



Short Communication

Haematological alteration in fish *Heteropneustes fossilis* (Bloch) on exposure to fly ash

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Abstract

Study on the changes in haematological parameter of air breathing fish *Heteropneustes fossilis* was done on exposed to different concentration of fly ash. After estimating the LC₅₀ of fly ash, fishes were exposed to different concentration of fly ash (200g/l, 400g/l and 600 g/l) for a period of one week. The haematological parameters like haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV) and total leucocyte count (TLC) were estimated. The absolute values like mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were calculated from the above parameters. There was significant alteration in all the haematological parameters on exposure to fly ash. Erythrocyte indices showed decrease from the control value whereas there was significant increase in total leucocyte count indicating stressful condition.

Keywords: Fly ash, Haematology, Fish.

Introduction

Thermal power station is the major consumer of coal and produces fly ash due to combustion. Fly ash produced enters to the ecosystem and affects the flora and fauna¹. Fly ash is known to contain many heavy metals which inflict stress on aquatic organism^{2,3}. Fish being the important part of trophic level in aquatic ecosystem, pollution due to fly ash brings changes in their physiology. Fly ash produced from Hindalco thermal power plant located near Hirakud reservoir contains heavy metals like Mn, Co, Pb, Zn, Cd, Ni etc⁴. Heavy metals are the major pollutants of aquatic body and gets accumulated in fish through food chain⁵. Stress on fishes is reflected in the fish haematology as changes in the parameters like Hb, TEC, PCV and TLC⁶. As the fly ash from power plant reaches to the aquatic ecosystem both lotic and lentic, this study was carried to study the effects of fly ash on the haematology of fish *Heteropneustes fossilis* (Bloch).

Materials and methods

Live and healthy fresh water fish *Heteropneustes fossilis* varying length 14-16cms and weighing 30-40gms were collected from the pond and acclimatized in laboratory condition for a period of one week. Care was taken to avoid stress and injury during capture and transportation. During the acclimation period fishes were fed with commercial fish food twice a day. Before the start of the experiment injured or inactive fishes were removed from the aquarium. After the determination of L.C.₅₀ value⁴, three aquariums with 80litre

capacities was set with different concentration fly ash (200g/l, 400g/l, 600g/l) collected from the Hindalco Power Plant located near Hirakud reservoir. One aquaria was used with normal water as control. Ten number of fish irrespective sex was released to the experiment aquaria for a period of 7 days. At the end of exposure period blood sample was collected for investigation. EDTA was used as anticoagulant. Haematological parameters like Hb, TEC, PCV, and TLC were estimated as per the standard methods⁷. Absolute value like MCH, MCHC and MCV were calculated from the above parameter⁸.

Results and discussion

After exposure to different concentration of fly ash for a period of 7 days, blood sample was collected from all the fishes and was analysed for the study of Haemoglobin Concentration (Hb), Total Erythrocyte Count (TEC), Packed Cell Volume (PCV) and Total Leucocyte Count (TLC). The absolute values like Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Volume (MCV) were done by calculating from the above value. All the datas are presented in Table-1.

The Total Haemoglobin Concentration (Hb) was found to be 12.8±0.46g/l for the control group. When exposed to different concentration of fly ash (200g/l, 400g/l, and 600g/l) there has been a significant decline in the haemoglobin level in all the three groups of fishes from the control. In 200g/l concentration of exposed fish, the haemoglobin content was found to be 10.2

$\pm 0.34\text{g/l}$, whereas it was $9.2\pm 0.39\text{g/l}$ in 400g/l and $8.5\pm 0.25\text{g/l}$ in 600g/l fly ash concentrations (Table-1).

The Total Erythrocyte Count (TEC) was found to be 3.65 ± 0.13 ($\times 10^6.\text{mm}^{-3}$) for the control group. When exposed to different concentration of fly ash (200g/l , 400g/l , and 600g/l) there has been significant decline in the TEC level in all the three groups from the control. In 200g/l concentration of fly ash exposed fish, the TEC was found to be 2.45 ± 0.11 ($\times 10^6.\text{mm}^{-3}$), whereas it was 1.95 ± 0.10 ($\times 10^6.\text{mm}^{-3}$) in 400g/l and 1.9 ± 0.12 ($\times 10^6.\text{mm}^{-3}$) in 600g/l fly ash concentrations (Table-1). The decline in TEC level is in correlation with Hb concentration.

The Packed Cell Volume (PCV) was found to be 48 ± 0.94 (%). When exposed to different concentration (200g/l , 400g/l , and 600g/l) there has been significant decline in the PCV level in all the three groups from the control. In 200g/l concentration of exposed fish, the PCV was found to be 38.7 ± 1.57 (%), where as it was 44.2 ± 0.82 (%) in 400g/l and 36.4 ± 0.94 (%) in 600g/l fly ash concentrations (Table-1). The decline in PCV is in correlation with Hb and TEC level.

The Mean Cell Haemoglobin (MCH) is an absolute index calculated from the results of Hb and TEC. The MCH was found to be 35.06 ± 0.74 (pg) in fish *Heteropneustes fossilis*. There has been an increase in the MCH of fish exposed to fly ash. In 200g/dl concentration of exposed fish, the MCH was found to be 41.63 ± 0.64 (pg), whereas it was 47.1 ± 1.0 (pg) in 400g/l and 44.7 ± 1.25 (pg) in 600g/l fly ash concentrations. The increase in MCH from the control is significant ($F=309.79$).

The Mean Corpuscular Haemoglobin Concentration (MCHC) in control fish was calculated to be 26.66 ± 0.61 (%). However, in lower concentration of fly ash (200g/l), no significant changes occurs. But in other two concentrations (400g/l and 600g/l) there has been a significant decline in MCHC of fish from the control value. The concentration of MCHC was recorded as 26.35 ± 0.55 (%), 20.9 ± 0.63 (%), 23.61 ± 1.22 (%) in 200g/l , 400g/l , and 600g/l concentration of fly ash exposed fish respectively. The Mean Cell Volume (MCV) in control fish was calculated to be 131.5 ± 3.17 (μ^3). There has been significant increase in MCV of fish after exposed to fly ash. In fly ash treated fish (200g/l , 400g/l and 600g/l) there was significant increase in MCV levels. The concentration of MCV was recorded as 157.95 ± 3.17 (μ^3), 225.64 ± 3.40 (μ^3), 189.47 ± 4.38 (μ^3) in 200g/l , 400g/l , and 600g/l concentration of fly ash exposed fish respectively.

However, the Total Leucocyte Count (TLC) shows a different trend in fish exposed to fly ash. The total number of TLC per cubic millilitre of blood of fish *Heteropneustes fossilis* was found to be 9.6 ± 0.52 ($\times 10^3.\text{mm}^{-3}$). On exposure to fly ash for a period of one week, there was significant rise in TLC number in the treated fish of all the three concentration of fly ash. The number of TLC was recorded as 22.5 ± 0.85 ($\times 10^3.\text{mm}^{-3}$), 26.0 ± 0.67 ($\times 10^3.\text{mm}^{-3}$), 25.6 ± 0.84 ($\times 10^3.\text{mm}^{-3}$) in 200g/l , 400g/l , and 600g/l concentration of fly ash exposed fish respectively.

After exposure to different concentration of fly ash there has been significant alteration of haematological indices like haemoglobin concentration (Hb), total erythrocyte count (TEC), packed cell volume (PCV) and total leucocyte (TLC). The absolute values like mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume were also in response to above value. On seven days exposure to fly ash, the Hb, PCV and TEC decreased significantly indicating anaemia. Fly ash is known to for its oxidative stress in fish *Channa punctatus*⁹. Fly ash contains heavy metals like Mn, Co, Pb, Zn, Cd, Ni etc⁴. Heavy metals are known to effect fish haematology either directly or synergistically^{6,10}. This may be due to disturbances in haematopetic tissue¹¹. Causes of anaemia may be due to lack of iron uptake by the fish during the exposure period¹². There was significant decrease in erythrocyte, haematocrit and haemoglobin of fish *Pleuronectes flesus* after Cd exposure¹³. There was also decrease in above erythrocyte indices like changes of nucleus and shape of RBC fish *Garragotyla* on exposure to MnSO_4 ¹⁴. Pb is also a heavy metal when available in aquatic environment effects the fish haematology¹⁵. It inhibits Na^+/K^+ ATPase activity there by affecting the haeme synthesis in erythrocytes¹⁶. The decrease in above parameters may be due to increased haemolysis. This may also be attributed to decrease in absorption of iron leading to less haemoglobin synthesis¹⁷. Manganese is present in higher concentration in the fly ash of the Hindalco thermal plant⁴ and there is also evidence that it affects the physiology of fish like *Garragotyla*¹⁸. The mean cell haemoglobin (MCH) is an absolute index calculated from the results of Hb and TEC. That is why it is dependent upon the above two parameters. There has been an increase of MCH of fish exposed to fly ash. The increase in MCH and decline in MCHC from the control is a clear indication of macrocytic and haemolytic anaemia. It has been reported on many occasion that due to heavy metals toxicity MCH and MCHC level alters significantly^{6,8}. The mean cell volume was increased significantly both in short term as well as long term exposed fish. The increase in MCV of fish is an indication of pernicious anaemia which may be due to deficiency in vitamins responsible for erythropoiesis. Hence it is case of macrocytic, haemolytic and pernicious anaemia in fish *Heteropneustes fossilis* due to fly ash toxicity.

However the total leucocyte count shows a different trend in fish exposed to fly ash. The total number of TLC per cubic millilitre of blood of fish *Heteropneustes fossilis* was significantly increased in the treated fish of all the three concentration of fly ash in both short term and long term exposure. This increase in TLC indicates stressful condition of fish. This reflects a physiologically disturbed immune system. This is an immune response to the fly ash toxicity. Heavy metals are known to induce the malfunctioning of immune system thus prone to mortality risk¹⁹. Similar results was also obtained by various worker while working on effects of pollutant on fishes²⁰⁻²². This results also agrees with all other workers and indicate that fly ash does have an effect on fish haematology²⁴.

Table-1: Alteration in the Haematological parameters of fish *Heteropneustes fossilis* on exposure to Fly ash.

Parameter	Control	200 g/l	400 g/l	600 g/l	F value	Significance
Hb (g/dl)	12.8± 0.46	10.2±0.34	9.2±0.39	8.5±0.25	259.7	1.957E-24
TEC (x10 ⁶ .mm ⁻³)	3.65±0.13	2.45±0.11	1.95±0.10	1.9±0.72	603.6	7.993E-31
PCV (%)	48.0±0.94	38.7±1.57	44.2±0.82	36.4±0.94	221.71	2.933E-23
MCH (pg)	35.06±0.74	41.63±0.64	47.1±1.0	44.7±1.25	309.79	9.33E-26
MCHC (%)	26.66±0.61	26.35±0.55	20.9±0.63	23.61±1.22	116.62	1.34E-18
MCV (μ ³)	131.5±3.17	157.95±3.17	225.64±3.40	189.47±4.38	1299.6	9.817E-37
TLC (x10 ³ .mm ⁻³)	9.6±0.52	22.5±0.85	26.0±0.67	25.6±0.84	1108.9	1.659E-35

Conclusion

Study of haematology indicates the physiological condition of fish on a given environment. In this piece of work it was found that there was a decline in the erythrocyte indices like total erythrocyte count, haemoglobin and haematocrit due to fly ash stress. However the immune system trying to cope with the stressful condition which is reflected in the increase of total leucocyte count. Further research is required to know the safe value of fly ash in the environment to the fish.

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