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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 ± 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

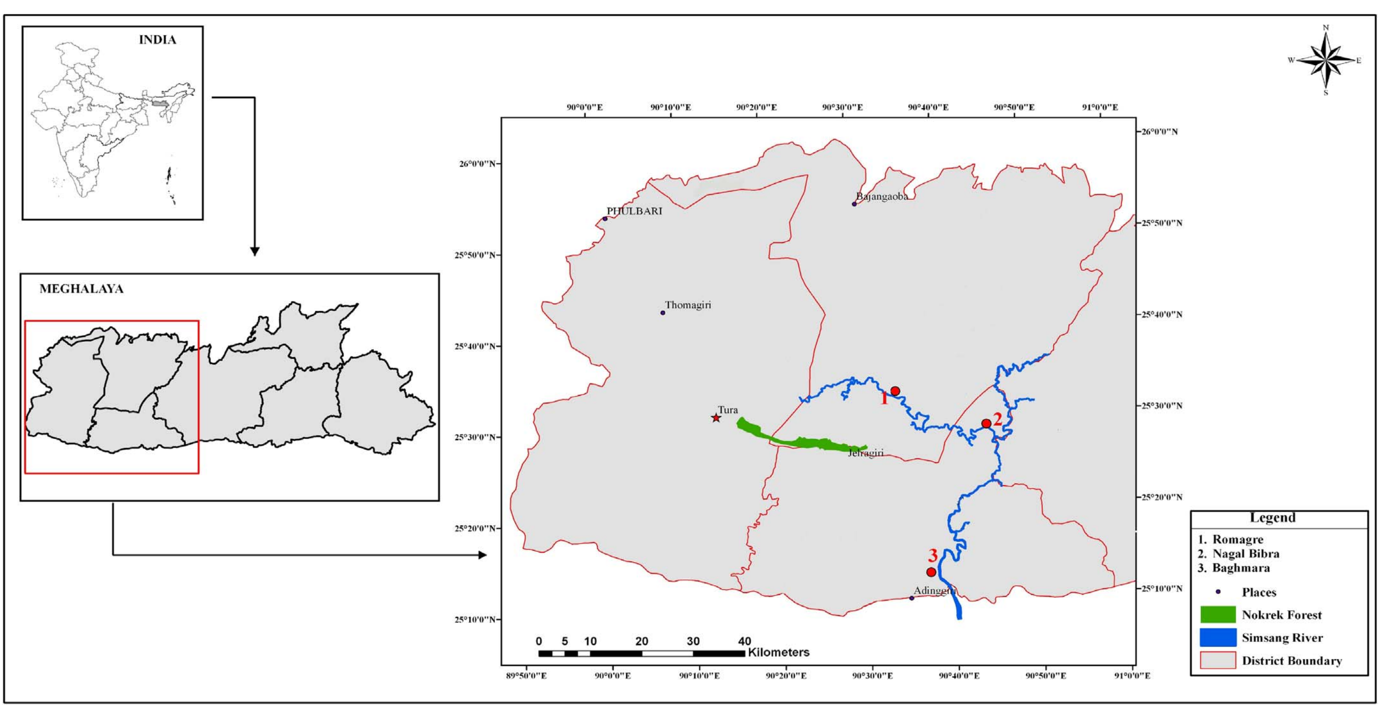

Fig. 1. Map showing flow route of Simsang River along with sampling site.