large plastic containers. In this case no animal ethical approval is required. The stock of animals was maintained in glass aquaria of 20/liter capacity containing 10/liter of dechlorinated water having physico-chemical characteristics [25] (Table 1).

Stock solution of Lead nitrate ($Pb(NO_3)_2$), AR grade molecular weight 331.21 gm/mole, (E-Merck (India) Ltd. Worli Mumbai-400018) was prepared by dissolving weight amount of salt in the double-distilled water. Lead nitrate was dissolved in water by adding 0.3 mL L-1 of concentrated Nitric acid [4].

Adult inter-moult staged *M. dayanum* (Average length - 5.64 ±0.42cm; weight - 3.262±0.68gm) were utilized in experiments after 5-7 days of acclimation to laboratory conditions. The LC50 values were calculated according to the trimmed Spearman-Karber's method [26] with the software on P.C. Acute and sub-acute exposure was carried out on 96h LC_{50} (116.46 mg/litre) and 25% of 96 hour LC_{50} 29.12 mg/litre for 24, 48, 72 and 96h and 10, 20 and 30 day respectively. One aquarium containing diluents water and 0.3 mL L-1 conc. nitric acid only served as control. During acute exposure, feeding was suspended 24h before and throughout the exposure, while in sub-acute exposure, change of exposure medium and food supply was maintained on an alternate day throughout the experiment. Continuous air Supply was provided by air diffusers and aerators in both controls as well as experimental aquaria during the experiment. The experiment was carried out according to the guidelines of APHA et al.[24].

Table 1. Physicochemical characteristics of experimental water used for the study

S.N.	Physico-chemical parameters	Characteristics
1.	Temperature	26.6 ±0.6 °C
2.	Biochemical Oxygen Demand	6.6 ± 0.74 mg/litre
3.	Total hardness	268 ±2.67 mg/litre
4.	Alkalinity	25±1 1.36 mg/litre

The experiments were replicated thrice. Samples of haemolymph were collected from both controls as well as experimental animals of *M. dayanum*, by direct and indirect methods for insects and arachnids [27, 28, 14]. In the direct method, prawns were wiped and semi dried with the help of Whatman No. 1 filter paper. Semi-dried prawns were pricked by sterilized needle between the carapace and abdomen and then inverted quickly to form an oozing droplet of haemolymph. This oozing drop of haemolymph is either directly taken on the slide or it was sucked by WBC diluting pipette. In the direct method, haemolymph was withdrawn either in glass capillaries or sterilized paraffinized syringes, following the method described by Hardy [29]. Both phase contrast and light microscopic studies were made on fresh and fixed haemolymph of both control and experimental animals to study morphological changes. For the live cells study, fresh haemolymph was observed, but for light microscopic studies, both fresh as well as fixed haemolymph preparations were stained with Harri's haemotoxyline and eosin [32] and other bloodstains like Leishman's and Giemsa stains [33].

3. RESULTS

Haemocytes of experimental animals showed pronounced morphological and behavioural effects after acute and subacute exposure. Lead-induced marked morphological changes in haemocytes of *M. dayanum*. Haemocytes of control prawns (Plate 1 and 2- Figs. 1, 2, 3 respectively) appeared normal in shape and behaviour under phase contrast and permanent preparations.

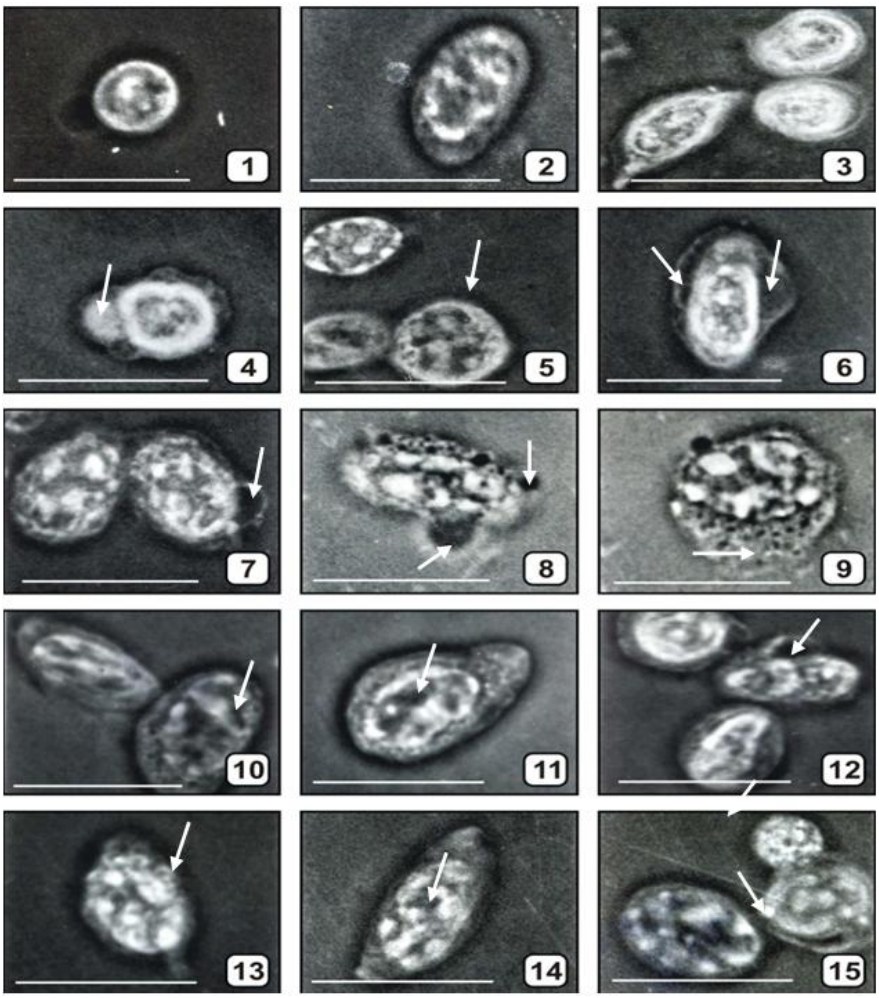

Plate 1. Photomicrographs of Haemocytes of M. dayanum under phase contrast microscope showing effects of acute exposure of Lead nitrate (Figs. 1-3: Control; Figs. 4-6: after 24 hr; Figs. 7-9: after 48 hr; Figs. 10-12 after 72 hr and Figs. 13-15 after 96 hr exposure showing blebbing, cone formation, nuclear pycnosis, dark granular depositions, breaking of plasma membrane; (arrows) Scale bar=50µ)

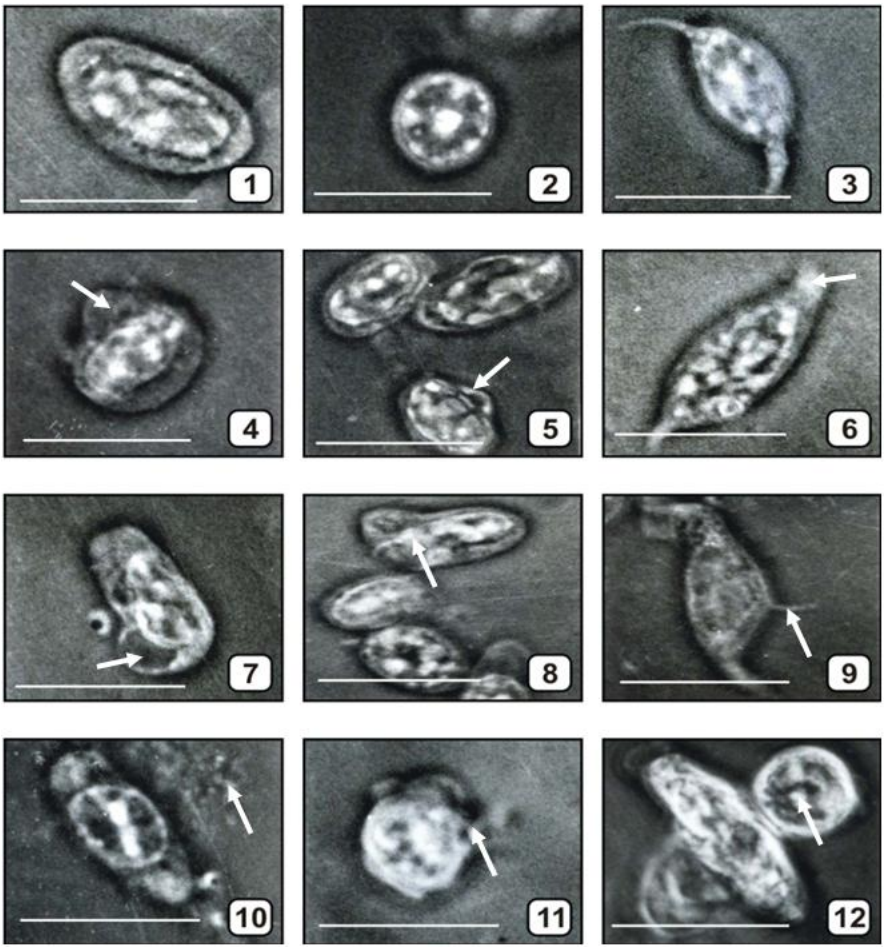

Plate 2. Photomicrographs of Haemocytes of M. dayanum under phase contrast microscope showing effects of sub-acute exposure of Lead nitrate (Figs.:1-3: Control; Figs. 4-6. after 10 days; Figs. 7-9 after 20 days; Figs. 10-12 after 30 days exposure showing blebbing,cone formation, nuclear pycnosis, dark granular depositions, breaking of plasma membrane; (arrows) Scale bar= 50µ).

3.1 Acute Exposure

After 24h exposure (Plate 1- Figs. 4, 5, 6), hypertrophy was observed in all cells. Surface blebbing in granulocytes and plasmatocytes and mild vacuolization and cytoplasmic darkening in granulocytes were evident in most haemocytes. After 48h exposure (Plate 1- Figs. 7, 8, 9), increased vacuolization, dark granular deposition in the cytoplasm, fragmentation of nuclear material and eccentric nucleus was observed in some cells. Most of the cells were deformed due to increased blebbing and cone formation. After 72h exposure (Plate- 1, Figs. 10, 11, 12), pronounced blebbing and nuclear pycnosis were observed in most cells, particularly in plasmatocytes and granulocytes. Most of the cells were found stuck together with their pseudopodial outgrowths. After 96h exposure (Plate- 1, Figs. 13, 14, 15), most of the cells were deformed and breaking of the plasma membrane along with nuclear pyknosis was observed. In some places, karyorrhexis and karyolysis were also observed. About 40% of cells were found ruptured and deformed.

3.2 Subacute Exposure

After ten days of exposure (Plate- 2, Figs. 4, 5, 6), the vacuolization, hypertrophy, surface blebbing and deposition of black granules in the cytoplasm below the plasma membrane of most of the haemocytes were noticed. Nuclear pyknosis and fragmentation in nuclear material was observed in granulocytes plasmatocytes and spindle cells. In some cells, cone-like projections were also observed. After 20 days of exposure (Plate 2-, Figs. 7, 8, 9), most of the cells were observed with the pseudopodia projections and blebbing of the plasma membrane. Cells were deformed and stuck together. Nuclear fragmentation and karyorrhexis were observed mostly in granulocytes and plasmatocytes. Plasmatocytes and

spindle cells showed cellular deformity due to more pronounced pseudopodial outgrowth. After 30 days of exposure (Plate 2-, Figs. 10, 11, 12), breaking of the outer covering of most of the cells was observed. Deformed cells formed aggregates. Nuclear pyknosis, karyorrhexis, achromatia was observed in prohaemocytes, granular haemocytes and spindle cells. About 72 to 75% of haemocytes were found deformed and broken.

4. DISCUSSION

The haemolymph of arthropods contains mesodermal origin haemocytes, basophilic and circulating in blood channels to perform various functions like transport of food phagocytosis capture of foreign particles, defense and coagulation of haemolymph [34, 35, 36]. Generally, blood is the main transporter between the internal and external environment and serves as physiological reflector of organelles [37]. For assessment of health status, investigation of disease and physiological disorders, haematological parameters can be used as a diagnostic tool. Lead-induced marked alterations in the haemocyte morphology of freshwater prawn, *M. dayanum* in the present study were surface blebbing, vacuolization, cytoplasmic darkening, fragmentation of nuclear material cone formation, and nuclear nuclear pycnosis, breaking of plasma membrane after acute exposure (Plate 1: Figs. 4, 5, 6 (24hr); 7, 8, 9 (48hr); 10, 11, 12 (72hr) and Figs. 13, 14, 15 (96hr), while black granular depositions, nuclear pycnosis and fragmentation of nuclear material, pseudopodial projection, blackening of the plasma membrane, achromatia were observed after sub-acute exposure (Plate 2: Figs. 4, 5, 6 (10 days); 7, 8, 9 (20 days) and 10, 11, 12 (30 days). These changes were pronounced in prohaemocytes, granulocytes, plasmatocytes and spindle cells. All these changes in cell morphology are due to the involvement of granular haemocytes in detoxification of metals. Almost similar changes were observed in various fishes after lead and other heavy metal exposure [7,8,38,39,40,41,42]. and in invertebrate like gastropod [43,44]. Metallic compounds like copper, lead and cadmium. have also been reported to affect circulatory haemocyte number and morphology in crustaceans like *Palaemon elegans*, *M. lamarrei* and *M. dayanum* [45, 16, 45, 46, 24].

It is well known that metal affects the cell permeability as a replacement of calcium in the phospholipids group of the plasma membrane [45] and oxidation of biological cell membrane, thereby interfering with simple and facilitated diffusion [46,47,47]. As observed in the present study, Haemocyte damage may be through direct toxic effect of metals inducing lysis or degranulation or through generalised stress response to physiological disturbance [48,49,50]. Changes observed in the nucleus of haemocytes after exposure to lead are in accordance with the findings of earlier workers on metals which are suggestive of disturbed RNA synthesis, and ribosomal abnormalities and may be the possible reasons for the changed nuclear-cytoplasmic ratio [51, 52, 53,25].

In the present study, surface structure of haemocytes of freshwater prawn, *M. dayanum* was found to be sensitive to the environmental fluctuations and contaminants, particularly the lead. Haematological parameters, can serve as a tool to assess the health status of economically important freshwater prawns as well as assess the worsening status of aquatic bodies mainly in relation to heavy metals.

5. CONCLUSION

The present study indicated that lead nitrate, acute (116.46 mg/l, 96h LC_{50}) and subacute (29.12 mg/l, 25% of 96h LC_{50}) were highly toxic to freshwater prawn, *M. dayanum*, characterised by marked alterations in the haemocyte morphology and behaviour of exposed animals. Haemocyte morphology can serve as a good biomarker of metallic pollution without sacrificing the animals. Thus, *M. dayanum* can serve as a better bioindicator of environmental pollution.

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COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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