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Evaluation of genetic toxicity caused by acid mine drainage of coal mines on fish fauna of Simsang River, Garohills, Meghalaya, India

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ABSTRACT

Fishery ecology of the Simsang River, Meghalaya is being threatened by large scale environmental degradation due to acid mine drainage (AMD) of coal mines. In the present paper, effort has been made to evaluate the genotoxicity caused due to AMD of coal mines on *Channa punctata* under laboratory condition through comet assay, micronucleus and chromosome aberration tests. Water samples were collected seasonally from affected and unaffected sites of the River and physico-chemical quality of water indicated low pH (4.6), high concentration of sulphates (270 mg L^{-1}) and iron (7.2 mg L^{-1}) beyond permissible limits. Polycyclic aromatic hydrocarbon (PAH) showed highest concentration of 4-ring PAH and Benzo[a]anthracene was the most important pollutant in the water collected from affected sites. The highest and the lowest mean concentrations of PAHs were estimated in monsoon and winter season, respectively. The index of DNA damage assessed by comet assay, micronucleus and chromosome aberration tests demonstrated significant differences season wise in different sampling sites. Frequency of DNA-damaged cells was found highest in the water samples collected from affected site in monsoon season.

1. Introduction

The biodiversity of freshwater constitute a valuable natural resource in economic, cultural, aesthetic, scientific and educational terms and their conservation and management are critical to the interests of all humans, nations and governments (Dudgeon et al., 2006). Simsang River, the longest river of Garohills, originated from Nokrek Biosphere Reserve of West Garohills, Meghalaya (altitudes of 1412 m MSL) and routed through south Garohills (350 m MSL) before entering into the plains of Bangladesh. The river harbours a rich variety of cold water fish species along with plain varieties. However, in the last few decades habitat ecology of Simsang River has been severely affected due to open cast coal mining. As a result, some areas of the river are now devoid of aquatic organism either completely or seasonally. In Meghalaya, Coal mining is done through rat hole-mining techniques to exploit shallow reserves with severe environmental impact. Rat hole mining of coal can deteriorate land because of the presence of chemical wastes or physical hazards such as abandoned shafts, boreholes and tunnels. Mine wastes generated in huge quantities are mostly flammable and ready to spontaneous combustion. They may also contain heavy metals capable of leaching out into rivers, streams and groundwater which may bio-accumulate along the aquatic food chain. Coal washing also generates similar waste problems. Sulphuric acid, created when exposed coal gets wet, dissolves toxic metals highly threatened to aquatic life as well as contaminating drinking water sources. The largest water quality problem associated with coal mining is undoubtedly AMD. AMD consists of many interrelated problems. The pyrite in the rock gives rise to water with a low pH which in turn mobilizes heavy metals from the environment, in the mine or in the river courses from the sediments (Munnik et al., 2010). In the present investigation, it has also been observed that many discarded dumps, stockpiles as well as abandoned pits remain closely associated with the river water course without any water barriers. River water near the coal mining area receives huge amounts of AMD waste, derived directly or indirectly from the atmospheric deposition of airborne emissions, which gets contaminated with complex, ill-defined mixtures of chemicals. Trucks which are used to transport coal may all affect air and water quality of certain area of the river. Most freshwater organisms will be exposed, to varying degrees, to this contamination and little is known about whether or not species are adversely affected by the chemicals present in their environment (Sumpter, 2009). One of the important consequences of incomplete coal combustion is formation of polycyclic aromatic hydrocarbons (PAHs). Many PAHs are carcinogenic, mutagenic, and/or toxic for reproduction (Crone and Tolstoy, 2010). In coal mining areas, PAHs mostly enter the environment

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through dusts. In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, there is a suite of fish biomarkers which may be examined. Genotoxic parameters are currently among the most valuable fish biomarkers for environmental risk assessment (Van der Oost et al., 2003). The count of MN has served as an index of chromosome breaks and mitotic spindle dysfunction (Bombail et al., 2001). The advantages of micronucleus test are its simplicity, reliability, and sensitivity. It is widely employed to assess the biological impacts of aquatic pollutants (De Flora et al., 1993; Minissi et al., 1996; Ayllon and Garcia-Vazquez., 2000; Vigano et al., 2002). Different types of chemicals and radiations have been reported to be responsible for various types of aberration in chromosomes by which clastogenic properties can be detected. The comet assay or single cell gel electrophoresis has also found wide application as a simple and sensitive method for evaluating DNA damage in fish exposed to various xenobiotics in the aquatic environment (Dhawan et al., 2009 and Frenzillli., 2009).

The advantage of fish as a suitable model for monitoring aquatic genotoxicity is their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Cordeiro., 2000). Genotoxicity assays in fish have not yet been reported to evaluate the genotoxic impact of AMD from coal mining affected areas of the Simsang River. Several eco-toxicological characteristics of the air-breathing freshwater fish *Channa punctata* have already been reported due to its wide distribution and abundance throughout the year, easy maintenance in the wet laboratory as well as presence of 32 well-differentiated diploid chromosomes making this species an excellent model for toxicity studies (Kumar et al., 2010). Therefore, the present study aims to assess the genotoxic effects on fish (*Channa punctata*) exposed to the water of Simsang River contaminated by AMD of coal mines through comet assay, micronucleus and chromosomal aberration test.

2. Materials and methods

2.1. Sampling sites

Three sampling sites were selected in the Simsang River, (Fig. 1) with different degree of coal mining impact. Sampling site 1, Near Rombagre, (S_1, {longitude 90°34′21″E and latitude 25°32′41″N} free from coal mining activities and was used as the reference site). The two other sites were Nangalbibra (S_2, {longitude 90°44′39″E and latitude 25°28′22″N} maximum coal mining activities are practiced (Fig. 2) in the hills of the vicinity) and Baghmara (S_3, {longitude 90°37′9″E and latitude 25°12′1″N} coal dumping activities are found at the bank of the River) in the downstream. Samples were collected four times in each season from each sampling site.

2.2. Test organism

Fresh water fish, *Channa punctata* (Bloch) 17.5 ± 2.2 g (mean $\pm$ SD) was selected for the exposure tests because of its easy availability in the river and its high tolerance capacity to extreme harsh environmental condition. The specimens were obtained from a local fish farm. Prior to experiments, the fishes were acclimatized for 7 days in 300 L tanks with non-chlorinated water at room temperature. During acclimatisation, the fishes were fed with commercial food at two days interval and feeding was suspended 24 h prior to the toxicity tests and they were then released into the aquariums with water from each sampling site within 4 h after collection of the sample water. Static toxicity tests were performed for a period of 20 days. All tests were carried out for three times and number of fishes used for each test was 10.

2.3. Physico-chemical characteristics of water

Immediately after collection, water samples were analyzed to determine the value of pH, salinity, dissolved oxygen (DO), free carbon-di-oxide (FCO_2), alkalinity, conductivity, total dissolved solid (TDS), sulphate, lead, chromium, copper, nickel, iron and zinc

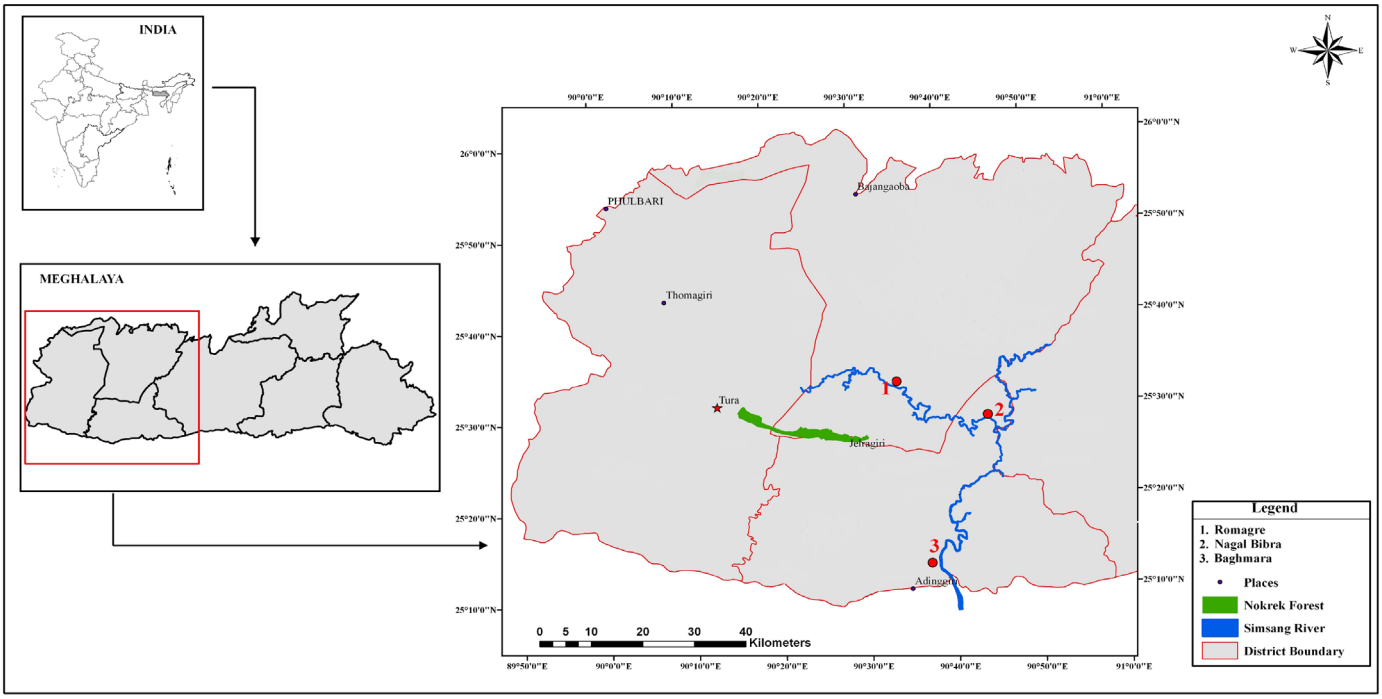

Fig. 1. Map showing flow route of Simsang River along with sampling site.

Fig. 2. Coal deposits on bank and bed of the river (a, b and c); one of the coal excavation point near bank of Simsang River at Nongal Bibra.

followed after APHA (2005). After acid digestion of water sample followed after the method of APHA (2005), heavy metal concentrations were quantified using Atomic Absorption Spectrophotometer (Varian SpectrAA-220 AAS). For PAH analysis, water samples (2.5 L) were collected in glass bottles from the surface and 50 cm below the water level. The bottles were covered with screw caps and were immediately transported to the laboratory for analysis. In the laboratory, processing of water sample was done using liquid-liquid extraction (LLE) as described in APHA (2005), the total amount of surface water sample (800 ml) was filtered with Whatman filter paper (i.d. 70 mm) to remove debris and suspended materials and then poured into a 2 l funnel. For the first LLE, the mixture of 100 ml n-hexane and dichloromethane (1:1 v/v) was added and shaken for about 2 min before two phase separation. The water-phase was drained from the funnel into a 1000 ml beaker. The organic-phase was carefully poured into a glass funnel containing 20 g of anhydrous sodium sulphate. Following the second and third LLE, the water-phase was poured back into the separatory funnel to re-extract with 50 ml of the same solvent mixture. The extract was concentrated upto the volume of 2 ml under a gentle stream of nitrogen using rotary evaporator and then analyzed with Gas Chromatograph (Siriwong et al., 2009). Saturated aliphatic hydrocarbons were eluted with 20 ml of n-hexane and then aromatic hydrocarbons were eluted with 30 ml of a mixture of hexane and dichloromethane (90:10) (v/v). The volume of the eluted fraction was reduced to 1 ml and then the aromatic hydrocarbon fraction was injected into a gas liquid chromatography equipped with a flam ionization detector (GC/FID) (Nasr et al., 2010). GC analysis was conducted on a fused silica capillary column of 60 m length, 0.25 mm id and 0.5 μm film thicknesses to detect 16 PAH components. The following PAHs were used for quantitation: naphthalene acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo [a] anthracene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, dibenzo [a,h] anthracene, benzo [g,h,i] perylene, Indeno [1,2,3-cd] pyrene. Recoveries were carried out by the addition of PAHs standards mixture at the three levels of 1, 5 and 10 μg. All data were corrected according to the recovery percentage values. Ratio of phenanthrene to anthracene (pH/An) and fluoranthene to pyrene (Fl/Py) have been widely used to distinguish petrogenic and pyrogenic (pyrolytic) sources of PAHs (Magi et al., 2002, Chen et al., 2006; Yunker et al., 2002). PAHs of petrogenic origin are generally characterized by pH/An values >10, whereas combustion processes often result in low pH/An ratios (<10). For the Fl/Py ratios, values greater than 1 have been used to indicate pyrolytic origins and values less than 1 are attributed to petrogenic source (Qiu et al., 2009).

2.4. Micronucleus test (MN)

Analyses of MN were performed (Fenech., 1993) with the erythrocytes of fish after exposures. Blood was collected at different time interval and was smeared, dried overnight, fixed with methanol for 10 min and stained with 8% Giemsa in phosphate buffer. A total of 3000 erythrocytes per fish were examined under Leica make (DM3000) bright field microscope (1000 × magnification). MN scoring was obtained following the method of Fenech et al. (2003).

2.5. Comet assay

After the exposure period, alkaline (pH >13) comet assay was performed adopting the method of Singh et al. (1988) with certain modifications. Clean conventional microscopic slides were prepared by covering them with a thin layer of 1% (w/v) NMP agarose. In each slide, cell suspension mixed with LMP agarose gel (1:10v/v) was then spread on the base layer of the slide and a cover slip was added above it, the agarose gel being allowed to solidify by placing the slide in refrigerator for 10 min When the gel got solidified, the cover slips were gently removed and a second layer of LMP agarose (0.05%) was placed over it and cover slip was added and allowed to solidify. After removing the cover slips, slide were placed in lysis solution at 4 °C for 1 h in dark to remove cellular proteins. After lysis, slide was placed in an electrophoresis box containing an alkaline solution (pH >13) for 15 min at 4 °C and then subjected to electrophoreses at 25 V, 300 mA for 15 min Then the slide was removed, neutralized, fixed in methanol and stained with ethidium bromide (20 g ml^{-1}) and was examined with the help of Leica make Microscope (DM 3000) equipped with a green excitation filter (450–490 nm) and a barrier filter of 515 nm at 400 × magnification. The extent of DNA damage quantified by the length of DNA migration was visually determined in 100 randomly selected and non-overlapping cells per fish. The DNA damage quantified by visual classification of cells into five categories 'comets' corresponding to the tail length: undamaged (Anderson et al., 1994), Type 0; low-level damage, Type 1; medium-level damage, Type 2; high-level damage, Type 3 and complete damage, Type 4, as demonstrated in Fig. 3. The extent of DNA damage has been expressed as the mean percentage of cells with medium, high and complete damaged DNA, which has calculated as the sum of cells with damage Types II, III and IV (Palus et al., 1999). From the arbitrary values assigned to the different categories (from Type 0=0 to Type IV=4), a genetic damage index (GDI) was calculated for each subject (Pitarque et al., 1999).

2.6. Chromosomal aberration

Chromosomal aberration test was performed following the method of Bertollo et al. (1978). After exposure for specified time of treatment, colchicine was injected intramuscularly to each fish. After 2 h of colchicine treatment, the kidney tissue was homogenized in hypotonic solution to prepare cell suspension. The cell

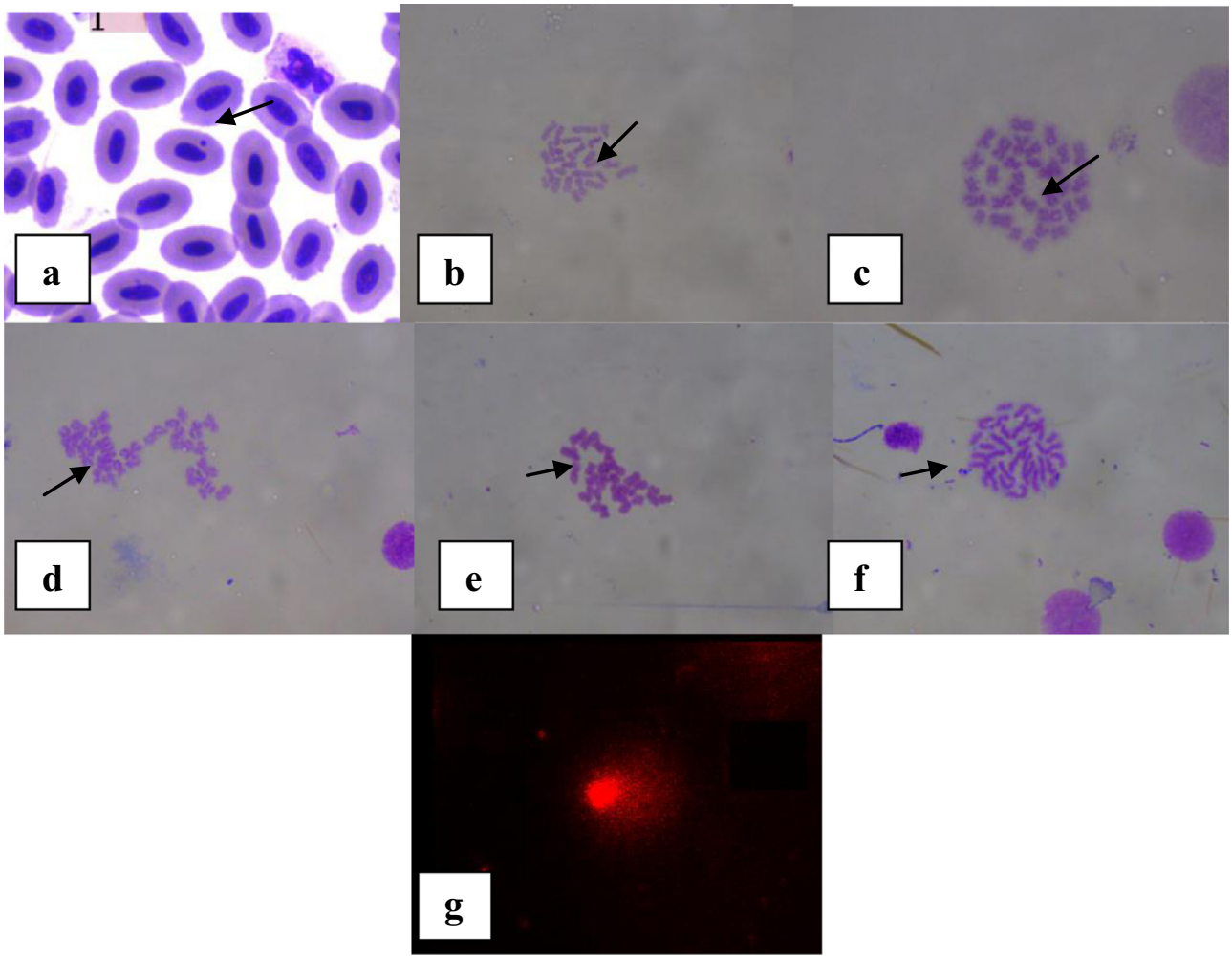

Fig. 3. Arrow indicates **(a)** Micronucleus**; (b)** chromatid gape; **(c)** chromatid breaks; **(d)** Ring formation; **(e)** Decondensation; **(f)** End to end joining; **(g)** Type 3; high-level damage, observed in comet assay.

suspension was poured in 15 ml centrifuge tube and incubated for 1 h at room temperature for optimum swelling of cells. Hypotonic action was stopped by adding freshly prepared chilled Carnoy's fixative. Cell suspension was centrifuged at 1200–1500 rpm for about 10 min at room temperature to get cell pellet. 2 or 3 washings were required to obtain clear cell suspension (Parveen and Shadab., 2012). Flame drying technique was used for slide preparation. After ageing of slides for about one night at room temperature, slides were stained in 5% Giemsa in phosphate buffer (pH 6.8). Detectable and finely spread 30 metaphase plates (under 1000 × magnification) from each individual were scored for alterations.

2.7. Statistical analysis

Statistical analyses were performed using ANOVA, followed by Duncan's multiple comparison tests when appropriate. All analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 18.0 at $p \leq 0.05$ significance level.

Table 1
Physico-chemical parameters present in the water samples collected from Simsang River in different sampling periods (November 2013–January 2015).

Sampling period	Sampling site	Temp (°C)	pH	DO (mg L^{-1})	FCO_2 (mg L^{-1})	Sulphate (mg L^{-1})	Fe (μg L^{-1})	Ni (μg L^{-1})	Pb (μg L^{-1})	Mn (μg L^{-1})	Cd (μg L^{-1})	Zn (μg L^{-1})	Cr (μg L^{-1})
Pre-monsoon	S1	24	7.9	9.2	3.0	33	0.06	0.003	0.007	0.03	ND	0.02	0.004
	S2	25	5.6	5.3	10	139	2.3	0.02	0.06	0.3	0.009	1.9	0.03
	S3	27.9	7.2	7.8	4.2	30	0.07	0.006	0.01	0.01	0.001	0.9	0.02
Monsoon	S1	23.7	7.2	8.2	4.0	23	0.1	0.002	0.02	0.02	0.001	0.05	0.001
	S2	25.6	4.6	4.8	24	270	7.2	0.09	0.23	0.9	0.08	5.1	0.07
	S3	28	6.8	6.7	4.0	97	0.13	0.005	0.03	0.12	0.009	0.07	0.01
Ret. monsoon	S1	25	7.3	8.9	2.8	25	0.03	0.002	0.003	0.06	ND	0.003	0.006
	S2	26	4.9	4.9	12	150	3.9	0.07	0.07	0.35	0.01	2.9	0.05
	S3	28.3	6.9	7.5	3.4	76	0.1	0.008	0.005	0.003	0.002	0.02	0.03
Winter	S1	21	7.8	8.6	2.6	45	0.05	0.005	0.004	0.02	0.001	0.009	0.009
	S2	23	5.2	4.6	10.5	190	3.6	0.08	0.003	0.4	0.025	2.9	0.06
	S3	24.5	7.2	6.5	3.4	87	0.4	0.007	0.006	0.09	0.002	0.2	0.004

3. Results

Physical–chemical parameters of water: The results of physico-chemical parameters of water for the three sampling sites are depicted in Table 1. Results showed low pH, DO and relatively high level of sulphates and some heavy metals in the S_2. The heavy metals Fe, Pb, Ni, Mn and Zn were detected above the permissible limit in site 2 and concentration was found higher in monsoon. The occurrence of Zn, Cr, and Ni was less frequent. At site 1 and 3, these heavy metals showed lower concentrations than the permissible levels established by WHO guidelines (WHO, 2008). The concentrations of the 16 detected PAHs in surface water of Simsang River are shown on Table 2. In terms of individual PAH composition in water, most compounds analyzed were detected in site 2. Benzo[a]anthracene was detected in water sample with mean value (ng L^{-1}) in three sites were 1.0, 22.7 and 1.8, respectively. It has been observed that the concentration of low molecular weight (4–6 ring) polycyclic aromatic hydrocarbons (HPAHs) was maximum than that high molecular weight (2–3 ring) PAHs (LPAHs).

Micronucleus test: Micronuclei were observed in the site 2 water treated group (Fig. 3a) and there was a significant difference in micronucleus frequency among fish populations exposure to affected river water in different sampling period. Increase in micronucleus frequency was observed in fish populations exposed to affected river water collected from site-2, but statistical significance at $P < 0.05$ was noted mostly in monsoon (Table 4).

Comet assay: Results of comet assay were depicted in Table 3 shows the comparison of Comet assay damage index in different sampling sites along with results of ANOVA. In site 2, fish group shows a significant increase in DNA migration (Fig. 3g), maximum observed in monsoon season. However, significant difference has been estimated in DNA migration in different sampling season at sampling site 2 ($p=0.34$).

Chromosome aberration: A significant increase in clastogenic damage was found in water collected from site 2. Table 4 represents a summary of the results of chromosomal aberrations. Analysis of chromosome aberrations showed structural alterations (e.g. gaps and breaks) but no numerical changes were observed, although de-condensation of chromatin on chromatid arms and ring formation was observed (Fig. 3).

Table 3.
Damage index estimated by the comet assay (mean ± standard deviation) in *Channa punctatus* exposed for 20 days in water samples collected from the Simsang River.

Sampling site	Sampling period				
	Pre-monsoon	Monsoon	Retreating monsoon	Winter	*p*
S_1	0.1 ± 0.05	0.22 ± 0.02	0.35 ± 0.04	0.17 ± 0.02	0.008
S_2	0.68 ± 0.01	1.67 ± 0.04	1.15 ± 0.04	0.69 ± 0.04	0.34
S_3	0.39 ± 0.03	0.8 ± 0.22	0.4 ± 0.17	0.29 ± 0.02	0.06
p	0.16	0.31	0.34	0.06	

P values referred to ANOVA between sampling sites in the same season and between seasons at the same sampling site.

4. Discussion

As a consequence of extraction of coal, a large quantity of toxic materials in the form of AMD is released into River waters. Due to the elevated concentrations of metals present in many types of wastewaters, metals are ubiquitous contaminants in aquatic ecosystems (Roche and Boge., 1996); so they are among the most intensely studied contaminants. They not only deteriorate the equilibrium of the aquatic body, but they also disrupt the food web and bring about morphological, physiological and cytogenetic changes in aquatic inhabitants. Genotoxic studies on aquatic organisms exposed to polluted waters containing heavy metals have implicated DNA damage (Vargas et al., 2001; Matsumoto et al., 2006, Yadav and Trivedi., 2009, Barbosa et al., 2009). Fishes are very sensitive to any change in their environment and can play significant role in assessing potential risk associated with contamination in aquatic environment (Lakra and Nagpure., 2009). As compared to mammals, the DNA repair was reported to be slower in fishes (Espina and Weiss., 1995). Therefore, fishes might be used as sentinel or surrogate species for the evaluation of genotoxic chemicals in the environment and their risk to human health (Sklarew, 1993).

The PAHs in water samples of site 2 originate from pyrolytic sources, while in first and third stations originate from both of pyrolytic and petrogenic sources (pyrolytic sources are more dominant). This was consistent with the results of the Langat River; Peninsular Malaysia (Riyahi Bakhtiari et al., 2009) might be

Table 2
Concentrations of PAHs (ng L^{-1}) in surface water of the Simsang River in different sampling periods (November 2013–January 2015).

	Site 1		Site 2		Site 3	
Components	Mean	Range	Mean	Range	Mean	Range
Naphthalene	1	0.03–1.5	8.8	3.1–15.2	1.4	0.9–2.16
Acenaphthylene	0.8	0.3–1.22	7.2	5.4–13.92	1.0	0.6–1.9
Acenaphthene	0.8	0.31–1.3	7.6	5.2–14.21	1.3	0.7–3.1
Fluorene	0.5	0.08–1.04	5.9	4.71–10.07	0.9	1.4–2.1
Phenanthrene	0.5	0.08–1.33	6.1	4.44–10.3	0.9	1.2–2.4
Anthracene	1.1	0.9–1.9	8.3	6.29–15.73	1.07	1.6–2.8
Total LPAH	4.7	1.7–8.3	44	29.14–79.4	6.6	4.8–14.4
Fluoranthene	1.8	1–2.1	18.9	12.3–34.8	2.3	0.8–3.6
Pyrene	1.6	0.8–1.9	12.3	16.45–39.4	2.1	1.1–3.5
Chrysene	0.9	0.08–1.5	15.8	11.02–22.8	1.6	0.8–2.9
Benzo[a]anthracene	1	0.9–1.7	22.8	16.71–32.1	1.8	1–2.7
Benzo[b]fluoranthene	0.3	0.04–0.5	20.3	16.3–24.9	1.0	0.6–1.8
Benzo[k]fluoranthene	0.2	0.06–0.8	13.9	9.1–17.5	0.9	0.1–1.4
Benzo[a]pyrene	0.33	0.06–0.8	17.5	12.04–25.6	0.9	0.2–1.6
Dibenzo[a,h]anthracene	0.07	0.01–0.3	19.7	11.02–26.3	0.8	0.2–1.7
Benzo[g,h,I]perylene	0.08	0.01–0.2	22.6	16.17–30.5	0.9	0.23–1.8
Indeno[1,2,3-cd]pyrene	0.03	0.01–0.06	6.7	4.07–10.1	0.6	0.1–0.9
Total HPAH	6.33	2.97–9.9	170.5	125.18–264	12.9	5.1–21.8
Total	11.1	4.67–18.21	214.5	54.32–343.5	19.5	9.95–36.2

Table 4.
Micronuclei frequency (mean ± standard deviation) and chromosomal aberration (mean ± standard deviation) in *Channa punctatus* exposed for 20 days in water samples collected from the Simsang River.

Site	Micronucleus test					Chromosomal aberration				
	Sampling period					Sampling period				
	Pre-monsoon	Monsoon	Retreating monsoon	Winter	*p*	Pre-monsoon	Monsoon	Retreating monsoon	Winter	*p*
S_1	0.2 ± .45	0.3 ± 0.2	0.4 ± 0.25	0.3 ± 0.2	0.11	0.6 ± 0.1	0.71 ± 0.2	1.76 ± 0.5	1.9 ± 0.35	0.46
S_2	3 ± 1	4.3 ± 1	4.9 ± 0.9	5.2 ± 0.8	0.45	13.3 ± 1.4	4.9 ± 1.98	18.73 ± 0.5	28.5 ± 6.8	0.59
S_3	0.3 ± .5	1.5 ± 0.4	0.6 ± 0.9	1.4 ± 0.4	0.004	3.89 ± 1.8	5.56 ± 1.24	4.8 ± 0.9	1.9 ± 0.4	0.35
p	0.008	0.002	0.001	0.0006		0.004	0.003	0.009	0.09	

P values referring to ANOVA between sampling sites in the same season (last line) and between seasons at the same sampling site (last column).

due to presence of coal mining activities near the river bank at these sites. The physico-chemical attributes of river water has also been estimated low pH, DO and high concentration of sulphates, and presence of some heavy metals above the permissible limit in the coal mining affected site of the Simsang River (S_2) which clearly indicates accumulation of AMD in the river. This results was reflected by the genotoxicity test estimated by the micronucleus, chromosomal aberration test and comet assay which showed a relation to the water parameters in different sampling period. Chromosomal damage represented as micronucleus formation resulting from inefficient or incorrect DNA repair and/or from the physical presence of metals around the mitotic apparatus, is expressed during cell division and represents an index of accumulated genotoxic agents (Kligerman., 1982). In the present investigation, differences in MN formation between and within the study sites in different season displaying different levels of AMD bioaccumulation. The variation in MN frequency is dependent on the environmental stress and could be related to the type and concentration of pollutants in that location (Kligerman., 1982). This significant variation could be attributed to the toxico kinetics of the pollutant and the speed of the haemopoietic cycle of the fish species (Kligerman., 1982). In this context, the increase of tailed DNA migration in comet assay in monsoon season reflect a faster metabolism in relation to the higher temperature with increase in other water chemical parameters, could have masked the effect of AMD in the other seasons. The frequency of aberrant cells was high in the water collected from the effluent discharge point (S_2) and downstream sites (S_3) (Table 2) which might be the results of higher concentration of AMD accumulation. In our experiment, most frequent aberrations observed were decondensation. AMD significantly increase DNA damage, principally by inducing single strand breaks that could possibly initiate double strand breaks, as a result of inactivation or alternation of repair mechanisms (Obe et al., 2002).

The result suggests that in monsoon season, the potency of genotoxicants in the river was maximum.it may be due to flowing of AMD mixed water from nearby coal field at the river bank which was reflected by the genotoxicity test. Fish treated with river water (S_2) scored statistically higher values in the piscine micronuclei and chromosome aberration in the affected site but showed a low level of tailed nucleus in the comet assay test. In this paper, attempt has been made to present results based on the use of three end-points to investigate the effects of coal mining on indigenous fishes of the river. The damage detected by the comet assay of the water collected from the S_2 was significantly different from that of S_1 of the river where maximum coal mining is being practised. Steps should be taken to modernise mining operations such as good planning and environmental management to minimise the impact of coal mining on the environment and help to protect biodiversity. Public awareness and clean coal technologies should be developed and used to limit particulate emissions, waste from coal production, trace elements, NOx, SOx and CO_2, limiting the negative effects of coal production and its use.

5. Conclusion

The findings highlighted the value of genotoxicity biomarkers as sensitive parameters of environmental pollutant contamination and their importance in the biomonitoring of aquatic ecosystem. The contaminants in aquatic environments seldom occur as single chemicals, but the mixture of some metals detected in our study and other classes of environmental contaminants are likely to induce genotoxic damage in the study area. Simsang River basin has been receiving continuous flow of untreated coal mining effluents for the last few decades which have threatened the aquatic biota of the river. Therefore; it is felt that necessary steps should be taken for conservation of the ecosystem.

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References

Anderson, D., Yu, T.W., Phillips, B.J., Schezer, P., 1994. The effect of various antioxidants and other modifying agents on oxygen-radical generated DNA damage in human lymphocytes in the comet assay. Mutat. Res. 307, 261–271.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater. 17th ed., American Water Works Association and Water Pollution Control Federation, New York, USA.

Ayllon, F., Garcia-Vazquez, E., 2000. Induction of micronuclei and other nuclear abnormalities in European minnow *Phoxinus phoxinus* and mollie Poecilia latipinna: an assessment of the fish micronucleus test. Mutat. Res. 467, 177–186.

Barbosa, J.S., Cabral, T.M., Ferreira, D.N., Agnez-Lima, L.F., Batistuzzo De Medeiros, S. R., 2009. Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals. Ecotoxicol. Environ. Saf. . http://dx.doi.org/10.1016/j.ecoenv.2009.10.008

Bertollo, L.A.C., Takahashi, C.S., Moreira-Filho, O., 1978. Cytotaxonomic considerations of Hoplias lacerdae (Pisces, Erythrinidae). Brazil. J. Genet. 1, 103–120.

Bombail, V., Gordon, E., Batty, J., 2001. Application of the comet and micronucleus assays to butterfish (*Pholis gunnellus*) erythrocytes from the Firth of Fourth, Scotland. Chemosphere 44, 383–392.

Chen, S.J., Luo, X.J., Mai, B.X., Sheng, G.Y., Fu, J.M., Zeng, E.Y., 2006. Distribution and mass inventories of polycyclic aromatic hydrocarbons and organochlorine pesticides in sediments of the Pearl River Estuary and the Northern South China Sea. Environ. Sci. Technol. 40 (3), 709–714.

Crone, T.J., Tolstoy, M., 2010. Magnitude of the 2010 Gulf of Mexico oil leak. Science 330 (6004), 634.

De Flora, S.L., Vigano, F., Agostini, A.D., Camoirano, M., Bagnasco, C., Bennecelli, F., Melodia, F., Arillo, A., 1993. Multiple genotoxicity biomarkers in fish exposed in-situ to polluted river water. Mutat. Res. 319, 167–177.

Dhawan, A., Bajpayee, M., Parmar, D., 2009. Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol. Toxicol. 25, 5–32.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque,

C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182.
Espina, N.G., Weiss, P., 1995. DNA repair in fish from polluted estuaries. Mar. Environ. Res. 39, 309–312.
Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E., 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis block micronucleus assay using isolated human lymphocyte cultures. Mutat. Res. 534 (1–2), 65–75.
Fenech, v, 1993. The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. Mutat. Res. 285, 35–44.
Frenzillli, G., Nigro, M., Lyons, B.P., 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat. Res. 681, 80–92.
Grisolia, C.K., Cordeiro, C.M.T., 2000. Variability in micronucleus induction with different mutagens applied to several species of fish. Genet. Mol. Biol. 23, 235–239.
Kligerman, D., 1982. Fishes as biological detectors of the effects of genotoxic agents. In: Heddle, J. (Ed.), Mutagenicity: New Horizons in Genetic Toxicology. Academic Press, New York, USA, pp. 435–456.
Kumar, R., Nagpure, N.S., Kushwaha, B., Srivastava, S.K., Lakra, W.S., 2010. Investigation of the genotoxicity of Malathion to freshwater teleost fish *Channa Punctatus* (Bloch) using the micronucleus test and comet assay. Arch. Environ. Contam. Toxicol. 58, 123–130.
Lakra, W.S., Nagpure, N.S., 2009. Genotoxicological studies in fishes: a review. Indian J. Anim. Sci. 79, 93–98.
Magi, E., Bianco, R., Ianni, C., Di Carro, M., 2002. Distribution of polycyclic aromatic hydrocarbons in the sediments of the Adriatic Sea. Environ. Pollut. 119, 91–98.
Matsumoto, S.T., Mantovani, M.S., Malaguttii, M.I.A., Dias, A.U., Fonseca, I.C., Marin-Morales, M.A., 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. Genet. Mol. Biol. 29, 148–158.
Minissi, S., Ciccotti, E., Rizzoni, M., 1996. Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, Pisces) from two natural environments: a bioassay for thein situ detection of mutagens in freshwater. Mutat. Res. 367, 245–251.
Munnik., V. (Mvula Trust), Hochmann., G. (Mvula Trust), Hlabane., M. (SA Green Revolutionary Council), Law., S. (Environmental Monitoring Group), 2010. The Social and Environmental Consequences of Coal Mining in South Africa – A Case Study.
Nasr, I.N., Arief, M.H., Abdel-Aleem, A.H., Malhat, F.M., 2010. Polycuclic aromatic hydrocarbons (PAHs) in aquatic environment at El Menofiya Governorate, Egypt. J. Appl. Sci. Res. 6 (1), 13–21.
Obe, G., Pfeiffer, P., Savage, J.R.K., Johannes, C., Goedecke, W., Jeppensen, P., 2002. Chromosomal aberration: formation, identification and distribution. Mutat. Res. 504, 17–36.
Palus, J., Dziubaltowska, E., Rydzynski, K., 1999. DNA damage detected by the comet assay in the white blood cells of workers in a wooden furniture plant. Mutat. Res. 444, 61–74.
Parveen, N., Shadab, G.G.H.A., 2012. Cytogenetic evaluation of cadmium chloride on *Channa punctatus*. J. Environ. Biol. 33, 663–666.
Pitarque, M., Creus, A., Marcos, R., Hughes, J.A., Anderson, D., 1999. Examination of various biomarkers measuring genotoxic endpoints from Barcelona airport personnel. Mutat. Res. 440, 195–204.
Qiu, Y.W., Zhang, G., Liu, G.Q., Guo, L.L., Li, X.D., Wai, O., 2009. Polycyclic aromatic hydrocarbons(PAHs) in the water column and sediment core of Deep Bay, South China. Estuar. Coast. Shelf Sci. 83, 60–66.
Riyahi Bakhtiari, A., Zakaria, M.P., Yaziz, M.I., Hj Lajis, M.N., Bi, X., 2009. Polycyclic aromatic hydrocarbons and n-alkanes in suspended particulate matter and sediments from the Langat River, Peninsular Malaysia. Environ. Asia 2, 1–10.
Roche, H., Boge, G., 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. Mar. Environ. Res. 41, 27–43.
Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.
Siriwong, W., Thirakhupt, K., Sitticharoenchai, D., Rohitrattana, J., Thongkongown, P., Borjan, M., Robson, M., 2009. DDT and derivatives in indicator species of the aquatic food web of Rang sit agricultural area, Central Thailand. Ecol. Indic. 9 (5), 878–882.
Sklarew, M., 1993. Toxicity tests in animals: aternative models. Environ. Health Perspect. 101, 288–291.
Sumpter, J.P., 2009. Protecting aquatic organisms from chemicals: the harsh realities. Philos. Trans. R. Soc. A – Math. Phys. Eng. Sci. 367, 3877–3894.
Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Vargas, V.M.F., Migliavacca, S.B., Melo, A.C., Horn, R.C., Guidobono, R.R., Ferreira, I.C. F.S., Pestana, M.H.D., 2001. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. Mutat. Res. 490, 141–158.
Vigano, L., Camoirano, A., Izzotti, A.A., Agostini, F.D., Polesello, S., Francisci, C., De Flora, S., 2002. Mutagenicity of sediments along the Po River and genotoxicity biomarkers in fish from polluted areas. Mutat. Res. 515, 125–134.
WHO (World Health Organization), 2008. 3rd ed.WHO Guidelines for Drinking Water Quality 1. World Health Organization, Geneva.
Yadav, K.K., Trivedi, S.P., 2009. Chromosomal aberrations in a fish, *Channa punctata* after in vivo exposure to three heavy metals. Mutat. Res. 678, 7–12.
Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 33 (4), 489–515.