to the site come from the local people themselves (Figure 3 *d–f*) because the site lies in the middle of an agricultural farmland (Figure 1). Also, the site is on the verge of destruction. Villagers use this site for grazing of their cattle and thus are destroying the eye-pleasing structures. However, proper preservation will help conserve this as a geoheritage site. It is proposed that concerned authorities declare this as a 'geoheritage' site for academicians, professionals and tourists. Time has come that we realize its importance and sustain our heritage of geological significance.

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Acid mine drainage, a potential threat to fish fauna of Simsang River, Meghalaya

Acid mine drainage (AMD) is formed when water and air come in contact with pyrite (FeS_2) present in coal and exposed rocks, to form sulphuric acid. The process of pyrite oxidation further leads to the formation of Fe^{3+} and some or all of this Fe^{3+} precipitates to cause red, orange or yellowish colour of the water. The Fe^{3+} precipitate also deposits at the bottom of the stream[1] to give black or orange coloured bed. Moreover, AMD is known to contain high levels of heavy metals, such as cobalt, aluminium, copper, nickel, manganese and lead[2]. Therefore, exposure of fishes to extremely low pH ($pH \leq 4.0$) and heavy metal causes mass fish kill, and eventually loss of fish biodiversity[3].

The coal deposits in Meghalaya, India along the southern fringe of Shillong plateau are distributed in Khasi, Garo and Jaintia hills[4]. Among these, the East Garo Hill region is a major producer of coal, and coal excavation is commonly done by primitive mining method known as 'rat-hole' mining[4].

In the present study, it has been observed that more than 100 km stretches of the Simsang River are severely affected due to coal mining (Figures 1 and 2). The Simsang River, while passing through Nongal Bibra, a small town in the East Garo Hill, receives a large amount of AMD. This river was once well known for its abundant fish faunal diversity, which has gradually declined over the years due to indiscriminate coal mining (estimated coal reserve is 359 million tonnes)[5]. The primary cause of degradation of water quality and decline in fish biodiversity in the water bodies of the mining area is attributed to AMD[6] that makes the water highly acidic and rich in heavy metal concentration. This 'acid flow' has altered the physico-chemical parameters of the environment, adversely affecting the health of rivers and streams. Many AMD-impacted water bodies have $pH < 4$, with high sulphur and as aluminium and iron contamination[7]. The contaminated water can be toxic to aquatic organisms except a few tolerant organisms. At low pH, the fish die due to acidaemia and toxicity of metals, especially aluminium that has been implicated as the primary toxicant[8]. Fish generally do not inhabit waters severely polluted by coal-mine drainage, because in the waters with $pH < 4.2$ CO_2 is present in its free form. Without buffering capacity from carbonates and bicarbonates, many aquatic animals would die due to acute acidaemia. Additional sources of toxicity of this water are the sulphate and salts of aluminium and iron. Recruitment failure is also a commonly reported cause of fish population decline associated with acidification[9].

In the present study, four sampling sites (William Nagar, Nongal Bibra, Siju and Baghmora) were selected in relation to drainage from upstream to downstream along the Simsang River, which flows to the south (Figure 2). William Nagar, situated upstream, is away from the coal-mining areas. But coal excavation is carried out adjacent to the river bank at Nongal Bibra and Siju. Though coal excavation is not carried out near the river bank at Bagmora downstream, it is one of the AMD receiving points, including Nongal Bibra and Siju along the Simsang River. Fish sampling was carried out 48 times (12 times at each site) at William Nagar (90°39′34″E and 25°28′44″N, 213 m amsl), Nongal Bibra (90°44′39″E and 25°28′22″N, 145 m amsl), Siju (90°45′22″E and 25°23′46″N, 138 m amsl) and Baghmara (90°38′22″E and 25°12′03″N, 20 m amsl). Cast net of similar weight and mesh size was operated 12 times at each sampling site by a single fisherman for 4 h. AMD samples were collected from Nongal Bibra. A total of 24 AMD samples were collected from August 2013 to December 2014. The pH, dissolve oxygen, temperature and conductivity were measured *in situ* using a multiparameter probe (HI

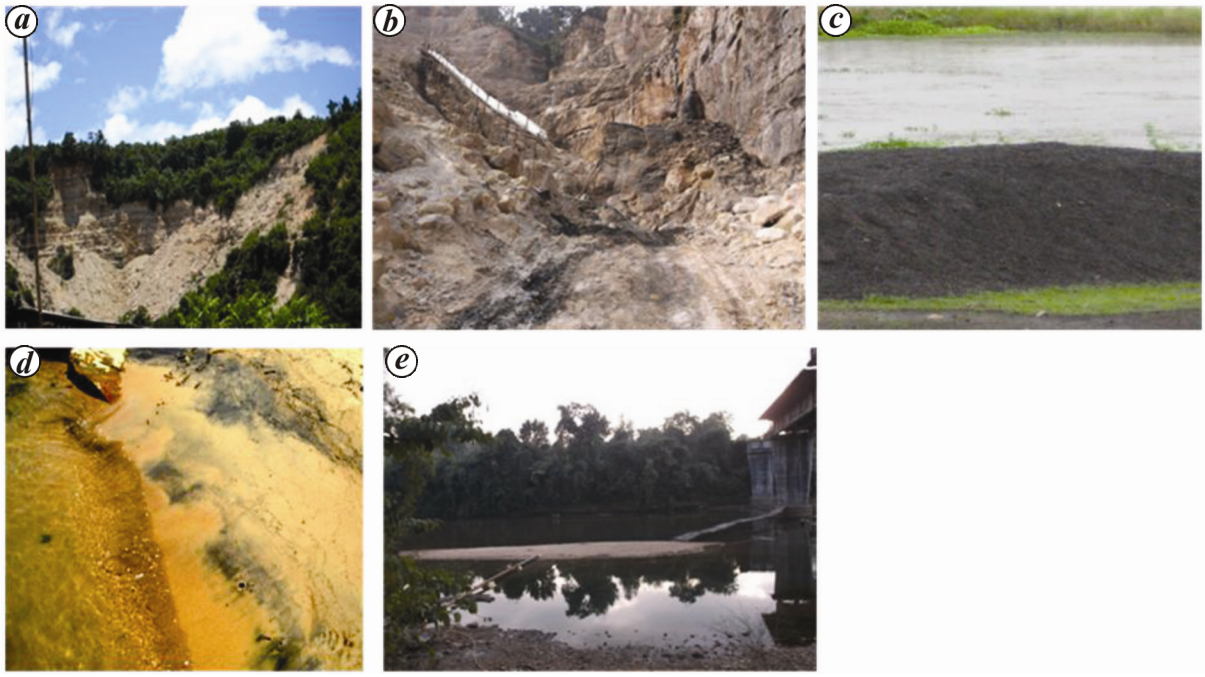

Figure 1. ***a***, ***b***, Coal excavation on the bank of Simsang River at Nongal Bibra. ***c***, ***d***, Coal deposits on bank and bed of the river. ***e***, One of the acid mine drainage (AMD) receiving points at Baghmara.

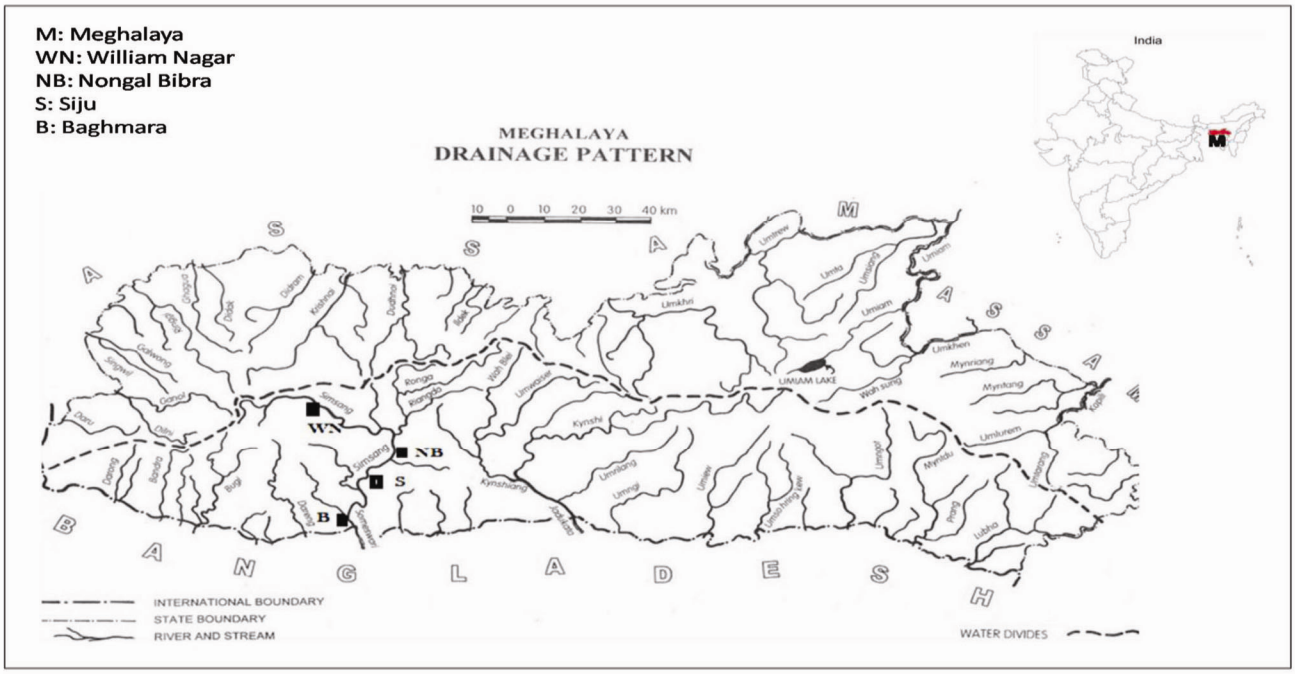

Figure 2. Location of sampling sites at Simsang River. WN, S and B, Sites for fish sampling; NB, Site for AMD sampling.

9828, Hanna Instruments). Biological oxygen demand was estimated by unseeded dilution method[10]. PO_4^{3-} and NO_3^- were estimated by ion chromatography and automated ascorbic acid methods respectively[11]. The concentrations of aluminium and iron were determined using an atomic absorption spectrophotometer (PerkinElmer Analyst 800). Sulphate concentration was determined following turbidimetric method[12], and K and Na were measured by flame photometer. The hardness and alkalinity were measured by titration method[13]. Total dissolved solid was measured by gravimetry method.

The concentrations of iron, aluminium and sulphate in AMD (Table 1) averaged 390, 46.21 and 1230 mg l^{-1} respectively. At this site, pH was very low (< 3.0) which explains the reason for such a high

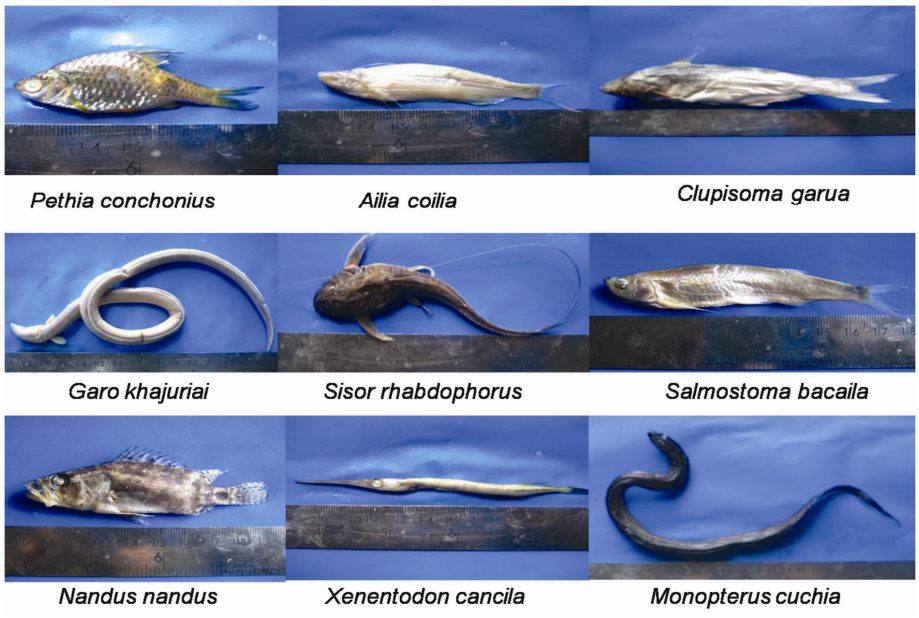

Figure 3. Fish species collected at sampling sites along the Simsang River.

Table 1. Water quality parameters of AMD at Nongal Bibra, Meghalaya. Sample size for parameters estimated (n) = 24

Parameters	Mean ± standard error	WQG*
Temperature (°C)	25.03 (± 3.20)	–
pH	2.40 (± 0.32)	6.5–8.5
Dissolved oxygen (mg l^{-1})	4.26 (± 0.93)	–
Conductivity (µm s^{-1})	136.00 (± 9.50)	–
Total dissolved solid (mg l^{-1})	68.00 (± 11.64)	500
NO_3^- (mg l^{-1})	4.18 (± 0.94)	45.0
PO_4^{3-} (mg l^{-1})	0.83 (± 0.08)	–
Alkalinity (mg l^{-1})	26.40 (± 3.61)	200
Hardness (mg l^{-1})	96.00 (± 4.82)	300
Sulphate (mg l^{-1})	1230 (± 98.32)	200
Potassium (mg l^{-1})	12.58 (± 0.59)	–
Fe (mg l^{-1})	390.16 (± 27.90)	0.3
Al (mg l^{-1})	46.21 (± 3.94)	0.03
Biochemical oxygen demand (mg l^{-1})	9.45 (± 2.80)	–

*National Water Quality Guidelines (WQG) values for drinking water[12].

level of trace metals in AMD and below the tolerable limit of fish fauna; thus at Nongal Bibra the fish fauna was completely absent. At low pH, heavy metals are more soluble and enter into the solution from limestone, clay, rocks and organic substances present in the mine drainage. In AMD from Nongal Bibra, the metal concentrations were equal to or greater than the levels usually considered toxic to most of the fish species.

In the present study, a total of 43 species belongs to 31 genera of 16 families were collected from both the AMD-affected sites and those not affected. Amongst the recorded species, 20 were identified as inhabitants of high-altitude streams and rivers along with some endemic species, *Garo khajuriai* and *Macrobrachium assamense*. But no fish catch was recorded from the major AMD-affected site, Nongal Bibra. The apparent low species diversity provides evidence that conditions in Simsang River are stressful and toxic. The IUCN Red list status of the fish species[14] (Figure 3) and fish catches at three sampling locations are given in Table 2.

A short-term exposure (8 and 10 days) of fingerlings of *Tor putitora* (Golden mahseer) as an experimental animal to 25% diluted AMD under laboratory conditions also shows its toxic effect at cellular and genetic level. For determination of hematology parameters, blood

Table 2. IUCN Red List threat status of fish species and their catch arranged in relation to drainage from upstream to downstream

Species/no. collected	WN	S	B
Endangered			
Tor putitora (22)	*	*	
Near threatened			
Ailia coila (15)		*	*
Tor tor (25)	*		
Garo khajuriai (13)		*	*
Vulnerable			
Leiodon cutcutia (11)	*	*	
Least concern			
Clupisoma garua (14)	*		*
Sisor rabdophorus (12)		*	
Schistura sikmaiensis (13)	*		
Glyptothorax cavia (11)	*		
Barilius barna (21)	*		*
Devario aequipinnatus (15)		*	
Monopterus cuchia (13)	*		
Puntius sophore (16)	*		
Xenentodon cancila (13)	*		*
Nandus nandus (9)	*		
Trichogaster labiosus (12)	*		*
Puntius terio (16)	*		
Channa striata (23)	*	*	*
Salmostoma bacaila (15)	*		*
Cabdia morar (9)	*	*	
Macrobrachium assamense (8)	*		
Data deficient			
Anabas testudineus (18)	*	*	
Channa gachua (14)	*	*	
Not assessed			
Pethia conchonius (9)	*		

*Recorded fish species at three sampling sites. WN, William Nagar; S, Siju; B, Baghmora.

Table 3. Blood parameters of golden mahseer, control (n = 12) and fishes exposed (n = 38) for 8 and 10 days to 25% of AMD

Blood parameters*	Fishes exposed		
	Control	8 days (mean ± SE)	10 days (mean ± SE)
Hematocrit (%)	37.4 (± 3.1)[a]	34.9 (± 2.0)[a]	35.9 (± 2.0)[a]
Erythrocytes ($\times 10^6$ μl^{-1})	1.59 (± 0.06)[a]	1.26 (± 0.04)[b]	1.03 (± 0.04)[b]
Immature cells (%)	0.20 (± 0.03)[a]	0.71 (± 0.06)[b]	0.86 (± 0.06)[b]
Thrombocytes ($\times 10^3$ μl^{-1})	5.43 (± 0.8)[a]	4.21 (± 0.72)[b]	3.31 (± 0.72)[b]
Total leukocytes ($\times 10^3$ μl^{-1})	5.31 (± 0.6)[a]	3.06 (± 0.14)[b]	2.61 (± 0.14)[b]
Neutrophils ($\times 10^3$ μl^{-1})	0.29 (± 0.09)[a]	0.09 (± 0.03)[b]	0.07 (± 0.03)[b]
Monocytes ($\times 10^3$ μl^{-1})	0.40 (± 0.16)[a]	0.31 (± 0.04)[a]	0.29 (± 0.04)[a]
Lymphocytes ($\times 10^3$ μl^{-1})	4.69 (± 0.18)[a]	2.09 (± 0.31)[b]	1.91 (± 0.31)[b]

Superscripts (a, b) in the same row show significant statistical difference ($P < 0.01$).

was taken from the caudal vein of randomly selected fish from both control and AMD-exposed groups using heparinized needles and syringe. Smear was prepared on cleaned glass slides immediately after sampling, air-dried, fixed with methanol and stained by May Grunwald Giemsa method. The slides were subsequently examined under a microscope (LEICA DM500). Erythrocytes, leukocytes and other blood cell counts were determined using a hemocytometer. Hemoglobin was determined within 2 h of sampling, using Drabkin's fluid (Qualigens, India). Differences among treatment groups were tested by one-way analysis of variance (ANOVA). A significant level of 0.01 was used and the statistical analysis was carried out using the software program SPSS, 19.0 (SPSS Inc, Chicago). AMD toxicity in genetic level was evaluated using DNA laddering assay, fluorimetric analysis of DNA unwinding[15] and comet assay[16], which are simple and sensitive techniques commonly applied to fish cells for detecting the genotoxicity over a wide range of chemicals. The metal-induced toxic effects mainly include DNA–protein cross-links, DNA strand breaks and oxidative DNA damage, etc. Moreover, heavy metals are positively charged ions and thus easily bind with DNA or nucleophilic sites to cause mutagenesis. The results show that total erythrocytes, thrombocytes, neutrophils, lymphocytes and leukocytes counts (Table 3) are significantly lower and the number of immature cells is significantly higher in the blood of AMD-exposed fish compared to control. Therefore, evaluating fish blood parameters might be a useful tool for understanding the impact of extreme water conditions on fish health. The presence of significantly higher number of circulating immature cells, as observed in the present study, suggests that AMD might be directly toxic to leukocytes, and it may mitigate immune function by reducing leukocyte population. Changes in size of the melano-macrophage centres (MMC) can occur in association with environmental pollutants. Liver of AMD-exposed fishes had apparent MMC that clearly indicates that even after dilution, the AMD water is highly toxic to fish. Therefore, MMC can provide sensitive indicators of stressful conditions in the aquatic environment of coal mine drainage area. Histological alterations were also observed at the level of the tubular epithelium and glomeruli. In the present study, tail length and percentage of tail DNA, the most frequent DNA damage indicators, were significantly increased when the fish were exposed to 25% AMD. The results suggest that AMD has the potential to induce DNA damage in the liver and kidney of fish at low dilution.

On the whole, the study establishes the deleterious effect of AMD generated from coal mine on the resident fish fauna of receiving water bodies through both field and laboratory observations. Specifically, the toxic properties of AMD have been demonstrated based on its negative impact on the abundance, diversity and distribution of fish species in the investigated field; and extensive alterations in blood parameters and DNA damage under simulated laboratory conditions. The findings underscore the need for policy makers to regulate coal excavation and other mining activities, and initiate efforts to restore degraded habitats and conserve native fish species.

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