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# Genotoxicity of Textile Dye Effluent on Fish (*Cyprinus carpio*) Measured Using the Comet Assay

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In the textile dyeing units, it is estimated that 10-15% of the dyes are lost in the effluent during the dyeing process. As the conventional wastewater treatment processes are not effective to remove the dyes from the effluent water, the unspent dyes are discharged into the aquatic systems along with other chemical substances in the effluent water. Due to the reported mutagenicity of some of the synthetic dyes (Rajaguru et al., 1999), disposal of untreated textile dyeing effluent water has raised concern about the safety of the aquatic environment.

Cytogenetic methods are probably the most sensitive and efficient way to detect the effects of genotoxins. Fish provide an excellent source of material for the study of the mutagenic and/or carcinogenic potential of water samples since they are aquatic vertebrate organisms that can metabolise, concentrate and store waterborne pollutants (Al-Sabti, 1991). Since the effects of genotoxic agents are often tissue and cell-type specific, techniques which can detect DNA damage on chromosomal characteristics and cell division are needed (Pandrangi et al., 1995). Among various techniques used to assess the genotoxicity of chemicals, the Single Cell Gel Electrophoresis (SCGE), also known as comet assay, has many advantages over the other DNA damage assays. This assay has been conducted on any nucleated cells, at the individual cell level and requires extremely small sample of cells. Therefore, in order to investigate the biological consequences of exposure to textile dyeing effluent on fish comet assay (SCGE) were conducted on erythrocytes and liver cells of Common carp (*Cyprinus carpio*), an omnivorous feeder, living on detritus in both the sediment and the water column.

## MATERIALS AND METHODS

Immature carp aged 3-4 months old, weighing between 8-12.5 g with a length ranging between 5-8 cm, were obtained from a fish hatchery near

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Coimbatore, India. The fish were kept in a 100 L aquarium and fed daily with commercial fish food. In this study, the effluent water collected from a textile dyeing industry, located in Tirupur, a textile centre in Tamilnadu state, India was tested for its genotoxicity. Effects of different concentrations (0,1, 2, 5, 10%) and treatment times (3, 9 and 15 days) were compared. The water containing the test solution was renewed every 24 hr. The fish were not fed during the exposure to the test solution. In order to evaluate the extent of recovery, after the treatment period of 15 days, the fish were transferred and maintained in dechlorinated tap water for another 15 days. Cyclophosphamide [CP (2 mg/L)] was used as a reference mutagen.

Blood samples were collected from the gills of the fish using 1mL heparinized syringes. Liver tissues were collected after sacrificing the fish by spinal incision and washed twice with cold PBS containing 10% DMSO. To the tissue samples cold PBS with 10% DMSO was added and made into a cell suspension so that three or four cells would be seen without crowding in a single field at 250x magnification. The tissue samples were further treated within 1hr of being maintained at 4 $^{0}$C. The comet assay was performed basically as described by Pandrangi et al., (1995). Briefly, 100 µl of cell suspension (~x$10^5$ cells) was mixed with 200 µl of low melting agarose and pipetted onto a base layer of 1% standard agarose. After polymerisation of the agarose, the cells were lysed for 2hr in 2.5M NaCl, 100mM EDTA, 10mM Tris, pH10, 1% TritonX-100, 10% DMSO. The slides are then placed in 300mM NaOH, 1mM EDTA, pH13, for 25min before electrophoresis at 25V, 300mA, for 20min. After electrophoresis and subsequent staining with ethidium bromide (10µg/mL), all slides were coded and examined blindly. Routinely, 50 cells were screened per sample. The length and width of the DNA mass was measured and used in all comparisons.

The results of the different treatment groups were compared using student's one-tailed 't' test. The extent of intercellular heterogeneity within each of the data sets was determined from the range to SD ratios. Values below 2 or above 6 indicated the data to be extremely homogeneous or heterogeneous (see Pandrangi et al., 1995). Multiple linear regression analysis were carried out to establish the correlation between effluent dosage and induced DNA damage.

## RESULTS AND DISCUSSION

In both the tissues, in all exposure concentrations of the effluent and CP, and in each of the treatment periods, all the treatment groups show a

**Table 1.** Distribution of DNA damage (based on length : width ratios and Range : SD ratios of DNA patterns) observed in the liver cells of fish (*C. carpio*) after exposure to textile effluent.

| Treatment | Control | CP | 1% | 2% | 5% | 10% |
|---|---|---|---|---|---|---|
| 3 days | 1.8 | 1.453* | 2.379* | 3.158** | 4.122** | 4.178** |
| | (0.061) | (0.023) | (0.1) | (0.023) | (0.099) | (0.115) |
| 9 days | 1.884 | 2.235** | 1.911** | 2.288** | 2.628** | 2.865** |
| | (0.02) | (0.035) | (0.119) | (0.066) | (0.044) | (0.104) |
| 15 days | 1.708 | 2.138** | 2.661** | 2.23** | 2.531** | 2.725** |
| | (0.03) | (0.019) | (0.114) | (0.045) | (0.072) | (0.109) |
| 21 days | 1.65 | 1.978* | 1.979* | 2.164** | 2.22* | 2.14** |
| | (0.021) | (0.041 | (0.036 | (0.009) | (0.124 | (0.038) |
| 28 days | 2.054 | 2.192** | 2.133 | 2.06 | 2.122 | 2.247 |
| | (0.05) | (0.043) | (0.01) | (0.037) | (0.025) | (0.063) |

Range : SD ratio

| Treatment | Control | CP | 1% | 2% | 5% | 10% |
|---|---|---|---|---|---|---|
| 3 days | 5.9422 | 6.432 | 3.913 | 3.4509 | 3.5324 | 4.5623 |
| 9 days | 3.6842 | 4.5866 | 5.2083 | 4.28 | 3.8563 | 4.5675 |
| 15 days | 4.0793 | 5.6213 | 3.6141 | 4.3353 | 3.8759 | 4.2827 |
| 21 days | 4.7619 | 4.5823 | 5.1975 | 4.444 | 3.4965 | 4.3103 |
| 28 days | 4.2857 | 3.1843 | 4.6763 | 3.8593 | 4.8309 | 5.0623 |

Data shown are mean length : width ratio ± SEM (N=6), *P<0.05, **P<0.01

significant increase ($P < 0.01$) in DNA ratios relative to the negative control. In all the study groups, the mean DNA length : width ratios were maximal at 3 days of exposure. In liver cells, a gradual decrease occurred after 3 days of exposure and continued until 28 days at which time the experiment was stopped. The decrease in DNA length : width ratios from 3$^{rd}$ day to 9$^{th}$ day was significant but it was not significant thereafter (Table 1). A similar trend was observed in blood cells also (Table 2). However, in fish exposed to CP, as the exposure period increased, there was a gradual increase in mean DNA length to width ratios up to a maximum of 2.787 and 2.594 at 15 days in both liver and blood cells respectively. A gradual decrease occurred after that time as the exposure to CP was discontinued and the animals were transferred to dechlorinated tap water. The range to SD ratios indicated that for the fish exposed to CP for 3 days, there was an extreme heterogeneity in both blood and liver cells.

The intercellular profile suggests that the heterogeneity was high because of a few cells with high DNA ratios. In all other cases, the range to SD ratios

**Table 2.** Distribution of DNA damage (based on length : width ratios and Range : SD ratios of DNA patterns) observed in the fish erythrocytes (*C. carpio*) after exposure to textile effluent.

| Treatment | Control | CP | 1% | 2% | 5% | 10% |
|---|---|---|---|---|---|---|
| 3 days | 1.798 | 1.433** | 2.197** | 2.682** | 3.31** | 4.516** |
| | (0.006) | (0.023) | (0.15) | (0.062) | (0.165) | (0.047) |
| 9 days | 1.825 | 2.145** | 2.032** | 2.103** | 2.181** | 2.2** |
| | (0.006) | (0.035) | (0.104) | (0.051) | (0.029) | (0.045) |
| 15 days | 1.645 | 2.787* | 2.661** | 2.135* | 2.509** | 2.592** |
| | (0.015) | (0.028) | (0.114) | (0.036) | (0.055) | (0.039) |
| 21 days | 1.778 | 1.753 | 1.891* | 2.254** | 2.284** | 2.169** |
| | (0.035) | (0.051) | (0.015) | (0.087) | (0.083) | (0.146) |
| 28 days | 1.753 | 2.019* | 1.955** | 2.024** | 1.957* | 2.137** |
| | (0.066) | (0.087) | (0.057) | (0.043) | (0.031) | (0.063) |

Range : SD ratio

| Treatment | Control | CP | 1% | 2% | 5% | 10% |
|---|---|---|---|---|---|---|
| 3 days | 5.642 | 7.082 | 4.768 | 3.876 | 3.296 | 3.924 |
| 9 days | 5.844 | 4.339 | 4.403 | 4.095 | 4.934 | 3.87 |
| 15 days | 4.306 | 5.025 | 3.596 | 4.404 | 4.882 | 4.05 |
| 21 days | 3.977 | 5.336 | 4.818 | 3.916 | 4.065 | 4.249 |
| 28 days | 2.793 | 3.173 | 3.294 | 4.052 | 4.164 | 4.189 |

Data shown are mean length : width ratio ± SEM (N=6), *P<0.05, **P<0.01

fell between 5.84 and 2.79 with an average of 4.212 for blood cells and between 5.64 and 3.18 with an average of 4.308 for liver cells. This suggests the intercellular distribution of DNA damage is neither extremely homogeneous nor extremely heterogeneous

In blood cells after 3 and 9 days of exposure, there was a strong linear correlation between DNA damage and dosage of the effluent ($r = 0.995 \pm 0.002$ SE of estimate and $r = 0.88 \pm 0.054$ respectively). The correlation between DNA damage and the effluent concentration was weak ($r = 0.304 \pm 0.312$) after 15 days of exposure. In liver cells, the correlations between

DNA damage and effluent dosage were strong after all 3 sampling days ($3^{rd}$ day $r = 0.847 \pm 0.069$, $9^{th}$ day $r = 0.925 \pm 0.034$ and $15^{th}$ day $r = 0.973 \pm 0.012$). The classic micronucleus (MN) test carried out on erythrocytes did not show significant difference in MN frequencies in fish exposed to CP and the textile dyeing effluent (data not shown) when compared with the controls.

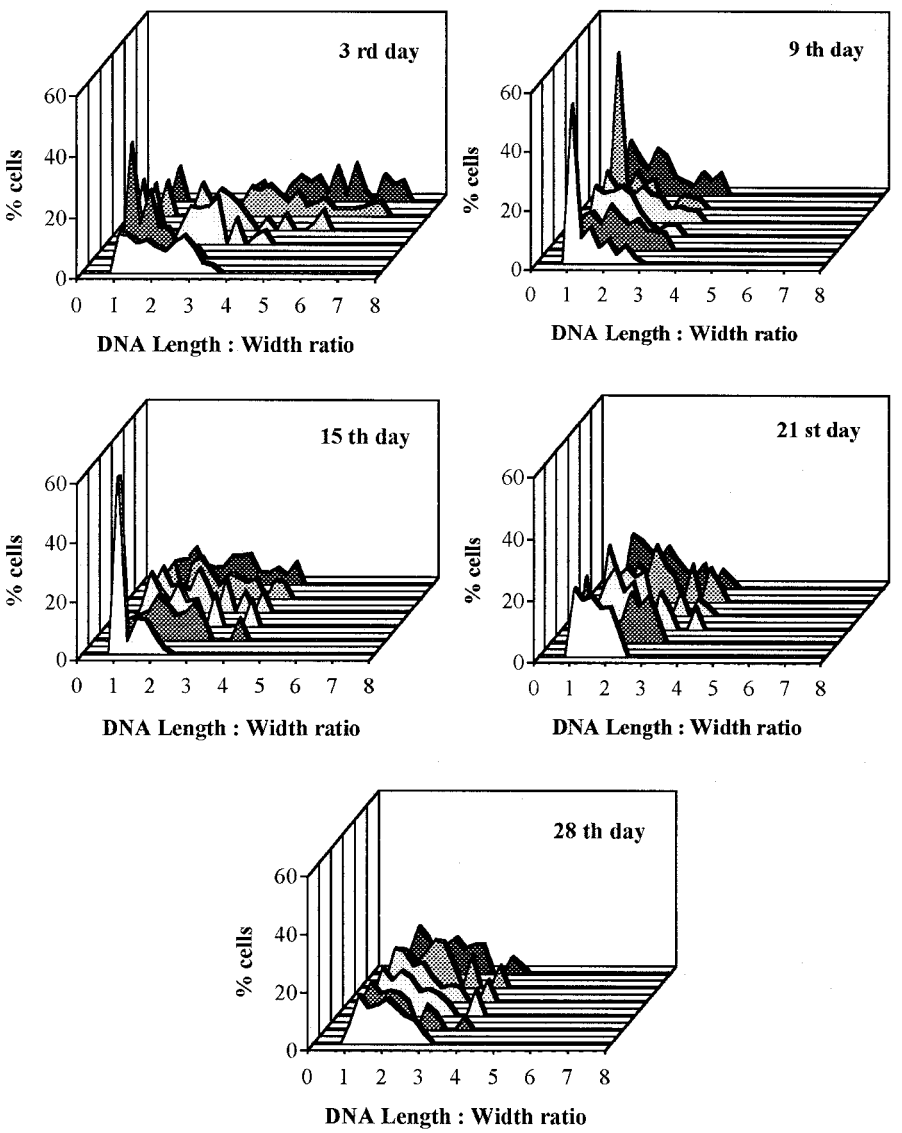


**Figure 1.** Distribution of DNA damage (based on length : width ratios of DNA patterns) observed in the liver cells of *C.carpio* after exposure to textile dyeing effluent. □ Control, ■ CP, ▨ 1% effluent, ▨ 2% effluent, ▩ 5% effluent, ▩ 10% effluent

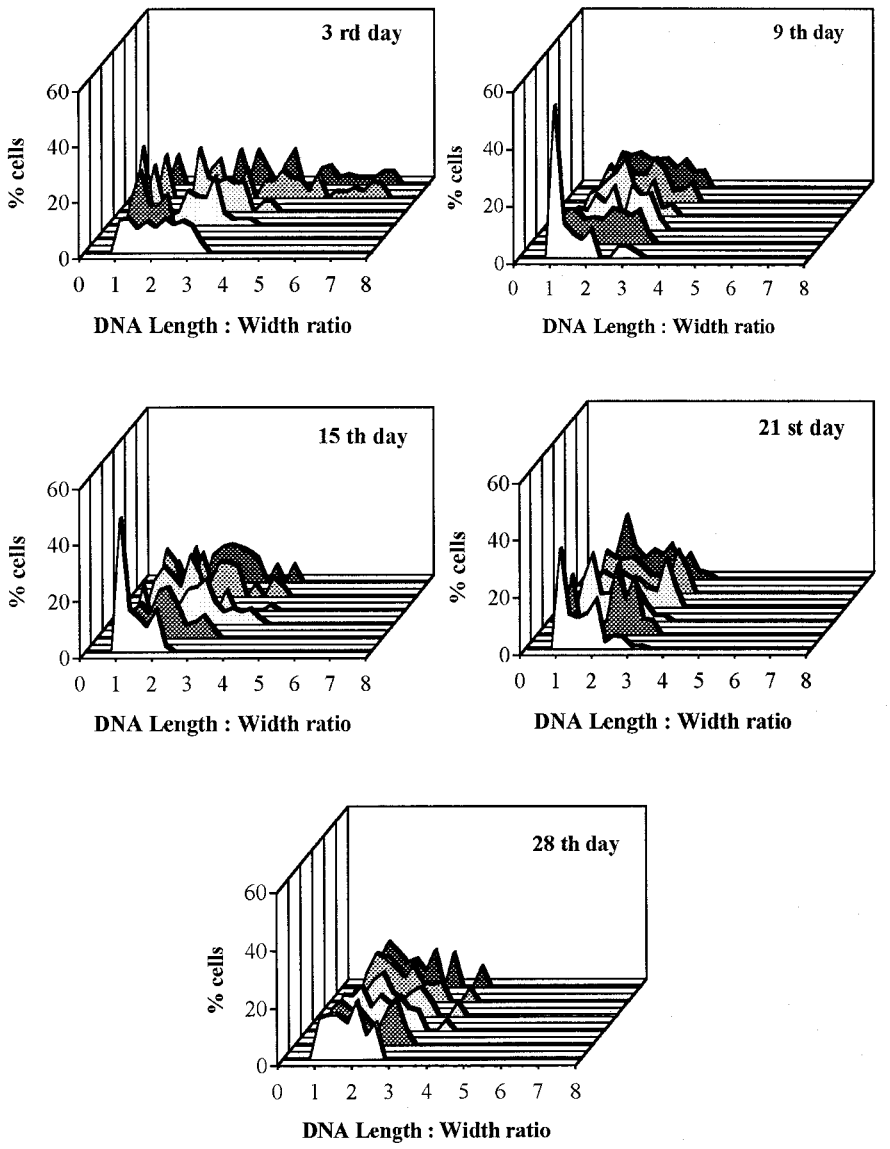


**Figure 2.** Distribution of DNA damage (based on length : width ratios of DNA patterns) observed in the erythrocytes of *C.carpio* after exposure to textile dyeing effluent. □ Control, ■ CP, ▨ 1% effluent, ▨ 2% effluent, ▩ 5% effluent, ■ 10% effluent

The SCGE assay has been shown to be sensitive to a number of mutagens using various tissues (see review by Fairbairn et al., 1995), on a number of organisms including fish (Pandrangi et al., 1995) and amphibians (Ralph and Petras, 1996) and environmental water samples (Fairbairn et al., 1994). The simplicity and relatively low cost of the assay makes it suitable for large-scale studies. Among the advantageous features of this technique, are that the size and number of chromosomes are not important and that mitotic activity is not required. The latter is especially important in fish because the metabolic rate and metabolic index fluctuate considerably with temperature and thus mitotically active tissue is difficult to isolate.

The evaluation of DNA damage in fish using the comet assay frequently involves the erythrocytes because of their ready availability and ease of collection (Moretti et al., 1998; Pandrangi et al., 1995). In the present investigation, in addition to blood cells, liver cells, an organ involved in the metabolism of xenobiotics, were also included. In both the tissues, there was a trend of increased DNA strand breaks at increasing concentrations of the effluent. Interaction of genotoxic agents with DNA forms alkaline labile adducts and other modifications, which can contribute to an increased level of DNA strand breaks via enzymatic removal of damaged nucleotides. If unrepaired, this damage to the integrity of the DNA may lead to mutation and neoplastic transformation.

Even at relatively low concentration (1%) a significant ($P < 0.01$) DNA damage was observed in fish erythrocytes and liver cells. Intercellular distribution of DNA ratios showed that even at the highest concentration (10%) some cells remained undamaged. The peaks of the distribution, however, did shift upward with the higher concentrations. For the most part, the intercellular distributions of DNA damage were not extremely homogeneous or heterogeneous. There were some differences noted in the mean DNA length to width ratios between blood and liver cells. Explanations for the observed difference may be due to the physiological activity distinctive to these two organs or the biological consequences and reparability of the different types of strand breaks.

The results of this experiment indicate that the textile dyeing effluent contains agent(s) capable of inducing DNA damage in liver cells and erythrocytes of fish (*Cyprinus carpio*). Therefore, the evaluation of the genotoxicity of the textile dyeing effluent in fish using the comet assay may be of value in the early warning systems for identifying and monitoring the effects of contaminants on aquatic biota.

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