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DETECTION OF DNA BREAKDOWN BY COMET ASSAY IN TWO TELEOST FISH EXPOSED TO WASTEWATER AND SODIUM FLUORIDE (NaF)

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Presently, with the advent of agricultural and industrial revolutions, most of the aquatic systems are becoming contaminated with different types of chemicals (Khare and Singh 2002) (Khare, S. and Singh S. 2002, *J. Ecotoxicol. Environ .Moni*t. **12**: 105-111). These toxic chemicals cause a variety of anomalies in aquatic animals (Anees) (Anees, M.A. 1975, *Pakistan J. Zool.***7**:135-141). Sodium fluoride (NaF) is an active ingredient of pesticide causing one of the biggest threats to an aquatic life when it enters in the aquatic ecosystem by agricultural run-off and other illegal uses (Sandipon *et al.* 2007) (Sandipon, P., Mukherjee, A.K. and Ghosh, A.R. 2007. *Inter. Conf. Ecotoxi. Envi. Sci.* 43 pp) Thus, the aquatic ecosystem is also faced with the threat of shrinking genetic base and biodiversity. Since the comet assay provides a very simple and sensitive method to detect DNA damage (Seonock *et al.* 2006) (Seonock, W., Sojung, K. and Seungshic, Y. 2006, *Mar. Poll.Bull* **52**: 1768-1775) the present study was undertaken to evaluate the DNA breakdown by wastewater and NaF followed by comet assay.

Healthy live (n= 200) adult cat fish (*H. fossilis)* and climbing perch (*A. testudineus)* were collected from the local market, Savar, Dhaka. Visually ascertained wastewater was collected from different spots of effluent ravaged Bangshi River located in Savar and NaF was purchased from VWR International Limited, England. *H. fossilis* were treated with wastewater for 30 and 90 days while *A. testudineus* was treated with NaF for 90 days. Group 1 and 2 were served as control while group 3 and 4 were regarded as treated.

Ten acclimatized fish were used in each aquarium containing NaF and wastewater as well as in the control as describe by (Rahman *et al.* 2002*)* (Rahman, M.Z., Hossain, Z., Mellah, M.F.A. and Ahmed, G.U. 2002. *The ICLARM Quarterly* **25**: 8-11).The fishes were fed with commercial floating pellets at 10% of their body weight. Unconsumed feed and faecal were removed and water replenished regularly as recommended by (Oyelese & Faturoti 1995) (Oyelese, O. A. and Faturoti, E.O. 1995. *J. Trop. Forest Resources* **11**: 71-81). A total of about 200 ml of blood was collected from the caudal vein into heparinized syringes.

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The comet assay was performed by the method introduced by (Olive 1989) (Olive, P. L. 1989, *Radiat. Res.,* **117**: 79-92.) The comet images were captured by the camera, transferred into computer and analyzed by using the software - CASP (Garci *et al.* 2007) (Garcia, O., Romero, I., Gonzalez. E., Mandina. T. 2007. *Mutation Research* **627**: 186-190). Head and tail length of comet in pixels and DNA were measured in percentage. Correlation coefficients between comet parameters were done using SPSS.

During the prolonging exposure period for both fish species, the percentage of comet tail DNA was significantly increased. The data suggested that wastewater and NaF could be responsible for DNA breakdown in fish and correlated with the exposure periods. Comet values for head and tail length and % DNA are presented in Table 1.

Table 1. Comet values for head and tail length and % DNA of *H. fossilis* and *A. testudineus* exposed to wastewater and NaF for various periods.

Fish species	Exposure day/ type	Head length	Tail length	Head DNA (%)	Tail DNA (%)
H. fossilis	30 control	105	3	100.006	0.00595
	30 wastewater	133	3	100.006	0.00635
	90 control	135	165	73.6523	26.3477
	90 wastewater	127	237	34.3105	65.6895
A. testudineus	90 control	100	3	100.007	0.00661
	90 NaF	101	243	27.3818	72.6182

In the present study, *H. fossilis* were exposed to wastewater for 30 days and comet assay showed no significant elevation in the length of head and tail and in percentage of DNA (Fig 1a, b) for the fish exposed to wastewater compared to control. But after 90 days exposure to wastewater the comet parameters such as head length, tail length, head DNA and tail DNA were increased (Fig. 1c,d). Interestingly, the percentage of tail DNA increased significantly ($p < 0.001$) in treated fish compared with control. *A. testudineus* were exposure to NaF for 90 days. During the study period it was observed that in treated fish the percentage of tail DNA increased significantly ($p < 0.0023$) compared to control (Fig. 1e,f).

(Seonock *et al.* 2006) (Seonock, W., Sojung, K. and Seungshic, Y. 2006. *Mar. Poll. Bull* **52**: 1768-1775) conducted an experiment on DNA damage in blood cells exposed to five types of polycyclic aromatic hydrocarbons (PAHs). The tail lengths of five PAHs-exposed groups at 50 and 100 ppb were significantly different from the non-exposed group, and the genotoxic effect of BaP correlated with both concentration and duration of exposure. Thus, our experiment showed similarity with their findings. According to Brook (1974) (Brooks, G.T. 1974.

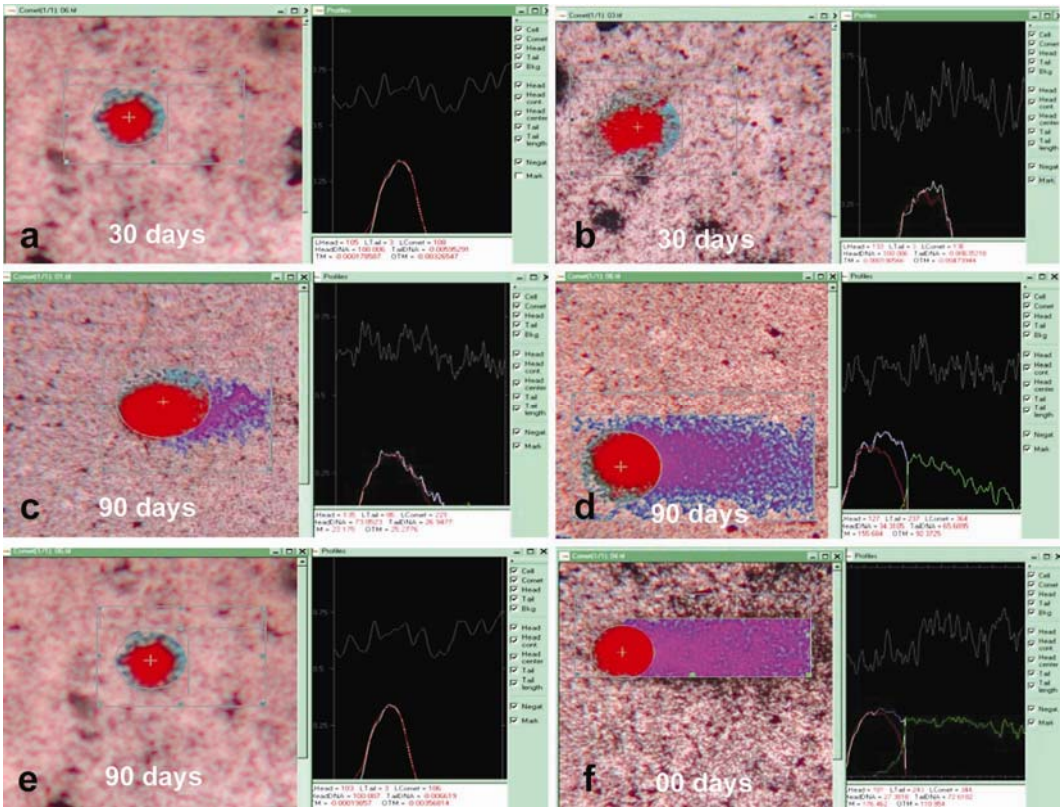

Fig. 1. Comet image of *H. fossilis* (a, b, c, d) and *A. testudineus* (e, f) exposed various periods to wastewater and NaF showing the degree of DNA damage (a, c ,e control and, b, wastewater, no damage. d, f wastewater and NaF with damage)

Chlorinated Insecticides **2**: 130-144) blood may serve as carrier of insecticides thus the bio accumulating pollutants acted as neurotoxins and subsequently damaged the blood DNA in lymphocytes. Therefore, the main prediction was the disaggregational effects of pollutants on membrane components leading to increased hemolysis of red cells and damage of DNA. Throughout the study, significant differences in DNA break were recorded between cells exposed to wastewater and NaF, and non- exposed control. It might be concluded that wastewater and NaF could be responsible for DNA breakdown in fish and correlated with the exposure period.

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