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Studies on Some Hematologic Values of *Oreochromis mossambicus* (Peters) Following its Sudden Transfer to Various Concentrations of Potassium Chlorate and Potassium Dichromate

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Abstract: Occurring huge number of fireworks, match factories, printing and large number of cottage industries are in Sivakasi (Lat 9° 27' N, Long 77° 49' E). The number of match factories is higher than other factories and they were expelling varies chemical compounds such as Sulphur, potassium chlorate, ferric oxide and potassium dichromate, among them potassium chlorate and potassium dichromate are used in greater quantities then directly mixed with near bond water hence affect aquatic fishes were affected y these chemical compounds. So there is no provision for the treatment of effluents from the match and firework industries. The various chemicals are discharged into the neighboring drains. During the showers these effluents are washed into water bodies posing problems to aquatic animals such as *Oreochromis mossambicus* (Peters). The present study is an attempt towards the analysis of the effects of these chemicals on the blood parameters of a fish.

Key words: Sivakasi % Oreochromis mossambicus % Sulphur % Potassium Chlorate % Ferric Oxide and Potassium Dichromate

INTRODUCTION

Pollution is undesirable change in physical, chemical or biological characteristics of our land; air and water that may or will harmfully affect human life or that of desirable species [1]. The air, water and land are the three basic amenities for living beings. But the heavy population explosion together with rapid industrialization for betterment of living standards and the subsequent urbanization have led to the contamination of these natural resources with undesirable and harmful substances.

Such water when contaminated with industrial wastes or other harmful to the human life [2]. Water ways are subject to entry of pollutants by direct discharge into the system, run off and/or sea page with subsequent transport, river flow transport, reaction and transport across the air water interface and reactions and transport across the water sediment interphase [3].

Now focus of concern especially in India is pollution by industrial wastes. Enormous quantities of minerals and chemical wastes degrade the quality of receiving water by imparting tastes, odour and colour. The long range effects of these minerals in water are so complicated that they have yet to be fully comprehended. The visible short range effects, however, suggest the severeness of this problem of water pollution. Fish kills are common in contaminated water and lakes are chocked with aquatic plants [3-6].

By the sewage and industrial wastes, the river and sea have been greatly contaminated. The organisms which are living in river and sea are mostly affected in such a way, fish standing first. Fish have been (mostly all) valued for many years as excellent indicators of water quality. It is an ideal biological system and very sensitive to the fluctuation of chemical parameters occurring in the aquatic environment *Oreochromis mossambicus* (Peters) offers interesting possibilities for research due to its prolific breeding habits and easy acclimation and acclimatization to the changing condition of the environment [7].

In recent years hematological studies are increasingly used to determine the systematic relationship to elucidate the physiological adaptation, to assess the health of the fish and to estimate the water pollution [8-10].

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The RBCs or Erythrocytes is one of the constituents of blood. The erythrocyte is a biconcave disc. Normally, it is opaque, because it is densely packed with hemoglobin. These erythrocytes are mainly produced by the bone marrow. The age, diurnal variation, exercise and emotion are some of the factors which regulate the total erythrocytes count (TEC). The main function of erythrocytes is transporting oxygen from respiratory organ to tissue and carbon dioxide from tissue to respiratory organ. They also maintain the blood pH and the viscosity of blood [11].

Effect of industrial effluents on the blood values of fish have been analyzed [12, 13]. Many reports on the effect of industrial effluents on the hematology of fish have been made. However, literature on the chemicals used in match factory on hematological values of fish is rare. The present investigation, therefore, is an attempt towards the study of hematologic values and related parameters of *Oreochromis mossambicus* (Peters) on sudden transfer to varying concentrations of Potassium chlorate separately and Potassium chlorate and Potassium dichromate combined.

Sivakasi is one of the industrial towns present in Virudhunagar District of Tamil Nadu. A number of factories, such as, match factories, fireworks, printing and large number of cottage is higher than other factories (~ 400). The chemicals used in match factories are potassium chlorate, sulphur, potassium dichromate, ferric oxide, red manganese, black manganese, phosphorus, potassium dichromate etc. In these chemicals potassium chlorate and potassium dichromate are used in greater quantities.

There is no provision for the treatment of effluents from the match and firework industries. The various chemicals are discharged into the neighboring drains. During the showers these effluents are washed into bodies of water posing problems to aquatic animals, such as, fish. The present study is an attempt towards the analysis of the effects of these chemicals on the blood parameters of a fish.

MATERIALS AND METHODS

Fish were collected from the local pond and fully acclimatized to laboratory conditions for two weeks in aquaria and were fed with groundnut cake daily. After two weeks the fish were divided into 13 groups with one control and exposed to various concentrations of potassium chlorate. Out of these experimental fish first eight groups were kept in varying concentrations such as 25, 50, 75, 100, 125, 150, 175 and 200 ppm. The other four groups of fish were kept in different concentrations of potassium chlorate and potassium dichromate, such as, 50, 100, 150 and 200 ppm for the study of synergistic effect.

The control fish were maintained in tap water all other conditions being exactly identical to those of experimental fish. Four fish with an average weight of 17.5 g were transferred to each of these aquaria. Observations were made after 24 hour of transferring from normal tap water to experimental water. The experiments were conducted to find out the effect of potassium chlorate and synergistic effect of potassium chlorate and potassium dichromate on the hematological values of the fish.

Collecting the Blood: Blood samples were collected from the gill region of the fish. The blood was collected into a micropipette rinsed with potassium oxalate solution. Great care was taken to avoid foaming when drawing the blood into micropipette as this readily resulted in hemolysis. The blood was carefully transferred to Neubaeur's counting chamber and hemocytometer tube for analysis.

RBCs Counting: The total erythrocyte count was made with the Neubauer's counting chamber. The blood was drawn up to 0.5 marks in the RBCs pipette and diluted to 101 marks with Hayem's RBCs diluting fluid. It was mixed thoroughly by rotating the pipette keeping it in horizontal position. The first few drops were discarded and the diluted blood sample was introduced into the counting chamber. Counting was done in all the 25 squares. Calculations were made taking into consideration the dilution factor and total erythrocytes count (TEC) exposed as $X \times 10^6/mm^3$.

WBCs Counting: The total leucocytes count was made with Neubauer's counting chamber. The blood was drawn up to 0.5 marks in the WBCs pipette and diluted to 11.0 marks with Turch's fluid. The principle of counting of leucocytes is the same as that of erythrocytes, but the counting is done in the outer four squares forming leucocytes area. Calculation were made taking into consideration the dilution factor and total leucocytes count (TLC) expressed as x/mm^3.

The Hemoglobin Content (Hb): The Hemoglobin content was estimated by Sahli's method using hemometer with permanent colored glass comparison standard. Hydrochloric acid N/10 was taken in the hemomter tube to the level of lowest graduation (0.02 g). First few drops of blood were wiped out and then little blood was sucked up to 0.02 ml mark of the capillary pipette. The blood in the capillary pipette was transferred to the hydrochloric acid already present in the hemometer tube and mixed thoroughly by repeated sucking and blowing. The hemometer tube was placed in the stand so that the scale was turned to the side and could not be seen. The acid haematin solution was diluted with distilled water until the color of the solution matched with that of the comparison standard glass. The results were read exactly 3 minutes after the blood had been added to hydrochloric acid and expressed as g/100 ml.

Mean Corpuscular Haemoglobin: Mean corpuscular hemoglobin of the blood sample was determined by dividing the hemoglobin content expressed in g/100 ml of the blood by the number of RBCs expressed in $10^6/mm^3$ [14]. The MCH value is expressed in Picograms (pg).

$$MCH\ (pg) = \frac{Hb \times 10}{RBCs \times 10^6/mm^3}$$

To Find out the Dissolved O_2: The oxygen content of both control and experimental samples was determined by Winkler's method [15].

To Find out Ph of the Samples: The pH meter was used to find out pH of the sample.

RESULTS

Fishes were active and there was no sign of pathological symptom to suggest any overt toxicity due to chemicals. The increase in the concentration of potassium chlorate in the medium was found to affect dissolved oxygen content and pH of water (Table 1) without fish. There was a linear increase in the oxygen concentration of water with increasing concentration of chemical.

Table 1: Effect of potassium chlorate on O_2 content and pH of medium

Medium (ppm)	O_2 content	pH
Control	3.1074	7.1
25	4.8024	7.3
50	5.9323	7.5
75	6.4973	7.7
100	7.0624	7.8
125	8.1923	7.9
150	8.7573	8
175	9.3223	8.2
200	10.1638	8.7

The effect of potassium chlorate and potassium dichromate on erythrocytes and leucocytes of *Oreochromis mossambicus*

Fig. 1: *Oreochromis mossambicus*

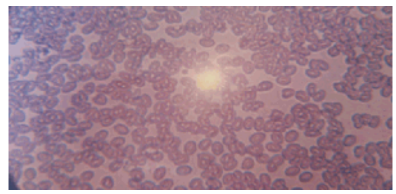

Fig. 2: KCLO3 BLD SMR Control

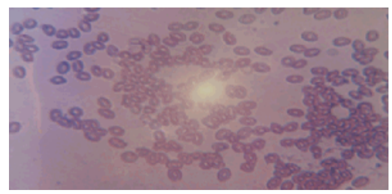

Fig. 3: KCLO3 BLD SMR 25 PPM

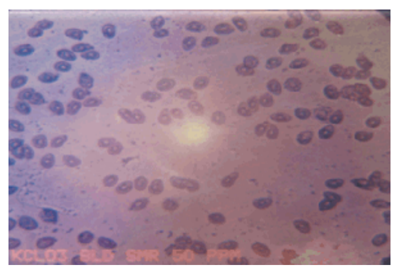

Fig. 4: KCLO3 BLD SMR 50 PPM

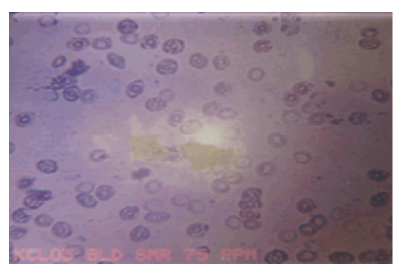

Fig. 5: KCLO3 BLD SMR 75 PPM

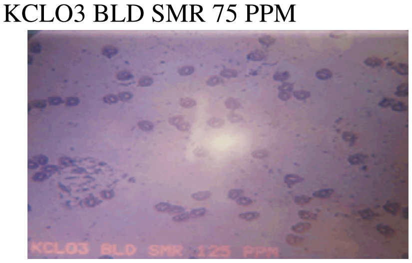

Fig. 6: KCLO3 BLD SMR 125 PPM

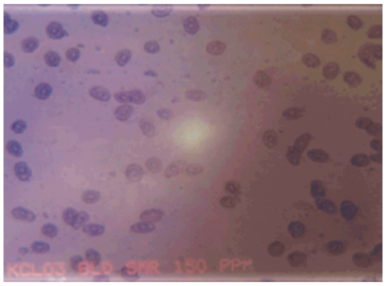

Fig. 7: KCLO3 BLD SMR 150 PPM

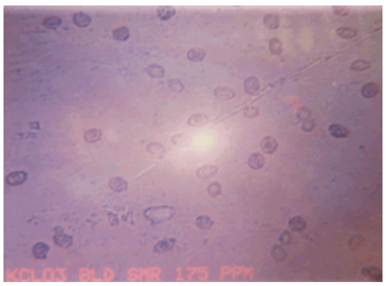

Fig. 8: KCLO3 BLD SMR 175 PPM

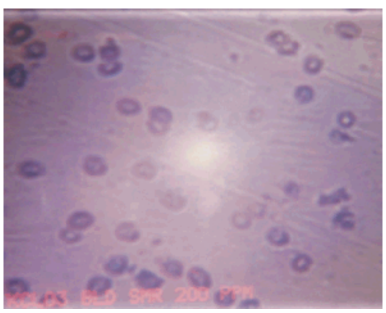

Fig. 9: KCLO3 BLD SMR 200 PPM

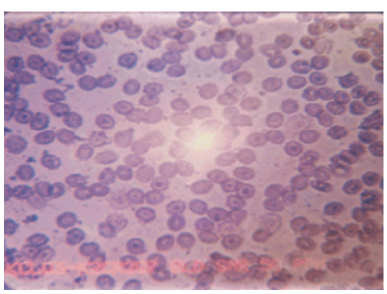

Fig. 10: KCLO3-K2CR207 BLD SMR 50 PPM

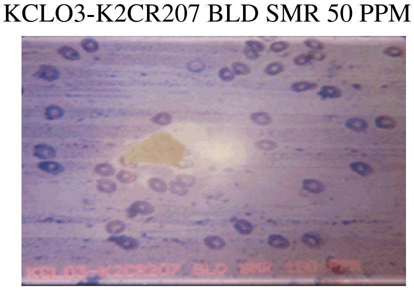

Fig. 11: KCLO3-K2CR207 BLD 100 PPM

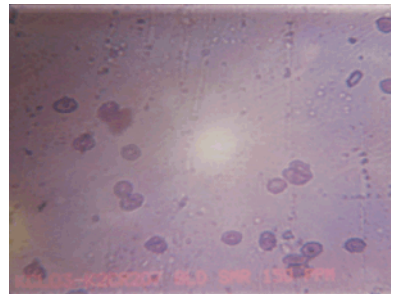

Fig. 12: KCLO3-K2CR207 BLD SMR 150 PPM

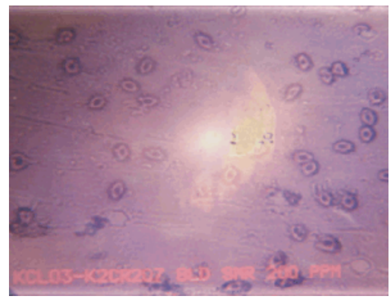

Fig. 13: KCLO3-K2CR207 BLD 200 PPM

The hematological values obtained of the various concentrations of potassium chlorate are shown in Table 2. With increase in the concentration of the chemical there was a progressive reduction in the total erythrocytes (TEC) and hemogloin (Hb) content. However, the total leucocytes count (TLC) and mean corpuscular hemoglobin (MCH) exhibited a gradual increase with the increasing n concentration of the chemical (Table 2; Figs. 2-5).

The sudden transfer of the fish from normal conditions to experimental situation brought about the following changes. The RBCs count fell significantly.

The effect of potassium chlorate and potassium dichromate on erythrocytes and leucocytes, Hb and MCH were found to show only a slight variation (Figs. 2 to 5). The erythrocytes count and leucocytes count showed sudden change from normal to 50 ppm concentration of the two chemicals. Beyond that the change was gradual.

Blood smear analysis (Figs. 2 to 13) reveals the change in the volume of the erythrocytes and bursting with increasing concentration of potassium chlorate separately and potassium chlorate and potassium dichromate synergistically.

DISCUSSION

Hematological values of fish fluctuate under varying ecophysiological conditions. The present investigation clearly reveals that salts in the medium cause disturbance

Table 2: Effect of potassium chlorate on blood values of *Oreochromis mossambicus*

CONC (ppm)	RBCs ($\times10^6$/mm^3)	WBCs	Hb	MCH
Control	3.23±0.036	9650±14.67	9.35±0.163	29.06±5.9
25	2.95±0.003	10000±52.06	9.05±0.117	30.66±1.23
50	2.57±0.007	11975±11.57	8.98±0.029	34.77±5.7
75	2.29±0.04	12650±75.67	8.63±0.36	37.81±13.15
100	2.02±0.005	12776±21.56	8.33±0.13	41.31±0.97
125	1.91±0.0009	13125±55.56	8±0.62	41.99±2.13
150	1.6±0.025	19650±55	6.8±0.6	42.44±5.13
175	1.43±0.005	20975±22.26	6.13±0.036	42.71±0.52
200	1.06±0.014	22225±77.69	5.93±0.015	55.79±1.22

in the process of haemopoiesis. The blood shows immediate response to the changing external environment which can be measured as change in the number of blood cells, differential count in the leucocytes, change in the Hb content and change in MCH value. These results corroborate with those of Rajendra Nayak [16] who has worked on the effect of a detergent on the blood cells of *Rasbora daniconius* (Hann.).

Madhyastha and Nayak [17] reported the progressive reduction in the total number of the erythrocytes in Rasbora daniconius (Ham.) as responses to the sodium lauryl sulphate (an anionic detergent). It has been later shown that there is a decrease in erythrocytes when the fish *Rasbore daniconius* (Ham) is exposed to detergent for 30 days [16].

The response of *Oreochromis mossambicus* (Peters) subjected to stress by increasing the concentration of pesticides seemed to vary with reference to erythrocytes counts. While a drastic increase has been reported for fish exposed to pesticides, such as aldrin [18and19] and industrial effluents [12, 13].

The influence of pesticides, detergents and toxic chemicals usually showed mostly an increase in leucocytes with reference to an increase in concentration and duration of exposure. This may be attributed to the induction of some defense mechanism in the body of fish to tide over the pollution stress [13].

It has been observed in the present study that the presence of potassium chlorate and potassium dichromate together in the medium may create some abnormalities in the blood because of the effect on haemopoiesis. The shape of erythrocytes of the control fish was oval with prominent nucleus, but in the potassium chlorate and potassium dichromate treated fish all the groups of erythrocytes became spherical, irregular with subsequent cellular hypertrophy and bursting (Fig. 2-13) with the increase in the concentrations thus indicating the effect of the toxic salts.

The various amount of salts dissolved in water affect the fish mainly through the changes in osmotic pressure and density of water. However, salts of water have a greater significance for the life of fishes. The salts dissolved in water permeate in to the body comparatively easily mainly through the gill causing stress [6].

CONCLUSION

The stressful condition encountered by aquatic organisms may be biological, physical or chemical. These results in weakening or death, of the individuals in the ecosystem thus altering the ecology of the communities so if these kinds' factories will increase totally not only affect fish community and human blood also chance to became haemopoiesis. So need to awareness to peoples and Factory management finally we save our societies.

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