

## DISTRIBUTIONAL PATTERN OF DIATOMS IN THE ACID MINE DRAINAGE IMPACTED STREAMS OF JAINTIA HILLS DISTRICT, MEGHALAYA, INDIA

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### ABSTRACT:

*Distributional pattern of diatoms have been considered as a tool to measure the status of water bodies in coal mine impacted streams of Jaintia Hills district of Meghalaya, a state in the North Eastern part of India. Streams in Jaintia Hills district, surrounding coal mine areas are seriously influenced by AMD (Acid Mine Drainage) due to unscientific and primitive "Rat Hole" method of coal extraction. To understand the extent of adverse effect of coal mining on the stream water quality, diatom communities have been used as biological indicator. Water parameter analysis showed significant differences in between unimpacted and impacted streams. In unimpacted stream, pH was 6.8 nearing neutral with high dissolved oxygen (11.2mg/l), low level of sulphate (0.16mg/l) iron (0.28ppm), manganese (0.05ppm), lead (0.01ppm) and zinc (0.02ppm) whereas in coal mine areas pH was low, varied from 2.9-4.06 with low dissolved oxygen (1.70-5.32mg/l), high level of sulphate (51.94-69.43mg/l), iron (12.40-17.88ppm), manganese (0.12-0.70ppm), lead (0.5-0.9ppm) and Zn (0.02-1.15ppm). The species richness and the diversity of diatoms also changed with the change in intensity of mining load. In unimpacted stream, the number of diatom species documented was 80 and only 16 - 27 diatom species could be recorded from mine affected streams. Dominant diatom taxa recorded were *Frustulia rhomboides*, *Navicula viridis*, *Navicula cryptocephala*, *Pinnularia biceps* and *Pinnularia viridis*. Canonical correspondence analysis (CCA) showed the presence of two clear major groups of streams one group with different species of diatom taxa characterized by higher pH, dissolved oxygen, low conductivity and the other group with few specific acid tolerant diatom taxa characterized by significantly low pH, high levels of toxic metals, conductivity and sulphate.*

**Key words:** Diatoms, Jaintia hills, Meghalaya, streams, dominant, impacted, Canonical correspondence analysis (CCA).

### INTRODUCTION

Diatoms are considered to be very sensitive to changes in water quality, particularly to pH, conductivity, dissolved oxygen and metal concentrations and were thus treated as exceptionally useful indicator of acidification in various mine affected water bodies of the world (Verb and Vis, 2000; De la Pena and Barreiro, 2009). A change in distributional pattern of diatoms thus could indicate the level of disturbance to the aquatic ecosystems (Patrick, 1973; Rosen, 1995). Acid mine drainage (AMD) is one of most extreme environment resulting from mining processes formed when pyrite minerals get exposed to atmospheric, hydrological or biological weathering followed by oxidation, low pH, dissolved metal ions, high sulphate contents, high conductivity, Fe, Mn and many other toxic metals (Luis *et al.*, 2008). Quality of AMD impacted streams pose complex problems that involve not only chemical but also physical and biological components (Karr and Chu, 1999). Earlier, Lackey (1938, 1939) was one of the first to examine the biology of streams affected by AMD and reported the presence of 'naviculoid diatoms'. In recent past Robb and Robinson (1995) reported presence of Diatoms in aquatic habitats with pH values <3.5 and with high concentrations of heavy metals, particularly iron in those streams. In many lotic systems,

increase in acidity lead to decrease in algal species richness and diversity. The decrease in species richness had often been linked to a variety of factors including lethal level of metallic salts and pH (Battarbee *et al.*, 1999; Stevenson and Pan, 1999; Verb and Vis, 2005).

Acidic seeps and streams are prevalent throughout Jaintia hills district of Meghalaya, a state in the north eastern part of India known for its floristic richness and mineral deposits. Coal is the major one among the minerals. Majority of the streams and rivers in those areas are affected by coloured discharge generated from primitive unscientific “rat hole” method of coal mining and their huge storage on the roadside for transportation. A drastic decrease in micro invertebrate’s diversity and fish diversity in impacted streams of Jaintia Hills had been reported (Sing, 2005; Myllemngap and Ramanujam ,2011) as well two filamentous green algae from AMD streams (Das and Ramanujam, 2011). The aim of the present study was to determine the distributional pattern of diatoms in different streams affected by AMD due to coal mining and huge storage of coal.

## MATERIALS AND METHODS

### Site selection

This study was carried out on monthly basis from April, 2010 to March, 2011. For the present study, a thorough survey was carried out and four different streams affected by coal mining at different levels were selected in Jaintia Hills of Meghalaya (Fig 1).The location of each site was determined with a Garmin 12 GPS receiver.

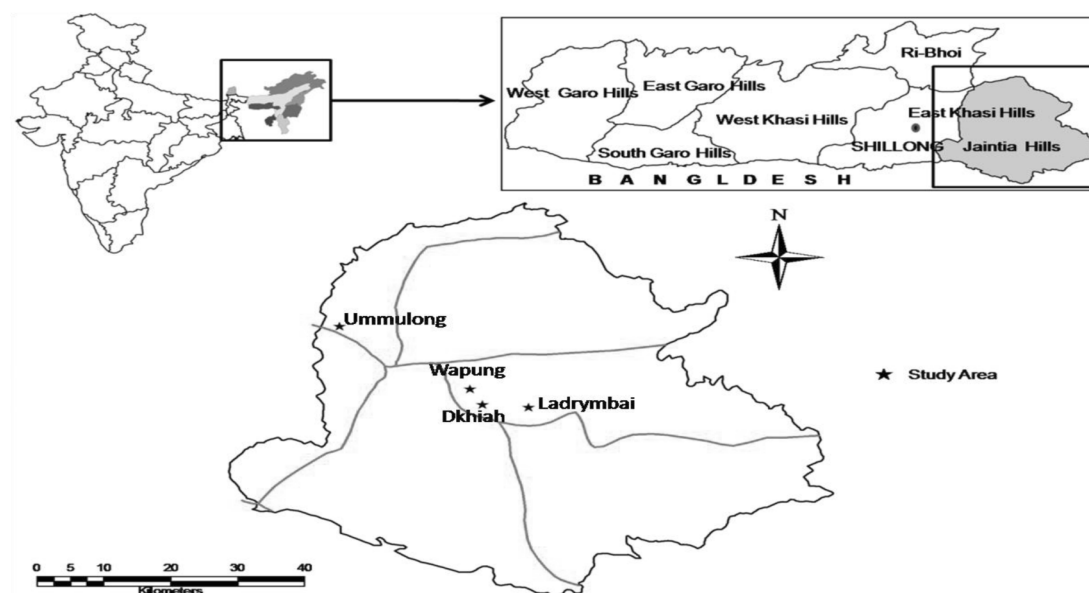


Fig 1: Study sites located in Jaintia Hills District of Meghalaya.

- 1) The first stream selected for this study was located in Ummulong far from coal mining areas and not affected by mining (lying between  $25^{\circ} 31' 21.06''\text{N}$  to  $092^{\circ} 08' 15.89''\text{E}$ ). This stream is considered as unimpacted stream.
- 2) The second selected stream was located in Wapung where the area was left abandoned for 5-7 years after active coal mining (lying between  $25^{\circ} 24' 30.72''\text{N}$  to  $092^{\circ} 18' 56.46''\text{E}$ ).
- 3) The third stream was located in Dkhiah receiving wastes from active coal mining areas (lying between  $25^{\circ} 22' 27.72''\text{N}$  to  $092^{\circ} 23' 22.86''\text{E}$ ).

4) The fourth stream was in Latrymbai receiving acid water through seepage from huge coal storage where mined coal is stored in on the road side for transportation to different places (lying between 25<sup>o</sup> 23'16.26"N to 092<sup>o</sup> 19'28.26" E).

#### **Collecting and Processing water Samples**

Water samples were collected from the selected streams every month in the year 2010-2011. For physico-chemical analysis of water- pH, conductivity, turbidity, dissolved oxygen, temperature were measured in the field using deluxe water and soil analysis kit (Model 191E). The mean current velocity was calculated by timing a bobber as it moved over a distance of one meter. The mean depth was calculated from 10 randomly selected points. Free CO<sub>2</sub>, acidity, sulphate from water samples were measured in the laboratory following the standard methods prescribed by APHA (2005). Estimation of silica was done by Molybdosilicate method. For heavy metal analysis, 5 ml of concentrated nitric acid was added to 500 ml of water ( samples were collected separately for metal analysis from five different points) boiled on a hot plate and brought the volume to lowest possible (15 to 20 ml). Filtration was done after digestion. The filtrate was then diluted to volume of 50 ml with distilled water in a volumetric flask (APHA (2005)). The concentration of heavy metals (iron, lead, zinc and manganese) was determined by running samples on AAS (Perkin Elmer, Analyst 700).

#### **Collection and processing algal samples**

Diatom samples were collected by scraping with tooth brush from various substrata such as rocks, twigs, leaves, plastic bags, pebbles etc and stored in 50 ml plastic containers where each sample was split in two, one kept alive (without preservation) and the other preserved with formalin solution (4 %). From the first sub-sample, an aliquot was cleaned using HNO<sub>3</sub> (65 %) and potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) at room temperature for 24 h, followed by several centrifugations (1500 rpm) to wash the excess of acid. Permanent slides were prepared using Naphrax. Taxa present were enumerated, quantified (No. of individuals/ml) using Olympus microscope fitted with a digital camera, drawn and photographed. Identification of diatom taxa to the possible lower taxonomic level (Tiffany and Britton, 1952; Desikachary 1988; Round *et al.*, 1990; Krammer and Lange-Bertalot, 1986-1991).

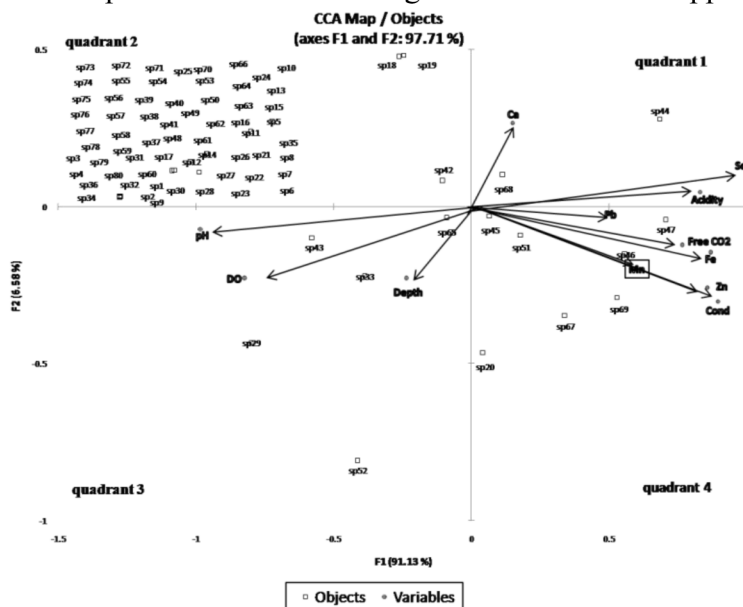
#### **Data Analysis**

Physicochemical parameters recorded from streams of different categories were compared using analysis of Variance at 5% level of significance. Canonical correspondence analysis (CCA) is used to study algal responses to the environmental variables. The used CCA original matrix was composed of 80 taxa and 12 environmental variables. CCA was performed using the computer XL-Stat program. The diversity index like Shannon diversity index, Shannon evenness, Species richness, was calculated following the formulae prescribed by APHA (2005).

### **RESULTS AND DISCUSSION**

The depth of the four streams varied from 16.4cm to 20.00cm and current velocity from (1.01-3.62m/s). Water quality varied significantly in all the parameters measured. pH value and dissolved oxygen was significantly higher in unimpacted stream compared to impacted ones. In unimpacted stream, pH was (6.32) nearly neutral as compared to 2.90-4.06 in impacted streams. Dissolved oxygen was recorded (11.20mg/l) in unimpacted stream compared to 1.70-5.32 mg/l in impacted streams. Conductivity was (0.07ms/cm) in unimpacted stream whereas in impacted streams it varied from 0.39-0.70 ms/cm. Free carbon-di-oxide was 6.42mg/l, sulphate 0.16mg/l, iron 0.28ppm, manganese 0.05ppm, lead 0.01ppm and zinc 0.02ppm in unimpacted stream and were significantly higher in impacted streams where free carbon-di-oxide varied from 28.20-39.04mg/l, sulphate varied from 51.94-69.43mg/l, iron 12.40-17.88, manganese 0.14-0.77ppm, lead 0.59-0.99ppm and zinc

0.47-1.15ppm. Likewise, metal concentration of iron, lead, zinc and manganese varied significantly within streams. It was minimum in unimpacted stream and was in higher concentrations in mine impacted streams. Mn ranged from 0.05 to 0.77 ppm, Pb ranged from



**Figure legends**

Fig 2: CCA ordination of the first two CCA axes for 4 stream samples showing abundance of species with environmental variables depicted by arrows. Each taxa is represented by a code no. Given in table 6. DO: dissolved oxygen; Free CO<sub>2</sub> : free carbon dioxide; Cond: conductivity; SO<sub>4</sub> : sulphate; Ca: calcium; Fe: iron; Pb: lead; Mn: manganese; Zn: zinc.

0.01 to 0.99ppm, Zn ranged from 0.02 to 1.15ppm and Fe ranged from 0.28 to 17.88ppm. (Table1). AMD streams in the present study exhibited the prominent environmental characteristics observed in other AMD streams studied elsewhere. Among the different parameters, the most influencing being pH, conductivity, acidity, sulphate, iron, lead, zinc and manganese which separated the AMD sites from the unimpacted stream. Comparable results were observed by Verb and Vis (2000) where AMD streams had significantly lower pH and higher concentrations of metallic salts like iron, manganese and sulphates. Harding and Boothroyd, (2004) reported that presence of high concentrations of As, Cu, Fe, Mn, Pb, Zn and sulphates and their solubility increased acidity of AMD water. Similar results were also demonstrated by Carter (1990) where AMD waters had significantly higher concentrations of metallic salts and low pH. Ganong *et al.*, (2007) reported very high concentration of iron, manganese and sulphate and high acidic water with pH level less than 2.5 from coal creek (AMD impacted site) of Tioga river, North Central Pennsylvania. Similar trend especially very low pH, high conductivity and high concentration of iron, zinc, lead, manganese and sulphate have been observed in the present study. High turbidity was caused by suspended matters such as clay, silt, sediment load owing to surface-runoff and mining activities from upstream. During the process of mining, soil and rock are exposed and processed. Fine particulate mineral matter enters surface waters increasing the turbidity and the rate of sedimentation. Changes in the water velocity of a stream or river might thus lead to the deposition or resuspension of particulate material remobilizing contaminants into suspension (Kelly, 1988). Fine inorganic suspensoids or clay particles are known to lead to extreme attenuation of light in stream water. They also significantly reduce dissolved oxygen,

bed permeability and cause particle entrapment within the matrix of periphytons and phytobenthos (Davies-Colley *et al.*, 1992; Rosemary, 2011). High silica content in stream near abandoned mining area and in stream near active mining in the range of 18.89 to 33.07 mg/l was due to depositions of diatoms. Silica used by diatoms in the formation of external cell wall thus affected biological productivity (Dutta, 2011). The variations recorded in different water parameters collected from four streams were analyzed statistically using one-way analysis of variance (ANOVA) and significantly different at 0.05 level. (Table2). Totally eighty diatom species were recorded from unimpacted stream where pH level was nearly neutral and only 16 diatom species could be recorded from active coal mining area (pH ranged from 2.90 to 3.15). It was 20 species from pH level (3.01 to 4.06) of coal storage area and 27 species from (pH level 3.13 to 3.83) of abandoned coal mining areas. The most species rich genus recorded was *Navicula* with 18 species. (Table 3).

Table1. Assessment of Physico- chemical characteristics of stream water in Jaintia Hill

Parameters	unimpacted stream	stream in mine abandoned area	stream in active mining area	stream in coal storage area
Water current m/s	1.01±0.45	1.20±0.60	1.65±0.15	3.62±0.76
Depth cm	20.00±1.46	16.40±1.35	18.60±2.58	19.60±1.05
Temperature °c	19.00±1.05	23.00±0.48	24.00±1.01	24.00±3.19
PH	6.32±0.27	3.31±0.30	3.10±0.13	3.75±0.39
Conductivity ms/cm	0.07±0.01	0.39±0.05	0.70±0.01	0.69±0.01
Turbidity NTU	2.27±0.25	4.36±0.12	10.45±0.10	3.53±0.09
Dissolved oxygen mg/l	11.20±4.41	1.70±0.56	2.40±1.16	5.32±1.29
Free carbon dioxide mg/l	6.40±2.60	28.20±16.85	39.04±9.16	33.60±17.16
Acidity mg/l	5.20±2.28	25.80±0.60	50.80±10.05	29.32±8.06
Sulphate mg/l	0.16±0.05	61.63±0.12	69.43±0.05	51.94±0.63
Silica mg/l	3.11±0.06	24.22±0.13	25.14±0.12	4.13±1.13
Iron ppm	0.28±0.18	12.40±0.18	17.88±0.36	16.88±0.54
Manganese ppm	0.05±0.07	0.14±0.06	0.27±0.21	0.77±1.15
Lead ppm	0.01±0.008	0.59±0.57	0.99±0.46	0.59±1.36
Zinc ppm	0.02±0.011	1.00±0.11	0.47±0.17	1.15±0.15

Table 2: One way Analysis of variance to show significant variation

	Sites	
	F-Value	P-Value
pH	4.009044	2.29E-13
Temperature	7.108987	0.000216
Depth	2.451511	0.063955
Current velocity	57.68408	5.1E-26
Conductivity	120.2371	1.4E-37
Turbidity	23.89162	2.05E-12
Silica	572.1764	2.54E-69
DO	25.83232	3.28E-12
Free CO2	39.29747	3.44E-19
Acidity	50.14963	9.48E-23
Hardness	37.78945	1.28E-18
Sulphate	271.3224	8.34E-61

\*P is significant at 0.05 level

Table 3: List of Diatom species recorded from streams of Jaintia hills

	Name of the Diatom species	Unimpacted stream	Stream in coal mine abandoned area	Stream in active coal mine area	Stream in coal storage area
1.	<i>Achnanthes inflata</i> (Kutzing) Grunow	+	-	-	-
2.	<i>Achnanthes lanceolata</i> (Brebisson) Grunow	+	-	-	-
3.	<i>Amphora elliptica</i> Kutzing	+	-	-	-
4.	<i>Amphora ovalis</i> Kutzing	+	-	-	-
5.	<i>Ampleura sp*</i>	+	+	-	-
6..	<i>Caloneis bacillum</i> Grunow	+	-	-	-
7.	<i>Craticula cuspidata</i> (Kutzing) Mann	+	-	-	-
8.	<i>Cyclotella menghiniana</i> Kutzing	+	-	-	-
9.	<i>Cymbella affinis</i> Kutzing.	+	-	-	-
10.	<i>Cymbella aspera</i> ( Ehrenberg) Cleve	+	-	-	-
11.	<i>Cymbella cuspidata</i> Kutzing	+	+	-	+
12.	<i>Cymbella lanceolatum</i> Ehrenberg	+	-	-	-
13.	<i>Cymbella naviculoformis</i> (Aueswald) Cleve	+	+	+	+
14.	<i>Cymbella tumida</i> ( Brebison) VanHeurck	+	+	-	+
15.	<i>Cymbella ventricosa</i> Kutzing	+	-	-	-
16..	<i>Diadesmis confervacea</i> Kutzing	+	-	-	-
17.	<i>Eunotia lunaris</i> (Ehrenberg) Grunow	+	+	+	+
18.	<i>Fragilaria biceps</i> (Kutzing) Lange Bertalot	+	+	+	+
19.	<i>Fragilaria capucina</i> Desmazieres	+	-	-	-
20.	<i>Fragilaria crotonensis</i> Kitton	+	-	-	-
21.	<i>Fragilaria pinnata</i> Ehrenberg	+	-	-	-
22.	<i>Fragilaria virescens</i> Ralfs	+	+	-	-
23.	<i>Frustulia rhomboides</i> (Ehrenberg) DeToni	+	+	+	+
24.	<i>Frustulia vulgaris</i> (Thwaites) DeToni	+	-	-	-
25.	<i>Gomphonema acuta</i> ( Gray)A.Nels	+	-	-	-
26.	<i>Gomphonema angustatum</i> (Kutzing) Rabenhorst	+	-	-	-
27.	<i>Gomphonema dichotomous</i> Ehrenberg	+	+	-	-
28.	<i>Gomphonema gracile</i> Ehrenberg	+	-	-	-
29.	<i>Gomphonema lanceolatum</i> Ehrenberg.	+	+	+	-
30.	<i>Gomphonema montanum</i> Schum var.genuinum	+	+	-	+
31.	<i>Gomphonema olivaceum</i> (Hornemann) Brébisson	+	+	+	-
32.	<i>Gomphonema parvulum</i> (Kutzing) Kutzing	+	-	-	-
33.	<i>Gomphonema telographicum</i> Kutzing	+	+	+	+
34.	<i>Gomphonema vibrio</i> Ehrenberg.	+	-	-	+
35.	<i>Grammatophora undulata</i> Ehrenberg	+	-	-	-
36.	<i>Gyrosigma acuminatum</i> (Kutzing) Rabenhorst	+	-	-	-
37.	<i>Hantzschia amphioxys</i> Ehrenberg	+	-	-	-
38.	<i>Mastogloia braunii</i> Grunow	+	-	-	-
39.	<i>Melosira granulata</i> Ehrenberg	+	-	-	-
40.	<i>Melosira varians</i> Agardh	+	-	-	-
41.	<i>Melosira italica</i> (Kutzing) Ehrenberg	+	-	-	-

42.	<i>Navicula borealis</i> Kutzing	+	+	+	+
43.	<i>Navicula cryptocephala</i> Kutzing	+	+	+	+
42.	<i>Navicula cuspidata</i> Kutzing.	+	-	-	-
43.	<i>Navicula dicephala</i> (Gregory) Cleve	+	-	-	-
44.	<i>Navicula exigua</i> (Gregory)Mueller	+	-	-	-
45.	<i>Navicula gracilis</i> Ehrenberg.	+	-	-	-
46.	<i>Navicula lanceolata</i> (Agardh) Ehrenberg	+	+	+	+
47.	<i>Navicula major</i> Kutzing	+	+	-	-
48.	<i>Navicula microspora</i> Kant and Gupta	+	+	+	+
49.	<i>Navicula oblonga</i> (Kutzing) Kutzing	+	-	-	-
50.	<i>Navicula platystoma</i> Ehrenberg	+	-	-	-
51.	<i>Navicula pupula</i> Ehrenberg	+	-	-	-
52.	<i>Navicula radiosa</i> Kutzing	+	-	-	-
53.	<i>Navicula reinhardtii</i> (Grunow) Van Heurck	+	-	-	-
54.	<i>Navicula sphaerophora</i> Kutzing	+	-	-	-
55.	<i>Navicula trimpunctata</i> (O.F. Muller) Bory	+	+	-	+
56.	<i>Navicula viridis</i> Kutzing	+	+	+	+
57.	<i>Navicula viridula</i> (Kutzing.) Ehrenberg	+	-	-	-
58.	<i>Neidium dubium</i> ( Ehrenberg) Cleve	+	-	-	-
59..	<i>Neidium iridis</i> ( Ehrenberg) Cleve	+	+	-	-
60.	<i>Nitzschia amphibia</i> Grunow	+	-	-	-
61.	<i>Nitzschia commutata</i> Grunow	+	-	-	-
62.	<i>Nitzschia palea</i> (Kützing) W. Smith	+	-	-	-
63.	<i>Pinnularia biceps</i> Gregory	+	+	+	+
64.	<i>Pinnularia borealis</i> (Ehrenberg) Rabenhorst	+	-	+	+
65.	<i>Pinnularia brounii</i> ( Grunow) cleve	-	+	+	-
66.	<i>Pinnularia brebissonii</i> (kutzing) Rabh	+	-	-	-
67.	<i>Pinnularia cardinalis</i> (Ehrenberg) Wm.Smith.	+	+	-	-
68.	<i>Pinnularia gibba</i> Ehrenberg	+	+	-	+
69.	<i>Pinnularia nobilis</i> Ehrenberg	+	+	-	-
70.	<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	+	+	+	+
71.	<i>Pleurosigma angulatum</i> (Quekett) Wm. Smith	+	-	-	-
72.	<i>Sellaphora purpula</i> (kutzing)Mereschkowsky	+	-	-	+
73.	<i>Stauroneis acuta</i> W.Smith.	+	-	-	-
74.	<i>Stauroneis anceps</i> Ehrenberg	+	-	-	-
75.	<i>Stauroneis phoenicentron</i> (Nitzsch) Ehrenberg	+	-	-	-
76.	<i>Surirella linearis</i> W.Smith	+	-	-	-
77	<i>Synedra dorsiventralis</i> O.Muller	+	-	-	-
78	<i>Synedra rumpens</i> Kutzing	+	-	-	-
79	<i>Synedra ulna</i> (Nitzsch) Ehrenberg	+	-	-	-
80	<i>Tabellaria fenestrata</i> (Lyngbye) kutzing	+	+	-	-
	Total	80	27	16	19

+ indicates present; - indicates absent; \* indicates unidentified species

Table 4 Different indices for Diatom taxa collected from four selected sites

Streams	Species richness	Population Density (individuals /ml)	Shannon diversity index	Evenness index
Unimpacted stream	80	8054	1.76	0.92
Stream in mine abandoned area	27	5592	0.90	0.63
Stream in active mine area	16	3708	0.76	0.64
Stream in coal storage area	20	4252	0.97	0.75

Table 5. Relative abundance of dominant diatom taxa from four selected sites

Stream	Species	Relative bundance (%)
Site I	<i>Eunotia lunaris</i>	7.6
	<i>Gomphonema montanum</i>	3.2
	<i>G. vibrio</i>	3.9
	<i>Navicula borealis</i>	2.4
	<i>N. cuspidate</i>	2.9
	<i>N. gracilis</i>	0.9
	<i>N. viridula</i>	5.2
	<i>Synedra ulna</i>	2.7
Site II	<i>Frustulia rhomboides</i>	35.76
	<i>Navicula viridis</i>	21.45
	<i>Navicula cryptocephala</i>	14.30
	<i>Pinnularia biceps</i>	3.9
Site III	<i>Pinnularia viridis</i>	7.5
	<i>Frustulia rhomboides</i>	37.75
	<i>Navicula viridis</i>	26.98
Site IV	<i>Navicula cryptocephala</i>	16.18
	<i>Frustulia rhomboides</i>	18.82
	<i>Navicula viridis</i>	28.75
	<i>Navicula cryptocephala</i>	15.05
	<i>Pinnularia biceps</i>	6.5
	<i>Pinnularia viridis</i>	9.4

Table 6: Code number of Diatom taxa used in CCA diagram

Sp 1	<i>Achnanthes inflata</i>
Sp 2	<i>Achnanthes lanceolata</i>
Sp 3	<i>Amphora elliptica</i>
Sp 4	<i>Amphora ovalis</i>
Sp5	<i>Ampleura sp*</i>
Sp6	<i>Caloneis bacillum</i>
Sp7	<i>Cocconeis sp*</i>
Sp8	<i>Cymbella affinis</i>
Sp9	<i>Cymbella ventricosa</i>
Sp10	<i>Cymbella cistula</i>



Sp11	<i>Cymbella cuspidata</i>
Sp12	<i>Cymbella lanceolata</i>
Sp13	<i>Cymbella naviculiformis</i>
Sp14	<i>Cymbella tumida</i>
Sp15	<i>Cyclotella michiganiana</i>
Sp16	<i>Diadesmis confervacea</i>
Sp17	<i>Eunotia lunaris</i>
Sp18	<i>Fragilaria biceps</i>
Sp19	<i>Fragilaria virescens</i>
Sp20	<i>Fragilaria crotonensis</i>
Sp21	<i>Fragilaria capucina</i>
Sp22	<i>Fragilaria pinnata</i>
Sp23	<i>Frustulia rhomboides</i>
Sp24	<i>Gomphonema abbreviatum</i>
Sp25	<i>Gomphonema constrictum</i>
Sp26	<i>Gomphonema dichotomus</i>
Sp27	<i>Gomphonema gracile</i>
Sp28	<i>Gomphonema lanceolatum</i>
Sp29	<i>Gomphonema montanum</i>
Sp30	<i>Gomphonema olivaceum</i>
Sp31	<i>Gomphonema parvulum</i>
Sp32	<i>Gomphonema telographicum</i>
Sp33	<i>Gomphonema vibrio</i>
Sp34	<i>Grammatophora undulata</i>
Sp35	<i>Gyrosigma</i> sp.*
Sp36	<i>Hantzschia amphioxus</i>
Sp37	<i>Melosira varians</i>
Sp38	<i>Melosira granulata</i>
Sp39	<i>Melosira italica</i>
Sp40	<i>Mastogloia braunii</i>
Sp41	<i>Navicula sphaerophora</i>
Sp42	<i>Navicula trim punctata</i>
Sp43	<i>Navicula borealis</i>
Sp44	<i>Navicula cuspidata</i>
Sp45	<i>Navicula graciles</i>
Sp46	<i>Navicula exigua</i>
Sp47	<i>Navicula lanceolata</i>
Sp48	<i>Navicula major</i>

Sp49	<i>Navicula microspora</i>
Sp50	<i>Navicula pupula</i>
Sp51	<i>Navicula radiosa</i>
Sp52	<i>Navicula reinhardtii</i>
Sp53	<i>Navicula viridis</i>
Sp54	<i>Navicula viridula</i>
Sp55	<i>Navicula dicephala</i>
Sp56	<i>Navicula cryptocephala</i>

*Eunotia lunaris* was the most dominant species in unimpacted stream whereas *Frustulia rhomboides* was the most dominant species in active and abandoned coal mine impacted streams. In coal storage area, *Navicula viridis* was the most widely distributed species, occurring at high density. *Frustulia rhomboides*, *Navicula viridis*, *Navicula cryptocephala*, *Pinnularia viridis*, *Pinnularia biceps* consistently dominated the diatom flora of AMD streams. The species richness and diversity was high in unimpacted stream (1.7) whereas lowest diversity was observed in active coal mine impacted stream (0.7). Other two levels of AMD impacted streams showed same diversity value of (0.9) lying between the unimpacted and most impacted stream. Total population density of diatom species was more in unimpacted site (8054 individuals /ml) followed by other three impacted sites (Table 4).

Relative abundance of *Frustulia rhomboides* was maximum in the coal storage region followed by stream in active coal mining area where *Navicula viridis* and *Navicula cryptocephala* were in abundance. Other dominant taxa from coal mine impacted streams were *Pinnularia biceps* and *Pinnularia viridis*. (Table 5). The AMD affected streams were dominated by *Frustulia rhomboides* and *Navicula viridis*, *Navicula cryptocephala*, *Pinnularia viridis* (DeNicola 2000). A decrease in species richness in different stream categories from non- impacted to heavily impacted AMD sites was observed. Diatom assemblages in the AMD streams in Jaintia hills district revealed low species richness and diversity but high abundance of few tolerant species compared to that in unimpacted stream. Reduction in species richness and diversity due to increase in metallic salts concentrations and acidification have been reported by many workers (Hargreaves *et al.*, 1975; Keating *et al.* 1996, Sabater *et al.*, 2003 Kwandrans *et al.*,2007).

High density of few diatom taxa in AMD impacted streams had been reported by Verb and Vis (2000). Joseph (1953) reported the abundance of diatoms (with six species of *Navicula*) in AMD streams primarily dominated by *Navicula viridis* and proposed that *Navicula viridis* could be used as a good indicator of acidity. *Pinnularia viridis*, another abundant taxa recorded along with many species of *Navicula* in this study was also reported from highly acidic streams of Aljustrel Mining area, Portugal (Luis *et al.*, 2008). Whitton and Satake (1996) and Gerhardt *et al.* (2008) reported the presence of diatoms like *Pinnularia*, *Eunotia*, *Navicula* and *Nitzschia* from AMD streams. *Frustulia rhomboides*, the most dominant taxa recorded in the present study was reported by many authors from AMD stream (Young 1976 ;Whitton and Diaz, 1981 ;Verb and Vis ,2000 where pH ranged between 2.6 to 3.5 forming extensive mats.

#### **Canonical correspondence analysis**

In total, 98.47 % of the relationship between species and environmental variables were explained by the first two axes of CCA. The sites data were linearly related to the

environmental variable data as the computed p value was lower than the significant level ( $P < 0.001$ ). The first two CCA axes were statistically significant (Monte-Carlo permutation,  $p = 0.0001$ ) and explained 97.71% of the total variance. The total variance explained by the species data and environmental variables (constrained CCA) was 99.68%. The total inertia (constrained CCA) for each axis was 91.12 % (axis 1), 6.58 % (axis 2), 2.20 % (axis 3) and 0.03 % (axis 4). The Eigen values of the first three CCA axes were 0.62, 0.04 and 0.01. The result of CCA indicated a relationship between diatom and environmental variables. Axis 1 had a strong relationship with almost all the variables except Ca which is explained by axis 3. Axis 1 had strong negative relationship with pH, DO and Depth and positive relationship with sulphate, free CO<sub>2</sub>, acidity, Mn, Pb, Zn and Fe. CCA grouped streams into two categories based on physical and chemical data. Group I consisted of the AMD impacted streams. Group II consisted of the unimpacted stream in the left end of CCA Axis 1 and 2. Quadrants 2 and 3 were represented with higher pH and dissolved oxygen data whereas quadrants 1 and 4 were represented with high acidity, free CO<sub>2</sub>, sulphate, conductivity and metal concentrations and with low pH and dissolved oxygen value. The relationship between environmental variables and abundance of diatom has been shown in (Fig 2). Among the abundant taxa recorded, *Cymbella naviculiformis*, *Eunotia lunaris*, *Fragilaria biceps*, *Gomphonema telographicum*, *Navicula trimpunctata*, *Navicula borealis*, *Navicula lanceolata* and *Pinnularia braunii* (total 8 species) found in unimpacted site were located on the left hand side of the CCA diagram and the second group of remaining species which were abundant in coal mine impacted streams were located on the right hand side of CCA diagram. Coal mine impacted sites were dominated by, *Frustulia rhomboides*, , *Navicula cryptocephala*, *Navicula viridis* and *Pinnularia viridis*, *Pinnularia biceps*, exhibiting highest relative abundance values and also showed that all taxa in quadrants 2 and 3 were correlated with high pH, DO and low metal concentrations. Most of those quadrant's taxa belonged to unimpacted stream whereas in opposition, quadrants 1 and 4 showed taxa which were correlated with low pH , DO and higher concentrations of acidity, free CO<sub>2</sub>, sulphate, Mn, Pb, Zn and Fe. Taxa found in those quadrants were the taxa which were found in high abundance from AMD impacted streams. Code numbers used in the CCA diagram are given for different diatom taxa (Table 7). There are reports which explained the twofold effect of AMD on algal species richness and diversity in aquatic ecosystems: (a) impacted communities experienced lethal levels of pH and metals, which lead to a decrease in algal species richness and diversity (Mulholland et al. 1986; Planas, 1996; Verb and Vis (2000 ) and (b) communities were restricted to tolerant organisms, which were able to survive in those conditions. In the present study, few tolerant diatom species have been found to be dominant. Shifts in species composition and in morphology of diatoms with metal pollution were noted by Cattaneo *et al.*, (2004). In metal-polluted rivers diatoms responded to perturbation at the community level through shifts in dominant taxa (Gustavson and Wangberg, 1995; Hirst *et al.*, 2004) as well diversity (Leland and Carter, 1984; Medley and Clements, 1998). Significant differences were observed in densities of diatoms among the streams of different level of AMD compared to unimpacted streams.

CCA diagram suggested that the few diatom species have substantial tolerance to different environmental variables. All the parameters used in CCA analysis might be important determinants of periphytic diatom assemblage. The most diverse genus recorded was *Navicula* with eighteen species and most of its dominant members were restricted to AMD streams. *Navicula* is generally found in).

## CONCLUSION

Freshwater with neutral pH (Rusanov *et al.*, 2009), but few species of *Navicula* could be found in extreme conditions as observed in the present study. CCA analysis showed axis I had strong relationship with pH, acidity and different metals as observed by other authors (Luis *et al.*, 2008; Verb and Vis, 2001

From the present study, it can be summarized that active unscientific coal mining, huge storage of coal on the road side for transportation and abandoned mining areas caused substantial degradation in the water quality of streams of Jaintia hills district and nearby areas resulting in highly acidic conditions of streams with the presence of toxic elements like Mn, Pb, Zn, Fe, SO<sub>4</sub> in high concentrations.

Diatom assemblages often used as good indicator of water quality demonstrated significant change in the distributional pattern when compared between unimpacted and impacted streams. In AMD impacted streams of the region, diatom taxa number reduced considerably from 80 (unimpacted) to 16 and were represented and dominated by *Frustulia rhomboides* and only few species of *Navicula* and *Pinnularia* which could tolerate high acidic environment. Survival strategy of these diatom species in highly acidic condition indicated acidophilic nature which can be used in biomonitoring of such degraded systems. In the abandoned area, an increase in the number of diatom species (27) indicated a slow recovery process.

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