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Sublethal Effect of Pickling Process Wastewater from a Steel Industry on the Haematology of *H.Fossilis* in Long Duration Experiments

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Abstract: *Industrial waste discharge is one of the major source of stream pollution. Waterborne wastes appear to be an inevitable result of nearly all manufacturing industries since water is used for many purposes by industries worldwide. Most and frequently all of the wastewater need to be discharged from plant premises. Such discharges are contaminated with varying amount of materials used in plant e.g. raw materials, unwanted substances accompanying raw materials, intermediate products, by products and other substances used in the processing. In this context a steel processing industry producing steel sheets is studied with respect to its pickling process wastewater at raw and neutralized levels using freshwater catfish H.fossilis. Sublethal effect of raw and neutralized wastewater are studied in long duration experiments of 10 days in laboratory level to assess the changes in haematological parameters of fish. The studied haematological parameters include total erythrocyte count, total leucocyte count, haemoglobin content, Packed Cell Volume, Clotting time, ESR, MCV, MCH and MCHC. Significant changes in haematological parameters of H.fossilis were noticed at sublethal levels too depicting the impact of wastewater on the haematology of the fish. The changes were more in raw wastewater*

Keywords: *H.fossilis*, Haematology, Sublethal Effect, long duration experiments, Pickling Process Wastewater

I. INTRODUCTION

Human eagerness to perform better and better with respect to production of food, energy and convenience products to enhance the standard of living resulted in increasing industrialization, urbanization and rapid development which in turn has led to tremendous growth in production of various industrial products. Pure chemicals and other toxicants do not enter the aquatic environment as single entities. The concern for environmental protection especially in the aquatic environment arises because industrial effluents contain complex mixtures of several chemicals which are discharged into surface water bodies and subsequently disturb the fragile ecology of receiving systems.

The disposal of industrial wastes in aquatic bodies poses serious problems due to their diverse chemical composition and complexity. The untreated industrial wastewaters often contain many toxicants which poses threat to aquatic life (David et al, 1988). The entry of these toxicants into aquatic environment triggers a series of events which directly or indirectly affect the aquatic life. The possible effects vary and affect metabolic and physiological functions in organisms or changes in properties of ambient medium that affect the aquatic organisms (Newman *et al*, 1992).

II. MATERIALS AND METHODS

Raw and neutralized pickling wastewater from a steel processing industry of Vidarbha region was collected and analyzed for its physic-chemical and heavy metal characteristics in NEERI Laboratory of Nagpur (APHA,1989). The industry manufactures cold rolled steel sheets in wide range of thickness. During cold rolling process hot rolled sheets are treated with hydrochloric acid in a pickling process to remove oxide scales and to impart clean surface properties to the steel sheets.

After a lot of processing spent hydrochloric acid and rinse water are the two major wastewater sources originating from pickling operation while alkaline wastewater stream originates from alkali cleaning. These are the wastewater sources which are collected and analyzed as raw and neutralized by lime. The characterization of raw and neutralized pickling wastewater was undertaken with respect to PH, conductivity, acidity, chlorides, TSS, TDS, oil and Grease & heavy metals iron, zinc, cadmium, chromium, nickel, manganese, lead, aluminium, copper, and silicon. The color of raw pickling wastewater was yellow and that of neutralized wastewater was pale greenish.

Freshwater catfish *H.fossilis* is used for studying the sublethal effect of raw and neutralized pickling wastewater. The fish size varies from 12-17 cms. While weight ranges from 50-60gms. Acute toxicity tests were performed for estimating LC50 values for raw and Neutralized wastewater. The concentration of $1/10^{th}$ of 96 Hrs LC50 was chosen for raw and neutralized pickling wastewater (Sprague, 1969; APHA, 1989; Mckim*et al*,1970). In case of raw pickling wastewater a sub-lethal dose of 0.055 was selected and in case of neutralized wastewater a sub-lethal dose of 1.1% v/v was used for experimentation. The exposed catfish was taken out after 10days interval and blood samples were obtained and analyzed (Wintrobe, 1973; Dacie and Lewis, 1975; Sood,1996).

The haematological parameters investigated include total RBC count, total leucocyte count, haemoglobin content, packed cell volume, clotting time, and ESR. The data was analyzed using students ‘t” test and significant differences established at 0.01,0.05 and 0.001 levels of significance.

III. RESULT AND DISCUSSION

Today no instrumental or chemical analysis is available to test the toxicity of pure chemical or complex wastewater to living organisms. The only real measure of toxicity is through test species whose dose response relationship can be obtained through time, either experimentally or through continuous field monitoring. The results of these toxicity tests ar used for establishing, predicting and deriving the maximum permissible concentrations at which the toxicants may be present in the environment without causing damage to the aquatic organisms (Sarkar*et al* ,1995).

Sublethal dose of raw (0.05%)v/v and neutralized (1.1%) pickling industry wastewaters produced marked changes in the haematological parameters of *H.fossilis* during 10 days duration. The changes in raw wastewater are shown in Table 1 and in case of neutralized it was shown in Table2.

The impact of the pickling wastewater in case of general haematological parameters was expressed as significant decrease in total erythrocyte count, haemoglobin content, packed cell volume and clotting time with a significant rise in total leucocyte count and erythrocyte sedimentation rate.

Significant decrease in erythrocyte count was observed in presen study supported by similar findings of Johansson-Sjobeck and Larsson (1978).The degree of impact was found to be considerably reduced on neutralization of raw wastewater as evident from the observations. The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Conentration (MCHC) were calculated with a view to designate the type of anaemia in *H.fossilis* during prolonged exposure to raw and neutralized wastewater.

Blood is an indicator of most of the vital physiological and biochemical functions. Many investigators have emphasized the need for the establishment of normal haematological values in fish from un polluted environments with a view to diagnose impacts of environmental stress due to pollution (Larsson *et al*, 1976; Thakur and Sahai,1986; Helawell, 1986).

An inherent need to develop rapid quantitative physiological methods to measure the impact of pollutants present at subacute levels in the aquatic environment has been expressed by these authors. The leucocytes are involved in the immune defense of an organism and any stress or damage to internal organs, parasitic infection, necrosis, injury or inflammation stimulates the production of leucocytes.

Acute toxicity end points are of use in screening of toxic wastewaters by regulatory agencies viz. pollution control boards to impose limit on the toxic waste discharge. However continued sub lethal exposures may produce a variety of chronic symptoms not observed in short term tests (Hassler*et al*, 1967) as evident from present studies on haematology.Many Indian scientists inferred that the haematological studies in fish could be a promising tool for investigating physiological changes caused by environmental pollutants (Panigrahi and Misra, 1980; Murty, 1986) and for monitoring fish health (Sinha et al, 1991).

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Table 1: Alterations in Haematological Parameters of *Heteropneustes fossilis* during 10 days exposure to Raw Pickling Wastewater at sublethal dose of 0.05%v/v

Sr. No.	Parameter	Observations		% Changes Observed
		Control	Exposed	
1.	Total erythrocyte Count ($x10^6$/cm)	3.18$\pm$ 0.12	2.50$\pm$ 0.08*	21.38(-)
2.	Total leucocyte Count ($x10^4$/cm)	4.30$\pm$ 0.11	6.30$\pm$0.08*	46.51(+)
3.	Haemoglobin Content (Gm/100ml)	11.50$\pm$0.21	10.10$\pm$0.16*	12.17(-)
4.	Packed Cell Volume (%)	41.00$\pm$0.84	38.60$\pm$0.93#	5.85(-)
5.	Clotting Time (sec.)	116.00$\pm$0.84	109$\pm$1.40#	6.03(-)
6.	Erythrocyte Sedimentation Rate (mm/Hr)	1.30$\pm$0.11	1.50$\pm$0.16^2	23.07(+)
7.	Mean Corpuscular Volume (cm^3/cell)	128.93$\pm$1.35	154.40$\pm$1.62*	16.50(+)
8.	Mean Corpuscular Haemoglobin(pg/cell)	36.16$\pm$1.09	40.40$\pm$1.02#	10.60(+)
9.	Mean Corupscular Haemoglobin Concentration (%)	27.80$\pm$0.54	26.16$\pm$0.47#	5.89 (-)

Results are expressed as Mean $\pm$ S.D.

* $P < 0.01$# $P < 0.05$

Table 2: Alterations in Haematological Parameters of *H.fossilis* during 10 days exposure to Neutralized Pickling Wastewater at sublethal dose of 1.1% v/v

Sr. No.	Parameter	Observations		% Changes Observed
		Control	Exposed	
1.	Total erythrocyte Count ($x10^6$/cm)	3.60±0.11	2.92±0.08*	18.88(-)
2.	Total leucocyte Count ($x10^4$/cm)	4.21±0.09	6.00±0.19*	42.85(-)
3.	Haemoglobin Content (Gm/100ml)	11.80±0.11	10.60±0.16#	10.16(-)
4.	Packed Cell Volume (%)	40.00±0.57	38.20±0.37#	4.50 (-)
5.	Clotting Time (sec.)	115.00± 0.84	109.00±1.20#	5.21(-)
6.	Erythrocyte Sedimentation Rate (mm/Hr)	1.30± 0.09	1.45±0.04$	11.53(-)
7.	Mean Corpuscular Volume (cm^3/cell)	111.00±2.01	130.82±0.90*	15.15(+)
8.	Mean Corpuscular Haemoglobin (pg/cell)	32.77±0.80	36.30±0.86	9.72(+)
9.	Mean Corupscular Haemoglobin Concentration (%)	29.50±0.44	27.54± 0.15	5.96(-)

Results are expressed as Mean ± S.D.

* P< 0.001 # P<0.01 $ P <0.05