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Assessment of Geochemical Characteristics and Geomicrobiology of Cave Spring Water from Jaintia and East Khasi Hills of Meghalaya, India

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Abstract. The present study was undertaken to know the concentration of various trace elements and the condition of water quality parameters in the cave water samples besides studying the role the microbes play in the precipitation of minerals in caves. The results revealed that the concentration of various trace elements such as copper, zinc, nickel and cadmium were low and below the water quality standard limits given by WHO (2006). While that of manganese it was exceptionally high, may be due to erosion of the manganese minerals deposits by the spring cave water. The results also revealed that phosphates are present in very low concentration while sulfates are present in high concentration which again may be due to erosion of secondary sulfate minerals. The co-relation matrices and one tailed analysis of variance of physic-chemical factors have been computed and analyzed. The positive correlation coefficient was observed between pH and alkalinity, hardness and conductivity, sulfates and turbidity. The one tailed ANOVA confirms that site spatial variations have less significant effect on concentration of trace elements. Microbial analysis showed that various types of microbes are present in cave sample which may play an important role in mineral precipitations.

Key words: geomicrobiology, water quality, speleothem genesis, mineral precipitation

Introduction

The Jaintia hills, one of the seven districts of Meghalaya, lies between latitude 25°5'N to 25°4'N and longitude 91°51'E to 92°45'E. The district is bound by the state of Assam on the north and east, the East Khasi Hills on the west and Bangladesh in the south. The district covers an area of 3819 km^2 constituting 17.03% of the total area of the state. The topography of the district is composed of undulating hilly landscapes dissected by numerous rivers and streams. On the northern and western borders, these hills take the form of tumbled ranges, running for the most part of north and south and ranging two to three thousand feet in height. Caves are formed in limestone areas and other rocks of similar composition by the process of weathering and erosion by water. The study of cave microbiology deals with the microscopic life that resides in cave. Without photosynthesis, caves are cut off from most energy that supports life on the surface. As a result, cave microorganisms must look for alternative sources of energy for their survival, such as those found in the

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atmosphere, or present in the very rock itself (BARTON *et al*., 2004; CHELIUS & MOORE, 2004; SPILDE *et al*., 2005). In adapting to these extremely starved environments, microorganisms produce elaborate scavenging mechanisms to pull scarce nutrients into the cell (KOCH, 1997). When these organisms are then exposed to the rich nutrients of a Petri plate, they cannot turn down these scavenging mechanisms and quickly gorge themselves to death (KOCH, 1997; 2001). As a result, microorganisms from starved cave environments may have a hard time adapting to rapidly changing nutrient status *in vitro*, and simply die from osmotic stresses (KOCH, 1997).The present study was undertaken to analyze the geochemistry of cave water samples and to analyze the geomicrobiology of speleothems.

Material and Methods

Physic-chemical parameters of water

The samples of caves spring water and rock samples were collected from Meghalaya by the Department of Environmental science and Engineering, Guru Jambheshwar University of Science and Technology, Hissar (Haryana).The physic-chemical parameters of water were carried out as per the standard methods.

The data collected were subjected to Pearson's correlation matrix to study the significant level at $p<0.05$ and $p<0.01$ (two tailed) to note the positive and negative correlation among physic-chemical factors. Similarly one way ANOVA was applied to know variation among trace elements. The SPSS version.16.0 statistical program was used for all statistical analysis throughout this research.

Geo-microbiology of caves

Rock samples were crushed to make powder. One gram of powdered rock sample was dissolved into 100 ml distilled water and agitated in a shaker for 15 minutes. Nutrient agar medium was prepared by dissolving 31 g of nutrient agar in one liter distilled water. The media, glassware like micro tips, 40 test tubes having 9 ml distilled water were autoclaved at 121°C and 15 psi pressure for 15 minutes. Then, the media was poured in the sterilized Petri plates in laminar flow and allowed to keep undisturbed until the media was solidified.

Table 1. Standard methods used for analyses of physic-chemical parameters of water

S .No.	Parameters	Methods
1.	pH	pH meter(APHA 1998)
2.	Dissolved Oxygen	Winkler's titration method (APHA, 1998; Wetzel and Likens, 2000)
3.	Conductivity	Conductivity meter(APHA 1998)
4	Water Temperature	Thermometer (APHA, 1998)
5	Total Alkalinity	Titrimetric method(APHA, 1998)
6	Total dissolved solids	APHA (1998)
7	Calcium Hardness	EDTA titrimetric method (APHA, 1998)
8	Magnesium Hardness	EDTA Titrimetric method (APHA, 1998)
9	Total Hardness	Complexometric method (APHA, 1998)
10	Chloride	Argentimetric method (APHA, 1998)
11	Nitrite	Spectrophotometric method (APHA, 1998; Wetzel & Likens, 2000)
12	Nitrate	Sodium salicylate (APHA, 1998; Wetzel & Likens, 2000)
13	Ammonias Nitrogen	Phenate method (APHA, 1998; Wetzel & Likens, 2000)
14	Ortho-phosphate	Ascorbic Acid method (APHA, 1998; Wetzel & Likens, 2000)
16	Trace Metals (Cu, Zn, Ni, Cd, Mg)	Atomic Absorption Spectrophotometer (AAS) (APHA, 1998)

After that, dilutions of the order of 10^{-2} of the rock sample were prepared and then inoculation was done. Then the Petri Best wishes, plates were wrapped with paraffin wax and were kept in the incubator at 28°C for five days. Then the colonies were identified using Gram Staining technique and Most Probable Number method (MPN).

Results and Discussion

The present study was undertaken to know the concentration of various trace elements in the cave water samples and to know the condition of water quality parameters. Geochemical analysis of cave waters revealed that there is a significant difference in the concentration of various trace metals from different sampling sites as shown in Fig. 1. The concentration of copper ranged from 0.2466 ppm (sample No. SI-3) to 0 ppm in most of the samples (Table 2). In case of zinc maximum concentration is found in sample No. S1-3 i.e. 3.9417 ppm, followed by MC1W–01 (3.1612 ppm) while minimum concentration is detected in sample No. S2-6 i.e. 0.0787 ppm. Nickel showed overall low concentration being maximum in sample No. MC2W-11 i.e. 1.8784 ppm and minimum in sample No. S3-9 i.e. 0.0247 ppm. In case of cadmium concentration ranges from 0.02423 ppm (sample No. MC2W-03) to 0.001287 ppm (sample No. MC2W–02). However, manganese showed highest concentration ranging from 5.7353 ppm in sample No. MC2W-03, while minimum i.e. 0.114 ppm in MC2W-07. It has been found manganese-oxidizing bacteria such as *Leptothrix* in a stream in Matts Black Cave, West Virginia, and attributed the formation of birnessite in this cave to the precipitation of manganese around sheaths of bacteria BROUGHTON (1971) and MOORE (1981).

The pH ranged from 7.6 to 8.2 indicating slightly alkaline nature. Acidity ranged between 10 to 40 ppm which may be due to free carbon dioxide, trace amount of sulfuric acid and nitric acid (Table 3). However, alkalinity ranged from 40 ppm (sample No. MC2W-02, S1 and S3) to 100 ppm (samples MC2W-01 and MC2W-11) which may be due to presence of free ions of hydroxide, carbonate and bicarbonates.

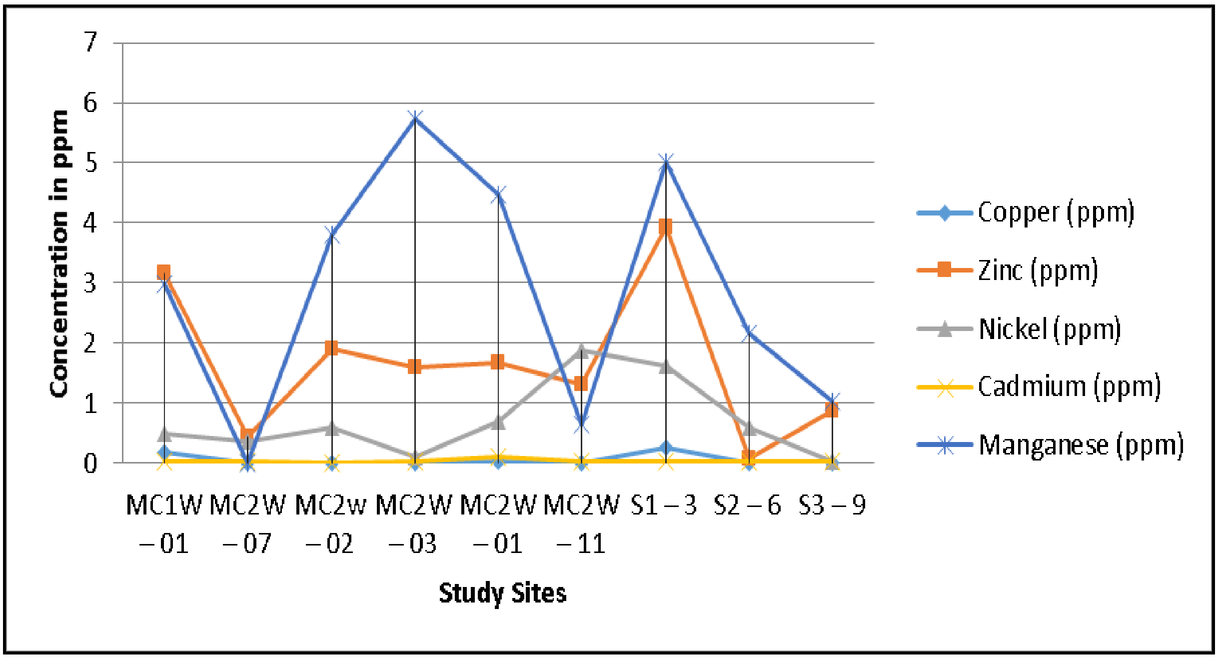

Fig.1. Showing variation in concentration of trace elements at different sites.

Table 2. Microbial colony characteristics.

Sample No.	Viable Count	CFU/g	Colour	Form	Elevation	Margin	Texture	Gram stain	Type
MCIR-01	15	15×10²	Pale yellow, Creamy white.	Circular, Irregular,	Flat, Convex.	Entire, undulate, erose.	Smooth, Slimy	+ve +ve	*Cocci, Cocci,*
MCIR-02	34	34×10²	Creamywhite, Slightly pink, Pale yellow	Circular, Irregular, Puntiform	Convex, Pulvinate, Raised.	Entire, Undulate, curled.	Powdry, Glutinous , Slight Slimy	+ve -ve -ve	*Cocci, ccoci cocci*
S-10	32	32×10²	Creamy white, Pale yellow.	Circular, Punctiform	Convex, Raised.	Entire, Undulate	Smooth, Slimy.	-ve -ve	*Cocci. Cocci*
S-15	19	32×10²	White, Yellow, Creamy white.	Circular, Irregular Filamentous	Convex, Pulvinate Flat	Lobate, Erose, Curved.	Glutinous, Powdry, smooth.	+ve +ve -ve	*Cocci Cocoi Cocci,*
MC2R-14	45	45×10²	Orange, Slightly Brown, Creamy white.	Irregular Filamentous. Punctiform.	Convex, Flat, Pulvinate.	Undulate, Lobate, Curved.	Glutinous, Smooth, Powdery.	-ve -ve +ve	*Cocci Cocci cocci*
S-17	14	14×10²	Orange, Translucent.	Circular, Irregular	Convex, Pulvinate.	Entire, Undulate	Smooth, Powdery,	-ve +ve	*Cocci cocci*
S-7	17	17×10²	Creamywhite, Greyish black, Pale yellow.	Punctiform, Circular, Irregular.	Convex, Raised. Flat	Entire, Undulate, lobate.	Slimy , Glutinous, Powdry	+ve +ve +ve	*Cocci Cocci Cocci*
S-9	14	14×10²	Red, Pale yellow	Circular, Irregular.	Raised, Convex.	Undulate, Filamento us	Rough, Slimy.	-ve -ve	*Cocci Cocci*
MCIR-13	17	17×10²	Pale yellow, grayish white	Circular, Irregular.	Raised, Convex	Entire, Undulate	Powdery, Smooth.	+ve +ve	*Cocci Cocci*
MCIR-24	Uncoun table	Uncounta ble	White, Creamy white.	Punctiform, Circular.	Convex, Pulvinate.	Entire , Erose.	Smooth, Slimy	-ve +ve	*Cocci, Cocci*

Table 3. Geochemistry of Caves wáter.

S. No.	Sample	pH	Temp. (⁰C)	Acidity (ppm)	Alkalinity (ppm)	Conductivity (µS)	Hardness (ppm)	Turbidity (ppm)	Phosphate (ppm)	Sulfate (ppm)
01.	MC1W-01	7.7	11.5	40	80	209.3	220	0.7	0.02	9.4
02.	MC2W 01	8.2	11.6	20	100	187.9	100	1.5	0.08	12.3
03.	MC2W 02	7.6	13	40	40	227.2	200	2.2	0.04	16.2
04.	MC2W 03	7.7	12.5	20	60	158.5	220	0.1	0.26	5.1
05.	MC2W 07	7.6	13	40	60	162.3	200	0.1	0.12	6.4
06.	MC2W 11	7.9	11	10	100	152	200	0.2	0.06	4.3
07.	S_1	7.6	8	20	40	253	220	0.1	0.07	1.2
08.	S_2	7.8	11.5	40	80	243.1	240	0.4	0.00	0.3
09.	S_3	7.7	12	40	40	74.16	100	0.2	0.05	9.8
10.	Mean	7.7	11.5	30	66.66	185.27	188.88	0.61	0.07	7.22
11.	S.E(±)	2.5	1.5	12.24	24.49	55.86	52.06	0.74	0.076	5.19
12.	CD (5%)	4.9	2.94	23.99	48.98	109.48	102.03	1.45	0.14	10.17

In case of conductivity it ranged from74.16 µs (sample No. S3) to 253 µs (sample No. S1) which may be due various free ions present in the sample. Hardness of caves water samples ranged from 240 ppm to 100 ppm which may be due to ions such as, carbonate and bicarbonates of calcium and magnesium. However the turbidity ranged from 0.1 to 2.2 which may be due to suspended matter ranging from pure inorganic substance to those that are organic in nature. In case of phosphate ions the concentration is extremely very low ranging between 0.02 ppm in sample No. MC1W-01 to 0.26 ppm in sample No. MC2W-03. However in case of sample No. S2 phosphate ions are absent. In case of sulfates the concentration varied from 0.3 ppm in the sample No. S2 to 16.2 ppm in the sample No. MC2W-02 thus being maximal in MC2W-02 cave sample. The results revealed that the concentration of various trace elements such as copper, zinc, nickel and cadmium were low and below the standard water parameter limits. While that of manganese it was exceptionally high, may be due to erosion of the manganese minerals deposits by the spring cave water. The results also revealed that phosphates are present in very low concentration while sulfates are present in high concentration which again may be due to erosion of secondary sulfate minerals.

Morphological characteristic of microbial colonies (Fig. 2-1 and 2-2, Table 4) revealed that both gram positive and gram negative microbes were existing in different forms. They play an important role in mineral precipitation (FRANKEL & BAZYLINSKI, 2003). Studies revealed that calcite was the dominant mineral and an abundant microbial community was detected by direct microscopic observation after DAPI staining which were indicative of microbial involvement in the speleothem genesis (BASKAR *et al.* 2005, 2006, 2007). The iron-oxidizing species *Gallionella ferruginea* and *Leptothrix sp*. has been recovered from cave samples (PECK, 1986). Further detailed investigations are required involving *in vitro* culture experiments and molecular techniques to quantify the extent of microbial participation in speleothem genesis. Progress in the field will depend on cross-disciplinary studies involving the abilities of biologists to recognize assuredly biological structures and measure these processes within the cave environment; and geologists, who can apply the complex tools of chemistry and geology to the problem.

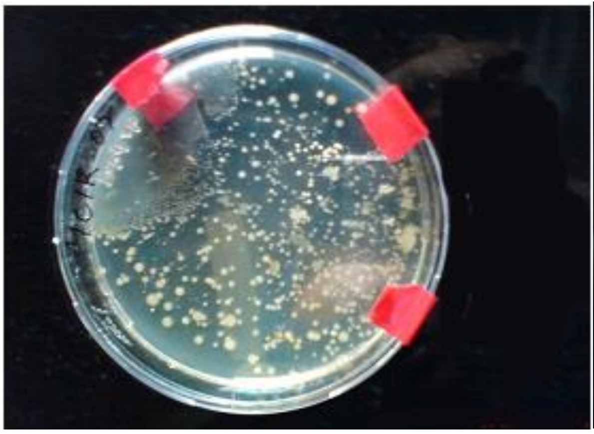

Fig. 2-1. MC2R-14

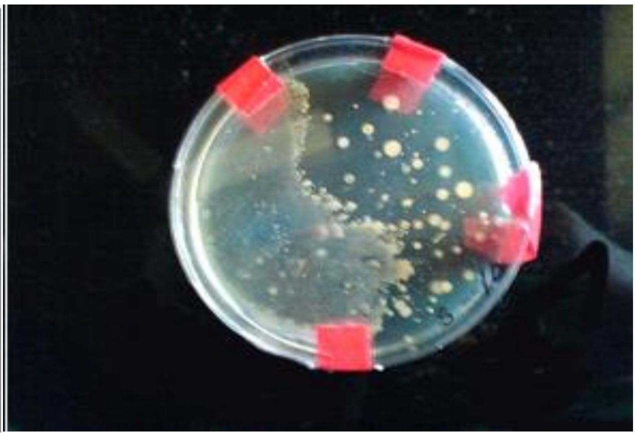

Fig. 2-2. S-10

Table 4. Geochemistry of Caves water

	A	B	C	D	E	F	G	H	I
A	+1								
B	-.014	+1							
C	-.300	+.431	+1						
D	+.866**	-.115	+.000	+1					
E	-.134	+.057	+.247	-.312	+1				
F	+.257	-.106	-.345	+.332	+925**	+1			
G	+.244	+.330	+.044	+.161	-.178	+.149	+1		
H	-.083	+.228	-.576	-.314	-.197	+.126	-.302	+1	
I	+.130	+.550	+.148	-.012	+.175	-.249	+.809**	-.097	+1

** = Correlation is high significant at $p < 0.01$ level, '-' indicate negative correlation, '+' indicate positive correlation,
Where A= pH, B= Temp., C= Acidity, D= Alkalinity, E= Conductivity, F= Hardness, G= Turbidity, H= Phosphate, I= Sulfate

The study of cave microbes has significant implications in the preservation of ancient marble monuments and statues, where microorganisms could be used to deposit a veneer of calcite to protect ancient structures from continued erosion (LAIZ *et al.*, 2003) and can be inoculated into contaminated environments to rapidly degrade pollutants and allow restoration of natural habitats in a process called bioremediation. Cave microorganisms also have the potential to harbour unique antibiotics, with properties that allow efficient ethanol production for fuel, enzymes for environmentally friendly paper processing and even the improved stonewashing of jeans (ONAGA, 2001).

The data collected were subjected to Pearsons's correlation matrix to study the significant level at $p< 0.05$ and $p< 0.01$ (2 tailed) to note the positive and negative correlation among the physic-chemical factors. The statistical analysis of Pearson's correlation coefficient is presented in Table 5. The study of correlation coefficient between various physic-chemical factors indicated that pH values varied with the variation of alkalinity. The rise of carbonate and bicarbonate concentrations increased the level of pH and hence alkalinity enhanced the decomposition of organic matter which in turn increases concentration of nitrite, phosphate and sulfate ions. The abundant of Ca and Mg in addition to nitrite, sulfates and phosphates are responsible for an increase of hardness and a perfect positive correlation with conductivity. The high concentration of sulfates increases turbidity of water.

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