Toxicity Evaluation of Wastewater Generated from Automobile Service Station through Micronucleus Test Using Freshwater Fishes

Pravin C. Singru [a], S. B. Zade[a], S.R. Sitre[b]

[a] PGTD of Zoology, R. T. M. Nagpur University, Nagpur (Ms), India- 440033.
[b] N. S. Science and Arts College, Bhadravati, Dist; Chandrapur (Ms), India-442902.

Abstract

Wastewater generated from vehicular servicing contains exhausted engine oil, Poly aromatic hydrocarbon (PAH), Polychlorinated biphenyl (PCB), heavy metals and other toxicants which contaminate inland water bodies when discharged indiscriminately. The toxicity of this wastewater was evaluated through two different freshwater fishes, Koi carp (*Cyprinus carpio*) and Catfish (*Clarias gariepinus*) using micronucleus test in erythrocytes under chronic exposure to sub lethal concentrations during 10 to 30 days. Sub lethal dose for Koi carp and for Catfish was 0.64mg/l and 24.832mg/l respectively, derived from acute bioassay test. On 10days exposure, poikilocytosis and micronucleus formation was less evident but micronucleus(MN) formation and bilobed nucleus was significant in erythrocytes of Koi carp during 20days exposure. Vacuolization in the nucleus of erythrocytes caused due to disintegration of chromatin was evident on 10days exposure and poikilocytosis, eccentric nuclei, clubbed erythrocytes were distinct with less micronucleus (MN) formation in Catfish on 20days exposure. Though the formation of micronucleus in erythrocytes was common in both the fishes but it was more evident in Koi carp compared to Catfish during 30days exposure to sub lethal concentration. The formation of micronucleus (MN) in erythrocytes is a marker of cytogenetic damage caused due to pollutants of wastewater from automobile service station. Thus it becomes imperative to treat this wastewater properly with planned treatment prior to its disposal into inland water sources to protect our precious aquatic environment from mutagens.

KEYWORDS: Koi carp, Catfish, Exhausted engine oil, Erythrocytes, Micronucleus test.

INTRODUCTION

Aquatic pollution caused due to indiscriminate discharge of wastewater from automobile service stations has become a serious issue of concern in developing countries like India. The servicing of vehicles generates large volumes of exhausted engine oil laden wastewater which contains PAH, PCB, heavy metals, detergents and other toxicants (1). When this wastewater is mixed with fresh water resources it not only causes surface water pollution but also contaminates ground water and tend to accumulate in flora and fauna. Majority of rural people living by the side of river and lakes depend largely on the fish as a source of protein and hence need to be protected.

Today scanty information is available on the toxic and mutagenic impact of wastewater generated from automobile service stations. It is therefore needed to evaluate its genotoxic effects on fish and to protect the biota from mutagenic effects of this wastewater. The spent engine oil is a toxic environmental contaminant, which is not naturally found in our environment. Toxicity of spent engine oil generated from workshops on juvenile Catfish, *Clarias*

gariepinus has been reported [2]. The formation of micronucleus (MN) is a measure of chromosomal aberration caused due to loss of whole chromosomes or acentric chromosome fragments in fish erythrocytes [3]. The micronucleus test in fish erythrocytes can be a sensitive indicator for aquatic pollution and useful for the study of mutagenic and carcinogenic contaminants of aquatic environment [4,5]. In this context the present study is undertaken on two different freshwater fishes to analyze the toxic effects of exhausted engine oil wastewater through micronucleus test.

MATERIALS AND METHODS

Fish species: Koi carp (*Cyprinus carpio*) and juvenile Catfish (*Clarias gariepinus*) of size 6.5cm to 9.8cm, of around 16-22 gm were used for toxicity evaluation. Fishes were procured from local fish supplier and treated with 1% $KMnO_4$ solution to remove dermal infection if any. Fishes were acclimatized for 15 days in laboratory aquaria prior to experimentation.

Dilution water: De-chlorinated tap water was prepared for experimental work and used as dilution water. This dilution water was analyzed as per the standard methods [6] and results are indicated in Table 1.

Table 1: Characteristics of dilution water

Parameters*	**Values (Range)**
pH	7.2-7.6
Temperature ^oC	24.8-27.0^oC
Alkalinity as $CaCO_3$	156-186
Dissolved Oxygen	6.9-7.5
Chlorides as Cl	150-166
Total Hardness as $CaCO_3$	142-176
Calcium Hardness as $CaCO_3$	62-78
Magnesium Hardness as $CaCO_3$	80-102
Calcium as Ca	24.8-31.0
Magnesium as Mg	18.6-19.0

Sodium as Na	36 - 46
Potassium as K	4 - 9

* All the values are expressed in mg/l except pH and Temperature

Wastewater collection: After detailed inventorization of automobile service stations located in Nagpur city of Maharashtra state (India), the wastewater was collected from an efficient and well organized service station on hourly basis for eight hours and a combined moreover homogenized wastewater was prepared for toxicity experiments. The collected wastewater was then subjected to physico-chemical analysis, PAH, PCB and heavy metals as per Standard Methods (APHA). Results are indicated in Table 2 and 3.

Table 2: Physico-chemical characteristics and Heavy metal analysis of wastewater of the vehicle servicing stations

Parameters*	**Values (mg/l)**
pH	7.45
Alkalinity as $CaCO_3$	375
Chloride as Cl^-	178
Phosphate as PO_4	0.82
Sulphide as S^-	<0.02
Sulphate as SO_4^-	35.0
Sodium as Na	173
Chemical Oxygen Demand (COD)	78
Oil and Grease	989
Magnesium as Mg	17.36
Alkyl benzene sulphonate	2.0
Heavy Metals	
Copper	0.024 - 0.279
Cobalt	0.0060 - 0.069
Iron	6.99 - 26.047
Nickel	0.002 - 0.043
Lead	ND
Cadmium	0.001 - 0.021
Chromium	0 - 0.025

Manganese	0.211 - 1.344
Zinc	0.117 - 0.530

* All the values are expressed in mg/l except PH.

Table 3: Presence of Poly aromatic hydrocarbons in the wastewater discharged from vehicle service stations

Name of the poly aromatic hydrocarbon*	**Values (Range)**
Naphthalene	0 - 32
Acchephthylene	0 - 0.27
Acenophthene	0.33 - 0.84
Fluorene	1.40 - 3.29
Phenanthrene	12.06 - 20.09
Anthracene	1.66 - 10.26
Fluoranthene	0.00 - 2.33
Pyrene	1.54 - 21.65
Benz(a) anthracene	0.43 - 1.69
Chrysene	0.51 - 0.53
Benzo(a)pyrene	0.03 - 2.05
Indenol(1,2,3,-cd)pyrene	0.17 - 0.81
Benzo(g,h,i)pyrelene	0.04 - 0.14

* All the values are expressed in µg/l.

Toxicity test and micronucleus (MN) test: Acute toxicity test was carried out as per the literature [7] to determine LC_{50} values for both the fishes. Based on LC_{50} values SAR (Safe Application Rate) and SAFE concentrations were calculated [8]. SAR was 0.64mg/l and SAFE was 0.08mg/l for Koi carp while SAR was 24.832mg/l and SAFE was 0.388mg/l for Catfish. Chronic toxicity test was carried out on both the test fishes separately over a period of 30 days in dosing unit, designed and fabricated by NEERI [9]. The dosing unit was filled with ten litre solution containing sub lethal dose of automobile service station wastewater and in ten litre of glass aquarium. The flow rate was so adjusted that a ten litre liquid flows through the aquarium in 24 hours which is delivered at the bottom of aquarium. Ten fishes were introduced into aquarium for toxicity testing. After every 24 hours fresh feeding was prepared and filled in the upper reservoir as per the details reported in literature [10]. Each concentration related to each fish was investigated for a period of 10, 20 and 30 days. Along with the experimental work, a parallel control was also run. Live fishes were removed on intervals of every ten days of exposure and blood samples were obtained from their caudal fin for further haematological investigations.

The blood smear was prepared, allowed to air dry and then treated with absolute alcohol for 15 to 20 minutes. Fixed smear was washed with distilled water for 5 minutes and later stained by Harris hematoxylin solution for 10 minutes. Excessive stain was removed by washing in running tap water for 2 to 5 minutes. The stained blood smear was treated with counterstain of 1 % Eosin for one minute, cleaned in xylene for 5 minutes and then mounted with DPX. The stained slides were observed under 400 X and 1000 X, oil immersion lens and photographed.

RESULTS

In the blood of control Koi carp (*Cyprinus carpio*), oval shape erythrocytes with oval nucleus in the center and defined cellular boundaries (fig-1a) was recorded. The blood of control Catfish (*Clarias gariepinus*) shown rounded nucleus in the center of erythrocytes and normal cellular boundaries (fig-2a). On 10days exposure in Koi carp, micronucleus (MN) in erythrocyte was evident. vacuolization in the nucleus of erythrocyte and angular erythrocytes were noticed in Catfish at same period of exposure (fig-1b & 2b). The formation of micronucleus (MN), bilobed nuclei and clubbed erythrocytes were significant in erythrocytes of Koi carp. While poikilocytosis, clubbed erythrocytes, micronucleus (MN) and ecentric nuclei were evident in Catfish on 20days exposure to sub lethal concentration of exhausted engine oil wastewater (fig-1c & 2c). When exposure period was increased to 30 days, the formation of micronucleus (MN), phagocytes and cytoplasmic fusion was more significant in erythrocytes of Koi carp but on same period of exposure, the formation of MN and poikilocytosis in erythrocytes of Catfish was more evident (fig-1d & 2d).

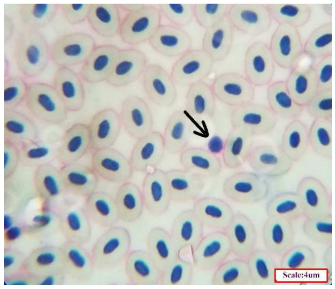

Fig.1a.Blood smear of control Koi carp (*Cyprinus carpio*) showing normal erythrocytes (1000x).

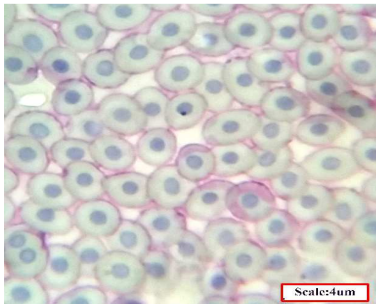

Fig.2a.Blood smear of contro Catfish, (*Clarias gariepinus*) showing normal erythrocytes (1000x).

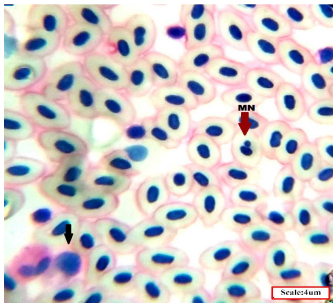

Fig.1b.Blood smear of Koi carp (*Cyprinus carpio*) showing formation of MN in erythrocytes on exposure to exhausted engine oil wastewater for 10days (1000x).

Fig.2b.Blood smear of Catfish, (*Clarias gariepinus*) showing vacuolization in nucleus and angular erythrocytes on exposure to exhausted engine oil wastewater for 10days (1000x).

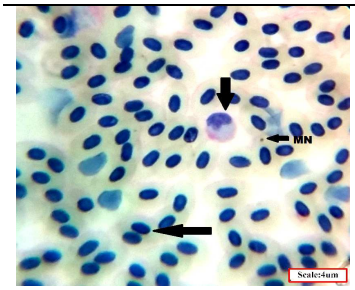

Fig.1c.Blood smear of Koi carp (*Cyprinus carpio*) showing formation of MN and bilobed nucleus in erythrocytes on exposure to exhausted engine oil wastewater for 20days (1000x).

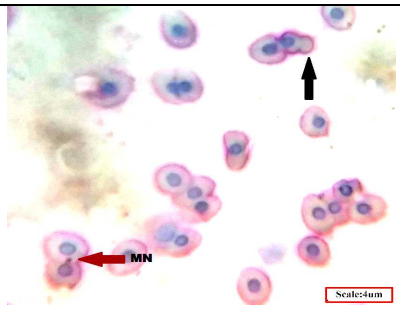

Fig.2c.Blood smear of Catfish, (*Clarias gariepinus*) showing formation of MN in erythrocytes and poikilocytosis on exposure to exhausted engine oil wastewater for 20days (1000x).

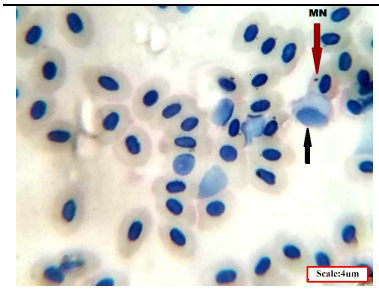

Fig.1d. Blood smear of Koi carp (*Cyprinus carpio*) showing formation of MN, cytoplasmic fusion in erythrocytes and phagocytes on exposure to exhausted engine oil wastewater for 30days (1000x).

Fig.2d.Blood smear of Catfish, (*Clarias gariepinus*) showing formation of MN in erythrocytes, vacuolization in nucleus of erythrocytes and poikilocytosis on exposure to exhausted engine oil wastewater for 30days (1000x).

DISCUSSION

Exposure of Koi carp and Catfish to automobile service station wastewater resulted in formation of micronucleus in erythrocytes. The formation of micronucleus in erythrocytes is a marker of clastogenic and aneugenic compounds present in aquatic environment. Thus it is used to study toxicity levels of wastewater.

Tendency of micronucleus formation(MN) increased during 10, 20 and 30days exposure to exhausted engine oil laden wastewater from automobile service station in Koi carp and Catfish, but vacuolization, poikilocytosis was more significant in Catfish during 20days exposure and bilobed nucleus was detected in Koi carp for the same exposure period. Vacuolization in erythrocytes of Catfish during 20days exposure to this wastewater was probably due to disintegration of chromatin in the nucleus. As exposure period increased to 30days to sub lethal concentration of wastewater, MN formation became more significant and fused, elongated erythrocytes were clearly seen in Koi carp.

Formation of micronucleus in erythrocytes was a common feature during thirty days exposure period in both the fishes. This is in agreement with findings reported in literature [2], supporting our findings.

Though minimal erythrocyte damage was observed in Catfish compared to Koi carp for same exposure period but in both the fishes, formation of micronucleus does exert genotoxicity of this wastewater.

CONCLUSION

From micronucleus (MN) test it can further be inferred that the exhausted engine oil laden wastewater from automobile service station exert genotoxicity on fresh water fishes after it`s discharge into inland waterbodies. It is hence very clear that the wastewater generated from automobile service stations needs proper and planned treatment prior to its disposal to safeguard our precious aquatic environment from aquatic mutagens and other toxicants.

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