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Haematological studies of *Anabas testudineus* Bloch 1792 on exposure to aquatic toxicants of Buckingham canal, Chennai, Tamil Nadu, India

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Abstract
Haematological parameters have been used to describe the health of a fish, monitor stress response and predict systematic relationship and physiological adaptations. They aptly reflect the poor condition of fish than other commonly measured parameters and are increasingly used as indicators of physiological stress response to endogenous or exogenous changes in fish. Therefore, in the present work, the haematological effects caused to *Anabas testudineus* on exposure to aquatic toxicants present in the Buckingham canal, Chennai, Tamil Nadu, India were studied. The results clearly indicated that the blood parameters, *viz*., The values of blood parameters, *viz*., red blood corpuscles, haemoglobin, haematocrit, erythrocyte sedimentation rate, mean corpuscular value, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood corpuscles, basophils, neutrophils, lymphocytes, monocytes and platelets values of *Anabas testudineus* from Buckingham canal had changed to 57.60, 21.10, 7.66, 68.42, 31.50, 23.16, 12.47, 144.26, 23.1, 5.71, 47.15, 32.30 and 50.00% respectively over control during the present study.

Keywords: *Anabas testudineus*, haematological parameters

1. Introduction
Aquatic organisms may take up pollutants from sediments, water, suspended particle and food items. Non-lethal exposure of fishes to pollutant results in various biochemical consequences. Effect on enzymes, level of steroids and haematological properties has received particular attention. Effects of sub-lethal level of pollutants also include changes in tissue architecture and various physiological processes, including sensory responses, ciliary and motor activity, metabolism, osmotic and ionic regulation, endocrine function, growth, respiration, reproduction and development, histological, neuromuscular and genotoxic parameters [1]. Haematological parameters have been used to describe the health of a fish [2], monitor stress response and predict systematic relationship and physiological adaptations. They more quickly reflect the poor condition of fish than other commonly measured parameters [3]. Haematological parameters are increasingly used as indicators of physiological stress response to endogenous or exogenous changes in fish. Haematological changes in some fishes exposed to various toxicants have been studied in *Tilapia mossambicus* [4], *Ctenopharyngodon idella* [5], *Oncorhynchus mykiss* [6] and *Salmo trutta fario* [7]. Therefore, in the present study, the haematological effects caused to *Anabas testudineus* on exposure to aquatic toxicants present in the Buckingham canal, Chennai, Tamil Nadu, India were investigated.

2. Materials and methods
2.1 Study area
Chennai situated on the eastern coast of India, on 13 0 4' north latitude and 8 0 15' east longitude has three water ways that flows through the city, *viz*., Cooum river, Adyar river and Buckingham canal (Figure 1). The Buckingham canal is a man-made water canal linking the above mentioned two rivers. The canal extends from Nellore in Andhra Pradesh to Marakkanam near Puducherry.

The length of this canal in Andhra Pradesh is 257km, and 163km is in Tamil Nadu. Within the city of Chennai, the canal is badly polluted from sewage and industrial effluents, and the silting up of the canal has left the water stagnant, creating an attractive habitat for mosquitoes.

2.2 Test organism

Taxonomic position:

Kingdom	:	Animalia
Phylum	:	Chordata
Sub Phylum	:	Vertebrata
Class	:	Pisces
Order	:	Anabantiformes
Family	:	Anabantidae
Genus	:	*Anabas*
Species	:	*testudineus*

Anabas testudineus commonly called climbing perch is an extremely hardy, small, brown or dark greenish-brown fish, native to Southeast Asia. It is highly adapted to life in a seasonal tropical environment. It inhabits the majority of drainage systems across its native range and has been recorded in many different habitat-types including swamps, marshes, lakes, canals, pools, small pits, rice paddies, puddles, tributaries and main river channels. It can tolerate very turbid and brackish water conditions and under extreme circumstances it can aestivate for several weeks by burying itself into moist ground [8].

2.3 Haematological assay

Blood sample of 1.5 to 2.0mL was collected from the experimental and control fish by piercing the ventrolateral side in the caudal region with a 2.0mL syringe. Heparinised syringe was used to collect blood samples for estimation of Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC) and haemoglobin percentage (Hb%). Subsequent sub-samples from these blood samples were used to analyze the required parameters. The TEC and TLC were studied by light microscopy (Olympus BX 51) following the procedure described by Hendricks [9] and Shaw [10]. Haemoglobin concentrations were estimated using QDx kit (Nicholas Piramal India Ltd, Mumbai) and FL kit (GL 150F CH-Chema Diagnostica, Italy), and absorbance measured using a UV1 spectrophotometer of Thermo-spectronic model at optical densities of 546 and 510nm.

3. Results

The values of blood parameters, *viz*., Red Blood Corpuscles (RBC), Haemoglobin (Hb), Haematocrit (Ht), Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Value (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Corpuscles (WBC), basophils (B), neutrophils (N), lymphocytes (L), monocytes (M) and platelets (P) in the experimental and control fish *Anabas testudineus* are presented in Table 1 and Figure 2. MCV, MCH, neutrophils and lymphocytes of the experimental fish were lower than those in the control. Hb, Ht, ESR, RBC, WBC, platelets, MCHC, basophils and monocytes values in the affected fish was found to significantly increase when compared to the control fish. But reverse was the case with MCV, MCH, neutrophils and lymphocytes. Significance differences between the haematological parameters of the control and the affected fish was observed in Hb, Ht and RBC. The haematological results clearly indicated that the blood parameters, *viz*., RBC, Hb, Ht, ESR, MCV, MCH, MCHC, WBC, B, N, L, M and P values of *Anabas testudineus* from Buckingham canal had changed to 57.60, 21.10, 7.66, 68.42, 31.50, 23.16, 12.47, 144.26, 23.1, 5.71, 47.15, 32.30 and 50.00% over control during the present study.

4. Discussion

The physiological and chemical properties of blood are very sensitive to environmental changes [11]. Toxicologists paid much attention to the impact of water pollution on blood chemistry of several fishes [12-17]. Atamanalp *et al*. [7] reported increased RBC, Ht, Hb and thrombocyte values with decreased parameters of MCV, MCH, MCHC and WBC in the treated fish, *Salmo trutta fario* than the control. Reduction of TLC and Hb and increase in TLC and plasma glucose are usually the immediate responses of an organism confronting stressed condition [18-20]. Nanda *et al.* [21] reported significant reduction in TEC and Hb in all the sub-lethal concentrations of rotenone exposed *Heteropneustes fossilis* at twelve hours, which indicated that entry of rotenone into the circulation which adversely affected the erythrocytes, reducing their population and the effect was dependent on the toxicant concentration. Nanda *et al*. [21] further stated that the reduction in Hb% was a usual response in organism confronting a stressed environment, but the body triggers the haemopoiesis process in the kidney in response to the stress to compensate the Hb loss. This probably explains the initial increase in the Hb% where stress levels are relatively low. In contrast, higher stress levels might have caused an immediate suppression of the haemopoietic activity in kidney due to damage of kidney tissue followed by late stimulation of the process [22]. Reduction of Hb may be attributed to the cumulative rotenone stress due to prolonged exposure which might have created hypoxic condition [23] and kidney tissue damage followed by exhaustion of its haemopoetic potential resulting in reduced Hb synthesis [18, 20, 24]. An increase in TEC generally occurs as an immunological response in an organism confronting a stressed condition [18-20]. Such stimulation of the protective response was also observed in the study of Nanda *et al*. [21] with rotenone exposure in *Heteropneustes fossilis.*

Aquatic pollutant exposure of *Anabas testudineus* resulted in disturbances of its haematology with elevation of Ht, Hb and RBC count. Larsson *et al*. [12] and Nair *et al*. [25] while studying the haematology of flounders exposed to titanium dioxide effluent, noted increased values for Ht, Hb and number of erythroblasts in effluent treated fishes. Increase in these parameters was noted in different fish exposed to low pH [26, 27]. It has been suggested that these haematological disturbances are a haematopoietic or erythropoietic mobilization response to hypoxemia induced by acid stress [28]. The fish exposed to aquatic pollutants in the present investigation showed leukocytosis and a decreased percentage of lymphocytes (lymphopenia). Occurrence of lymphopenia was reported in flounders exposed to titanium dioxide effluent [12, 25] and in mammals subjected to severe stress condition [29]. In teleosts, stress from exposure to industrial effluent caused a decrease in the number of circulating lymphocytes [30]. According to Barton [31] stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic state. If severe or long lasting, the response then becomes maladaptive and threatens the fish health and wellbeing. Therefore, in the presence of stressors (contaminants/pollutants), blood parameters and blood chemistry can be employed as standard

biomarker (bioindicator) to determine diseased conditions and metabolic disturbances in fish [32]. Haematological parameters are very sensitive to stress. Decrease in the value of Hb, Ht, RBC and MCV with increased concentration of the aquatic pollutants to which the fish was exposed was studied on *Clarias gariepinus* and *Oreochromis niloticus* exposed to crude oil [33, 34]. The decrease in the value of these parameters could be attributed to the haemolysis resulting in the haemodilution, a mechanism for diluting the concentration of pollutants in the circulatory system [35]. Erythropenia recorded in the exposed fish may also be accounted for the swelling of the erythrocytes, damage of haematopoietic tissue in the kidneys and aggregation of cells at the gills thereby causing a decrease in the number of the circulating cells in the stressed fish [36].

Exposure to various stressors elicit changes in WBC [37]. Leucopenia and/or leucocytosis are normal reactions to stressors or irritants like aquatic pollutants. Leukopenia has been reported in *Oreochromis mossambicus* exposed to 150 and 300ppm kerosene [38]; and *Clarias gariepinus* to copper [39]; and kerosene [34]. Changes in the value of WBC in the present study may be due to the level and duration of toxicant exposure. The different level of the toxicant may have exerted varying degree of stress on the defense mechanism of the exposed fish and hence the production of different amount of WBC [40]. Increase in TLC of *Anabas testudineus* in the present study has been attributed to several factors, *viz*., increase in thrombocytes and lymphocytes or squeezing of leucocytes in peripheral blood [41]. Leucocytes are involved in the regulation of immunological function of body [18]. An increase in TLCs thus occurs as a protective response to stress as observed in the present study [42]. Pollutants and other stressors as recorded in the present study and several other studies causes a change in the subpopulations of leucocytes [40, 43]. *Oreochromis mossambicus* exposed to kerosene suffered with neutrophilia [38]. RBC, thrombocytes and neutrophils of *Channa punctatus* were reported to be the most sensitive to starvation [44] and heavy metal (lead) poisoning [45]. The present study also recorded a significant neutrophilia and lymphocytosis in *Anabas testudineus* and erythropenia associated with hypochromasia, increased ESR, MCHC and large lymphocytes. Leucocytes, thrombocytosis and hypercoagulability of blood was observed in *Heteropneustes fossilis* exposed to mercury and zinc [46] and in *Oreochromis mossambicus* to kerosene [38]. Similar changes were recorded in *Anabas testudineus* in the present study on exposure to aquatic pollutants of Buckingham canal. Further, the decrease in the number of neutrophils might be due to the pathogenic action of the aquatic toxicants which inhibit the formation of neutrophils. The increase in basophil and monocyte percentages in *Anabas testudineus* in the present investigation represented the activity of the first and second lines of defense against the cellular damage after pollutant exposure and similar results has been reported previously due to mercury exposure [47]. According to Wedemeyer *et al*. [48] the suppression of the immune system increase the susceptibility to diseases in fish, a significant aspect considering the presence of heavy metal in natural ecosystems as a result of human activities.

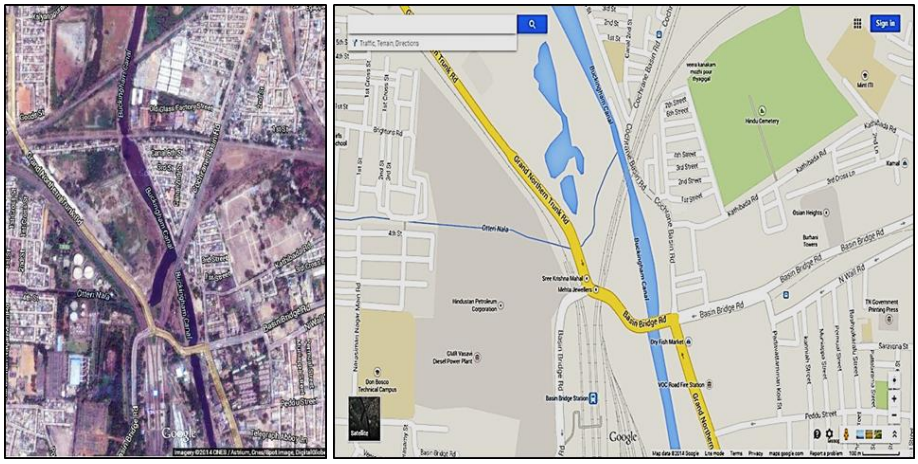

Fig 1: Study area – Buckingham canal

Table 1: Effect of aquatic toxicants on the blood parameters of *Anabas testudineus*

Parameters	Control fish	Experimental fish
RBC (cell mm 10)	2.83 ±0.14	4.46 ±0.18 (57.60%)**
Hb (g/100mL)	14.36 ±0.04	17.39 ±0.37 (21.10%)*
Ht (%)	42.85 ±0.16	46.13 ±0.23 (7.66%)*
ESR (mm/h)	1.14 ±0.11	1.92 ±0.08 (68.42%)**
MCV (cu µ)	151.54 ±0.32	103.80 ±0.06 (31.50%)*
MCH (µ g)	50.74 ±0.20	38.99 ±0.05 (23.16%)*
MCHC (%)	33.51 ±0.17	37.69 ± 0.06 (12.47%)*
WBC (cell mm 10)	11.50 ±0.28	28.09 ±0.42 (144.26%)**
Basophils (%)	23.08 ±0.09	31.50 ±0.26 (23.1%)*
Neutrophils (%)	32.23 ±0.16	30.39 ±0.21 (5.71%)*
Lymphocytes (%)	38.05 ±0.28	20.11 ±0.26 (47.15%)*
Monocytes (%)	0.0 ±0.0	3.23 ±0.15 (32.30%)*
Platelets ($x10^4/mm^3$)	8.00 ±0.23	12.00 ±0.27 (50.00%)*

Values are mean ±standard error of five individual observations; Values in parenthesis denotes per cent change; *and** denotes < 0.05 and < 0.001 level of statistical significance respectively

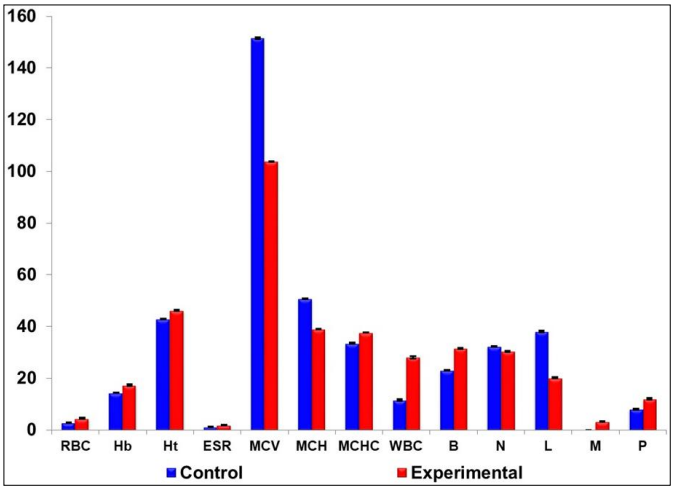

Fig 2: Effect of aquatic toxicants on the blood parameters of *Anabas testudineus*

5. Conclusion

The present study showed that under experimental conditions, the blood parameters were sensitive to different aspects of heavy metals/aquatic pollutants exposure. In conclusion, the findings of the present study is in confirmatory with work of various researchers and indicate that the sub-acute concentrations of heavy metals, may cause several changes in the haematological parameters of treated fish, therefore, estimation of these indices, could provide a useful indicator regarding the pollution of ecosystem. In addition, the results of the present investigation revealed that heavy metals cause immunological impairments in *Anabas testudineus*, which suggests that the heavy metal may weaken the immune system and may result in severe physiological problems, ultimately leading to the death of fish.

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