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Seasonal metal sequestration by acidophilic Algae in mine waste water of Simsang River, East Garo Hills, Meghalaya, India

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Abstract

The present study deals with an aim to observe how the plentiful growth of filamentous algal species and few diatoms which has an ability to absorb/adsorb dissolved metals in Acid Mine Drainage impacted water bodies. The findings indicated low pH, high conductivity, low DO, and higher levels of hazardous metals (measured above BIS/WHO standards). Despite the fact of high acidic and traces of dissolved toxic metals, mat of green filamentous algae was found abundantly growing in all the sites which increases a huge biomass. The water and algal mat was collected from three different sites (*i.e.* Site-I, Site-II & Site-III) of Simsang River near coal mining areas. Important Physico-chemical characteristics such as pH, DO, conductivity, acidity were done following the standard methods prescribed by APHA (2005) and sequestration of metals such as Fe, Zn, Pb, Cu, Ni & Mn by both water and algae were done by the use of atomic absorption spectrophotometer. A few resistant species proliferated quickly while damaged streams increased their biomass, which was mostly observed in Site-II. Significant seasonal fluctuations were seen in the metal concentration of the algal mat and water samples taken during the research. As per observation, metals present in the water were sequestrated effectively by dominant algae.

Key words : Simsang River • Water analysis • Identification of algae • Biomass content of algae • Sequestration of metals by algae

Meghalaya (derived from Sanskrit "the abode of clouds"), a home of three tribal population *i.e. Khasis, Garos* and *Jaintias,* one of the seven state of North East India of approximately 22,430 square kilometers, with a length and breadth in the ratio of about 3:1 is

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widely known for high rainfall, subtropical forests, biodiversity and minerals such as coal, limestones, sillimanite, Uranium, etc. Among these riches, coal is the most exploited of all (total coal reserve of Meghalaya being about 560 million tones) resulting in unscientific primitive rat hole extraction. Water (70% of our planet), being the most vital resources for mankind as well as other living organisms but few proportion are primarily confined to human[27]. Increasing human activities in and around aquatic systems and their catchment areas has resulted to decline in water quality leading to their accelerated eutrophication[4]. Biotic and abiotic components of aquatic ecosystem are affected as observed by limnological studies[14]. Temperature, turbidity and water current play a very important effect on the organism's distribution. AMD can have adverse effects on water ecosystem through various stressors *i.e.* acidity, heavy metals, metal oxide deposition[22]. Similar study was conducted on "Status of water quality in coal mining areas of Meghalaya" in which they found the physico-chemical and biological parameters which signifies the degradation of water quality like low pH, high electric conductivity, high concentration of ions of sulphate and iron, toxic heavy metals, low dissolved oxygen (DO) and high BOD[30]. When mining run-offs enters the water bodies it can result in the range of negative impacts[16]. Excavation of an inclined (a decline or drift) or horizontal shaft, followed by a network of perpendicular and parallel shafts (bord-and-pillar) enabling maximum extraction of the coal seam is the most commonly used method[16,35]. Algae are bio-indicators and even shows the types of pollution, such as many blue green algae occur in nutrient less water, while some grows organically polluted water[15,20]. Algae grow well in water containing a high concentration of organic wastes. Green algae, *Chlamydomonas, Euglena, & Microspora quadrata* (Fig. 5) Diatoms, *Navicula, Synedra* Blue green algae, *Oscillatoria* and *Phormidium* are emphasized to tolerate organic pollution. Filamentous algae usually float on the surface of water forming large mats, which are commonly known as "Pond scums" or "Pond mass. They can resemble mats of wet wool, hair, cotton, or slime and are often green, but can occasionally turn yellowish, greyish, or brownish. The majority of them are real algae, although a few are cyanobacteria. The present study is undertaken to study the ecology of green algae in selected stations of AMD polluted Simsang River, Garo Hills to analyze the physico-chemical characteristics of mine wastes water, to identify green algae from the mining capability of identified algae.

Study sites :

The present study was conducted in different points of Simsang River (Fig. 2), the longest river of Garo Hills located at South Garo Hills District, Meghalaya (Fig. 1). It originates from Nokrek Biosphere Reserve (longitude 90° 23/59/E and latitude 25° 31/21/N) located at the elevation of about 1412m above sea level. This river flows towards East Garo hills and South Garo hills Districts of Meghalaya and as it passed through Nongalbibra, a small town in South Garo Hills bordering East Garo Hills District, it was afflicted by unscientific method of coal mining for the last few decades and the quality of water has constantly being degraded due to human activities by Acid Mine Drainage (AMD). The District South Garo Hills has an area of 1,850 sq km, the District headquarters is located at Baghmara some 50 km from Nongalbibra, and the district vary in an elevation from 150-1200m above sea level.

Sampling and analysis :

Three sites of Simsang river (fig. 2) in and around Nongalbibra, (fig. 3&4) South Garo Hills were selected and the reading was undertaken (February 2022 to January 2023). 1km away from the coal mining area was selected as Site-I and Site-II and Site-III was selected near the coal mining area. To check the variations in biomass and metal sequestration, reading was taken during four seasons *i.e.* autumn, spring, monsoon and winter and evaluation was done using different methods prescribed by APHA in the laboratory by collecting 6 replicates from each sites of the river[2]. For Identification of algae: A tooth brush was used to scrape periphytonic algal samples off of a variety of surfaces, including rocks, twigs, leaves, plastic bags, and pebbles. Algal mat could be seen expanding a lot at the affected place, and samples from the system were taken for the subsequent analysis. Using a light microscope, the major components of the mat were counted, quantified (number of individuals/ml), then drawn and photographed using a phase contrast microscope. A part of the sample of the dominant algal mat was taken to the lab and fixed in 2% formalin before being viewed under a light microscope. To identify the filamentous algal mat that was gathered from the mine-impacted area, various monographs were used. With the aid of flora from many authors, taxonomic identification may have been accomplished up to the species level[12,17,27,31].

For biomass content : The mat samples came from AMD streams and were taken from five different locations along the river. They were then washed properly to eliminate any other species that may have been stuck to the filaments, dried at 105°C for 24 hours, and weighed.

For sequestration of metals by the algae : A hot plate, a strong nitric acid was used to break down 1g of dried green algae. Nitric acid, sulfuric acid, and perchloric acid were used in a tri-acid mixture to perform the digestion (9:1:1). Filtering was taking place with the solution. In a 50 ml volumetric flask, the filtrate was thinned with distilled water[2]. By adding additional distilled water, the final volume was increased to 100 ml. Using an atomic absorption spectrometer (Perkin Elmer), samples of digested algae were examined for the presence of Fe, Pb, Zn, Mn, Cu, & Ni.

The results of physico-chemical analysis of water are illustrated below in Table-1. Site-I has a very high pH, ranging from 5.6 to 6.9, whereas Site-II was in the range of 4 to 5.8 and Site-III was in the range of 4.3 to 5.7.

The water's conductivity varies from 0.01 µs to 0.036 µs at Site-I, 0.23 µs to 0.63 µs in Site-II, and 0.19 µs to 0.40 µs in Site-III.

In Site-I, the DO ranged from 3.94 mg/L to 12.23 mg/L; in Site-II, it ranged from 0.78 mg/L to 7.49 mg/L; and in Site-III, it ranged from 0.78 mg/L to 7.49 mg/L.

During the spring season, the acidity of the water were between 0.3 m/sec and 0.9 m/sec. Comparatively to two seasons, Site-I has an acidity range of 6 mg/L to 10 mg/L, Site-II has a range of 38 mg/L to 70 mg/L, and Site-III has a range of 24 mg/L to 58 mg/L.

Identification :

The most prevalent filamentous alga in all the AMD-impacted streams was the chlorophyte *Microspora quadrata* (Fig. 5). Many tolerant species of diatoms, such as *Frustulia rhomboides,* Kutz., *Navicula cryptocephala* Kutz., *N viridis* Kutz., *Pinnularia viridis* (Nitz.) Ehrenb., *Eunotia exigua* (Brebisson ex kuetzing), *Euglena mutabilis* Schmidtz, a member from Euglenophyceae and another filamentous green algae *Klebsormidium acidophilum* Novis dominated the AMD streams with high cell density.

Biomass content of algae: In the spring, the biomass content of the mat ranged from 137.52 to 205.81gm/m^2. During the monsoon, the biomass content of the mat varied from 36.68 to 62.31 gm/m^2, during the fall and winter it varied from 105.56 to 116.68 gm/m^2, and during the autumn it varied from 122.36 to 146.44 gm/m^2. S-II added the most biomass content among the locations (Fig. 6).

Sequestrations of metals : Fe levels in water ranged from 0.25 to 2.61 mg/l during spring, 0.77 to 2.06 mg/l during monsoon, 0.31 to 1.49 mg/l during autumn, and 0.04 to 0.55 mg/l during winter at different sites, but when compared, it was significantly higher in algal mat and ranged from 5.39 to 17.37 mg/l during spring, 3.77 to 18.45 mg/l during monsoon, 17.83 to 21.44 mg/l during autumn, and 17.89 to 18.

Pb concentration in water varied from 0.07 to 0.25 mg/l in water and 0.52 to 1.51 mg/l in algal mat during spring, 0.02 to 0.04 mg/l in water and 0.02 to 0.06 mg/l in algae during monsoon, 0.06 to 0.17 mg/l in water and 0.16 to 0.59mg/l in algae during autumn, 0.06 to 0.10 mg/l in water and 0.23 to 1.23 mg/l in algae during winter.

Zn concentration ranged from 0.21 to 1.18 mg/l in water while in algae it ranged from 0.13 to 0.36 mg/l during spring, 0.34 to 0.48 mg/l in water and 0.31 to 0.48 mg/l in algae during monsoon, 0.23 to 0.75 mg/l in water and 0.14 to 0.86 mg/l in algae during autumn and 0.62 to 0.93 mg/l in water and 0.43 to 0.63 mg/l in algae during winter.

Mn concentration varied from 0.01 to 0.18 mg/l in water while in algae in varied from 0.54 to 1.38 mg/l during spring, 0.03 to 0.16 mg/l in water and 0.07 to 0.99 mg/l in algae, 0.02 to 0.16 mg/l in water during monsoon, and 0.02 to 0.59 mg/l in algae during autumn, 0.03 to 0.61 mg/l in water and 0.08 to 0.91 mg/l in algae during winter.

Chromium was not detectable in many seasons from water but wherever present in minute quantity, it ranged from 0.01 to 0.05mg/l but a significant accumulation of the same was measured from algal mat where it ranged from 0.01 to 0.80 mg/l in different seasons. Ni concentration in water ranged from 0.02 to 0.09 mg/l in water while in algae it ranged from 0.02 to 0.55 mg/l during spring; 0.02 to 0.07 mg/l in water and 0.02 to 0.09 mg/l in algae during monsoon, 0.01 to 0.08 mg/l in water and 0.03 to 0.17 mg/l in algae during autumn, 0.02 to 0.05 mg/l in water and 0.06 to 0.21 mg/l in algae during winter. The metal contents

Fig. 1. Map showing study sites in South Garo Hills district of Meghalaya (https://www.mapsofindia.com/maps/meghalaya/districts/south-garo-hills.html)

Fig. 2. Parts of Simsang River

Fig. 3. Coal deposition in the water bodies of Nongalbibra

Fig. 4 . Mat formation in AMD impacted rivers of Nongalbibra

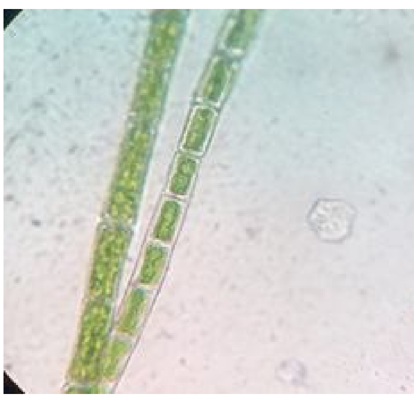

Fig. 5. *Microspora quadrata* under microscopic view.

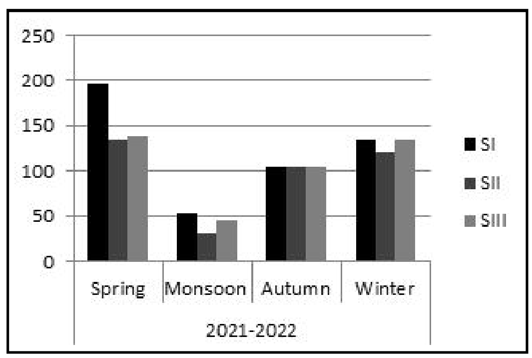

Fig. 6. Seasonal variations in the biomass content of the algal mat

Table-1. Table showing analysis of water chemistry

Water chemi-stry	Monsoon			Autumn			Winter			Spring		
	SI MEAN ±STD	SII MEAN ± STD EV	SIII MEAN ±STDE V	SI MEAN ±STDE V	SII MEAN ±STDE V	SIII MEAN ±STDE V	SI MEAN ±STDE V	SII MEAN ±STDE V	SIII MEAN ±STDE V	SI MEAN ±STDE V	SII MEAN ±STDE V	SIII MEAN ± STD EV
pH	6.61 ±0.23	5.54 ±0.19	5.47 ±0.1	6.4 ±0.14	4.44 ±0.20	4.48 ±0.23	6.18 ±0.08	5.06 ±0.18	4.94 ±0.18	5.86 ±0.20	4.24 ±0.14	4.6 ±0.21
Conduc-tivity (µS/cm)	0.02 ±0.006	0.58 ±0.04	0.28 ±0.064	0.02 ±0.006	0.24.018	0.24 ±0.03	0.02 ±0.007	0.26 ±0.02	0.24 ±0.04	0.0282 ±0.005	0.036 ±0.05	0.35 ±0.036
DO (mg/l)	4.41 ±2.33	1.4± 2.71	1.65 ±3.44	6.31 ±6.32	2.76 ±2.82	2.44 ±2.65	9.31 ±4.83	5.05 ±5.57	5.84 ±4.07	11.68 ±2.03	6.86 ±2.71	6.39 ±2.93
Acidity (mg/l)	10.8 ±3.70	60.4 ±5.42	38.8 ±12.3	9.2 ±2.03	66.6 ±5.7	66.4 ±7.2	21.2 ±8.99	71.2 ±6.27	94.8 ±6.52	7.64 ±0.82	50.4 ±7.63	46 ±7.69

of water and algae from various locations and seasons also showed significant variations when assessed at 0.05 levels (Tables 2 & 3).

The pH is a crucial factor to consider while assessing the water's quality. In streams near coal mines, the pH range was lower than the recommended range of 6.5 to 8.5 for household use[6,34]. Given that there are several open shafts and rat hole mines found next to streams, this may be directly related to the acidic drainage from mines and spoils that seep into water bodies[7,30]. It was found that S-I was more acidic with high pH ranging from 5.6 to 6.9 comparing to S-II and S-III.

The capacity of ions in a solution to convey electric currents is measured as conductivity. The presence of ions, their total concentration, and temperature all affect this ability. Water samples with a high conductivity level suggested electrolyte pollutants but provided no information on a specific chemical[1]. As higher conductivity has been shown to be a highly accurate predictor of mining-related impacts, mining operations are frequently linked to it[18,29]. Results showed conductivity ranged from 0.23 µs to 0.63 µs in Site-II whereas it was lesser in S-I and S-III.

When discussing the amount of free, non-compound O_2 in water or other liquids, DO is used because of its impact on the aquatic life and it is a crucial factor in determining the quality of water[13]. Highest DO ranged from 3.94 mg/L to 12.23 mg/L in S-I. among all the study sites.

Many authors have also reported finding highly acidic water in streams that have

Table-2. Metal concentration in water and algae from each site sampled seasonally (2021-2022)

2021-2022													
Seasons	Location	Fe (ppm)		Pb (ppm)		Zn (ppm)		Mn (ppm)		Cr (ppm)		Ni(ppm)	
		water	Algae	Water	Algae	Water	Algae	Water	Algae	Water	Algae	Water	Algae
SPRING	SII	0.45±0.05	7.90±0.03	0.07±0.01	**1.51±0.9**	0.68±0.04	0.31±0.92	0.09±0.02	0.54±0.55	0.02±0.02	0.05±0.02	0.09±0.08	0.20±0.2
	SIII	0.25±0.04	10.5±0.05	0.11±0.03	0.88±0.7	0.18±0.24	0.296±0.93	0.11±0.44	0.96±0.4	0.03±0.01	0.07±0.03	0.03±0.08	**0.55±0.82**
	SIV	2.61±0.08	17.87±0.09	0.25±0.05	0.52±0.09	1.01±0.28	0.13±0.15	0.18±0.92	**0.85±0.9**	0.05±0.03	**0.8±0.04**	0.03±0.12	0.09±0.12
MONSOON	SII	0.71±0.10	3.77±0.65	0.04±0.04	0.04±0.1	0.38±0.30	0.39±0.11	0.07±0.2	0.07±0.09	N.D	0.05±0.06	N.D	0.06±0.66
	SIII	2.06±0.55	9.54±0.34	0.03±0.09	0.06±0.15	0.34±0.33	0.310.16	0.07±0.31	0.85.14±0.82	N.D	0.09±0.08	0.03±0.03	0.08±0.68
	SIV	0.77±0.14	4.99±0.88	0.02±0.01	0.02±0.18	0.27±0.45	0.11±0.13	0.16±0.12	0.99.24±	N.D	0.02±0.9	N.D	0.19±0.88
AUTUMN	SII	0.58±0.68	17.83±0.91	0.08±0.01	0.24±0.65	0.23±0.54	0.14±0.25	0.18±0.15	0.02±0.87	0.01±0.02	0.01±0.7	0.04±0.1	0.08±0.96
	SIII	0.31±0.99	18.39±0.25	0.17±0.02	0.24±0.02	0.57±0.63	0.15±0.22	0.13±0.2	0.25±0.62	0.03±0.04	0.24±0.6	0.01±0.12	0.17±0.09
	SIV	1.49±0.34	21.44±0.33	0.06±0.03	0. 16±0.2	0.65±0.72	0.86±0.28	0.07±0.25	0.07±0.18	N.D	0.19±0.72	0.08±0.13	0.16±0.94
WINTER	SII	0.14±0.92	9.81±0.61	0.09±0.04	1.23±0.1	0.62±0.62	0.57±0.35	0.18±0.28	0.51±0.4	N.D	0.06±0.75	0.03±0.33	0.10±0.93
	SIII	0.13±0.85	7.89±0.48	0.09±0.03	0.27±0.3	0.93±0.61	0.45±0.55	0.61±0.32	0.14±0.42	N.D	0.039±0.03	0.05±0.45	0.11±0.3
	SIV	0.04±0.62	18.45±0.88	0.06±0.06	0.23±0.04	0.41±0.16	0.43±0.45	0.14±0.33	0.08±0.49	N.D	0.03±0.06	0.03±0.66	0.21±0.9

SII : Abandoned mining site ; SIII: Active mining site ; SIV: Storage site

Table-3. One way analysis of variance (ANOVA) showing significant difference between two media in different seasons:

2021-2022								
	Spring		Monsoon		Autumn		Winter	
	F- Value	P- Value	F- Value	P- Value	F- Value	P- Value	F- Value	P- Value
Fe	117.436266	*0.0006	102.988445	*0.0004	1447.013	*0.0001	140.0219	*0.0005
Pb	6.59279026	*0.014537554	2.40359722	0.129804	50.1589	*0.0002	201.6488	*0.0002
Zn	26.5553955	*0.0009	5.33172787	*0.026791	19.96511	*0.0007	3.572906	0.066801
Mn	20.6368685	*0.0006	9.32429147	*0.004373	0.13634	*0.714237	0.20913	0.650359
Ni	12.25855	*0.001575	8.3600267	*0.007523	26.44417	*0.0001	41.75307	*0.0005

been damaged by coal mines[10,19,26,32,33]. It was observed that during the spring season, the acidity of the water were more ranging between 0.3 m/sec and 0.9 m/sec comparing to other seasons.

The acidophilic nature of the algae was demonstrated by the fact that *Microspora* filament grew best in the pH range of 2 to 4. In every area that was affected, a mat developed, covering the whole stream bed. It was that noted seasonal variations in various metal accumulation patterns and the capacity of *Microspora quadrata* mats to accumulate metals, primarily Fe, Pb, and Mn, in coal mine damaged streams[10]. In algal mats vs water, there was less zinc buildup.

Out of all the seasons, spring is the time when mats contributed the most biomass content to the atmosphere, ranging from about 137.52 to 205.81gm/m^2. The biomass content of the mat revealed a substantial positive Pearson correlation coefficient. This study demonstrated how damaged streams increased their biomass while just a few resistant species multiplied rapidly[9].

Except for Zn, which was below allowed limits, the quantity of Fe, Mn, Pb, Ni, and Cr in coal mine-impacted streams surpassed the limits[6,11,34].

These results support past research from this area done by many scientists[8,30]. Metal concentration in algal mat and water taken from several places during the investigation showed significant seasonal variations. The maximum Fe buildup was predicted to occur in the autumn. When water and algae were compared as two media, Fe was considerably greater in algae in every season. Pb levels in water did not differ significantly when compared between different sites or seasons, however seasons did differ when compared between two media which ranged from 0.07 to 0.25 mg/l in water and 0.52 to 1.51 mg/l in algal mat during spring. When Zn levels were evaluated between medium (water vs algae), Zn levels in the algal mat varied dramatically depending on the season. At 0.05 levels, the concentration of Mn was statistically significant across a range of locations and seasons. When compared to the seasonally changing Mn concentration in water, the Mn accumulation by algae was noticeably higher. In many seasons, chromium was not visible in water, it was always present in traces but an algal mat measured a large accumulation of the metal. Ni could not be identified from various places during the study period during the monsoon and winter. Ni concentration in water and algae varied greatly between various sites and seasons when compared, as well as between distinct sites and locations. The peak metal accumulation was thought to occur in the spring (Table 2 & 3). Different sites and seasons were statistically different between two mediums (algae and water) when evaluated at 0.05 levels. The average amount of metals present in the water and algae, measured in mg/l are characterized as: Water= Fe> Zn> Mn> Pb> Ni > C and Algae= Fe> Pb>Mn> Ni>Zn> Cr[5].

When compared to operational mines (Site-II) and coal storage sites, the metal concentration in the water and algal mat from abandoned sites (Site-I) was the lowest (Site-III). Similar cases were also found by at Subarnarekha River at Swarnamukhi River at Ganga River and at Hindon River[3,21,23,24]. This could result in adverse effects on the health of local people.

It is clear from the current study that the unscientific, haphazard, and primitive "rat-hole" method of mining coal in Meghalaya's South Garo hills has seriously acidified several water systems nearby. Most stream waters turns brownish or reddish orange, which is a sign of contamination, that is one of the worst repercussions of coal mining is acid mine drainage. Typically, it relates to the leaching of harmful metals into water bodies and higher levels of hazardous metals are the major hallmarks of AMD. The chemistry of the water has been impacted by these actions. The river water has been contaminated by human induced pressures under various land-use patterns, as evidenced by the acidic pH and heavy metals measured above BIS/WHO standards. Hence, taking the economic and ecological effects of water into the account that has degraded the water quality along the Simsang River's stretch, it is important to develop strategies for resolving problems with river water. Abundant mat formation in the study area and their capability to sequester metals can be used to formulate recovery strategies of this AMD impacted water bodies. Therefore, this study demonstrates that metal sequestration was quite successful and gives us essential knowledge about how to employ the dominant algae to manage AMD wastewater recovery.

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Conflicts of interest: None

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