Global Journal of Science Frontier Research
Enviornment & Earth Sciences
Volume 12 Issue 2 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Geomicrobiology and Geochemistry of Cave Spring Water from Jaintia and East Khasi Hills of Meghalaya, India

By Gulzar Ahmad Sheikh, Idrees Yousuf Dar, R. Bhaskar, A. K. Pandit & Theophilus
Guru Jambheswar University of Science & Technology, Hissar

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 phosphate is present in very low concentration while sulphate is present in high concentration which again may be due to erosion of secondary sulphate 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, sulphate 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.

Keywords : Geomicrobiology, Water quality, Speleothem genesis , Mineral precipitation.

GJSFR-H Classification : FOR Code: 060504, 040699

GEOMICROBIOLOGY AND GEOCHEMISTRY OF CAVE SPRING WATER FROM JAINTIA AND EAST KHASI HILLS OF MEGHALAYA, INDIA

Geomicrobiology and Geochemistry of Cave Spring Water from Jaintia and East Khasi Hills of Meghalaya, India

Gulzar Ahmad Sheikh [α], Idrees Yousuf Dar [σ], R. Bhaskar [ρ], A. K. Pandit [ω] & Theophilus [¥]

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 phosphate is present in very low concentration while sulphate is present in high concentration which again may be due to erosion of secondary sulphate 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, sulphate 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.

Keywords : Geomicrobiology, Water quality, Speleothem genesis , Mineral precipitation.

I. Introduction

Caves are formed in limestone areas and other rocks of similar composition by the process of weathering and erosion by water. The rainwater reacts with carbon dioxide from the atmosphere to form a weak carbonic acid solution. This solution slowly dissolves carbonate rocks and forms cavities and passages. The cavities and passageways are formed where the maximum amount of continuous water flow occurs, right below the water table. Once the water table has lowered, leaving the caves in the aerated zone, the deposition of calcite occurs, creating different beautiful "cave decorations" called speleothems. Sulphuric acid, often found in water from bogs in very low concentration produces a significant amount of limestone solution by the oxidation of sulphide ore, thereby causing localized cavern formation (Caro, 1965). Organic acids such as formic, acetic and butyric formed in plants both during life and by decay after death are available in the soil and can be leached by water to dissolve limestone. 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 atmosphere, or present in the very rock itself (Barton *et al.*, 2004; Chelius and 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.

II. Material and Methods

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

Author α, ρ, ω : Department of Environmental Science & Engineering, Guru Jambheswar University of Science & Technology, Hissar (India).
Author ¥ : Department of Environmental Science, University of Kashmir Srinagar-190006- India.
Author σ : Terrestrial Ecology Lab., Department of Environmental Science, University of Kashmir, Srinagar -190 006, Kashmir, India.
E-mail : wilderness4@gmail.com

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 titrimetrc method (APHA 1998)
8	Magnesium Hardness	EDTA Titrimetric method(APHA 1998)
9	Total Hardness	Complexometric method(APHA 1998)
10	Chloride	Argentimeteric method(APHA 1998)
11	Nitrite	Spectrophotometric method (APHA,1998;Wetzel and likens, 2000)
12	Nitrate	Sodium salicylate APHA, 1998; Wetzel and Likens, 2000)
13	Ammonical nitrogen	Phenate method (APHA, 1998; Wetzel and Likens, 2000)
14	Ortho-phosphate	Ascorbic Acid method (APHA, 1998; Wetzel and Likens, 2000)
16	ace Metals(Cu, Zn, Ni, Cd, & Mg)	Atomic Absorption Spectrophotometer (AAS) (APHA, 1998)

The data collected were subjected to pearsons 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 programme was used for all statistical analysis throughout this research.

a) Geo-microbiology of caves

Rock samples were crushed to make powder. One gram of powdered rock sample was dissolved into 100ml distilled water and agitated in a shaker for 15 minutes. Nutrient agar medium was prepared by dissolving 31gm of nutrient agar in one liter distilled water. The media, glassware like micro tips, 40 test tubes having 9ml distilled water were autoclaved at 121^0C 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. 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 28^0C for five days. Then the colonies were identified using Gram Staining technique and Most Probable Number method (MPN).

III. Results and Discussion

The present study was undertaken to know the concentration of various trace element 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 figure 1. The concentration of copper ranged from 0.2466 ppm in sample No. SI-3 to 0 ppm in most of the samples (Table 1) 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 (in sample no.MC2W-03) to 0.001287ppm.(in 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 between 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 sulphuric acid and nitric acid (Table2).However alkalinity ranged between 40 ppm (in sample no.MC2W-02, S1 and S3) to 100 ppm. (in sample MC2W-01 and MC2W-11) which may be due to presence of free ions of hydroxide, carbonate and bicarbonates. In case of conductivity it ranged from74.16 µs in sample S3 to253 µs in the 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 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 sulphate the concentration varied from 0.3 ppm. in the sample no. S2 to 16.2 ppm. in the sample no. MC2W-02 thus being maximum 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 phosphate is present in very low concentration while sulphate is present in high concentration which again may be due to erosion of secondary sulphate minerals.

Morphological characteristic of microbial colonies (Plate1 & Plate 2, Table 3) revealed that both gram positive and gram negative microbes were existing in different forms. They play an important role in mineral precipitation (Frankel and 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.

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 harbor 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).

IV. Statistical Analysis

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 physico-chemical factors. The statistical analysis of pearson's correlation coefficient is presented in table-4.The study of correlation coefficient between various physico-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. Alkalnity enhanced the decomposition of organic matter which in turn increase concentration of nitrite, phosphate and sulphate ions. The abundant of ca and Mg in addition to nitrite, sulphate and phosphate are responsible for an increase of hardness and a perfect positive correlation with conductivity. The high concentration of sulphate makes water turbid and hence increases turbidity of water.

V. Acknowleedgement

The authors acknowledges the support and facility provided by Department of Environmental Science & Engineering, Guru Jambheswar University of Science & Technology, Hissar (India)

References Références Referencias

1. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association, Washington, DC.
2. Barton, H.A., Taylor, M.R., and Pace, N. R. (2004). Molecular phylogenetic analyais of a bacteria community in an oligotrophic cave environment: *Geomicrobiology Journal*., 21, 11-20s
3. Baskar S, Baskar R, Mauclaire L and McKenzie J.A. (2006). Microbially induced Calcite precipitation by culture experiments – Possible origin for Stalactites in Sahastradhara, Dehradun, India.*Current Science*, 90, 58 – 64.
4. Baskar S., Baskar R., Kaushik A. (2007). Evidences for microbial involvement in the genesis of

speleothem carbonates, Borra Caves, Vishakapatanam, India. *Current Science*, 92, 350 – 355.

5. Baskar S., Baskar R., Mauclaire L. and McKenzie J.A. (2005). Role of microbial community in stalactite formation, Sahastradhara caves, Dehradun, India. *Current Science*, 88, 1305-1308.
6. Broughton, P.L.(1971). Origin and distribution of mineral species in limestone caves: *Earth Science Journal.*, 5, 36–43.
7. Caro, P. (1965). La chimie du gaz carbonique et des carbonates, et les phěnoměnes hydrogěologiques karstiques. *Chronique d' Hydrogeologie,*7, 51 – 77.
8. Chelius, M.K., and Moori, J.C. (2004). Molecular phylogenetic analysis of Archaea and Bacteria in Wind Cave, South Dakota: *Geomicrobiology Journal,* 21, 123-134.
9. Frankel, R.B., and Bazylinski, D.A. (2003). Biologically induced mineralization by bacteria: *Reviews in Mineralogy and Geochemistry,* 54, 95–114.
10. Koch, A.L. (1997). Microbial physiology and ecology of slow growth: *Microbiology and Molecular biology Reviews*, 61, 305-318.
11. Koch, A.L.(2001) . Oligotrophs versus copiotrophs: *BioEssays.* 23, 657-661.
12. Laiz, L., pinar, G, Lubitz, W., and Saiz-Jimenez, C. (2003). Monitoring the colonization of monuments by bacteria: Cultivation versus Molecular methods: *Environmental microbiology,* 5, 129-138.
13. Moore, G.W. (1981) .Manganese deposition in limestone caves, in Beck, B.F., ed., *Proceedings of the 8th International Congress of Speleology* : 642–645.
14. Onaga, L. (2001).Cashing in on nature's pharmacy: *EMBO Reports*, 2, 263-265.
15. Peck, S.B., 1986, Bacterial deposition of iron and manganese oxides in North American caves: *Bulletin of the National Speleological Society.*, 48, 26–30.
16. Spilde, M.N., Northup, D.E., Boston, P.J., Schelble, R.T., Dano, K.E., Crossey, L.J., and Dahm, C.N. (2005). Geomicrobiology of cave ferromanganese deposits; A field and laboratory investigation: *Geomicrobiology Journal.*, 22, 99-116.
17. Wetzel, R.G. and G.E. Likens, 2000. Limnological Analysis, 3rd edition. Springer a. Verlag New York Publications, 429 pp.

Table 1 : Geochemistry of trace elements.

Sample No.	Copper (ppm)	Zinc (ppm)	Nickel (ppm)	Cadmium (ppm)	Manganese (ppm)
MC1W – 01	0.1695	3.1612	0.4827	0.01210	2.9899
MC2W – 07	0.0	0.4263	0.3543	0.01923	O.114
MC2w – 02	0.0	1.9085	0.5849	0.00128	3.8015
MC2W – 03	0.0	1.5806	0.1071	0.02423	5.7353
MC2W – 01	0.0154	1.6593	0.6898	0.1099	4.4588
MC2W – 11	0.0	1.3052	1.8784	0.0212	0.6384
S1 – 3	0.2466	3.9417	1.6065	0.01847	5.0080
S2 – 6	0.0	0.0787	0.5849	0.02377	2.1683
S3 – 9	0.0	0.8723	0.0247	0.01741	1.0280
Mean	0.0479	1.6593	0.7014	0.0275	3.2285
S.E(±)	0.0876	1.2397	0.0929	0.0316	1.8525
C.D	0.1716	2.4298	0.1820	0.0619	3.6309

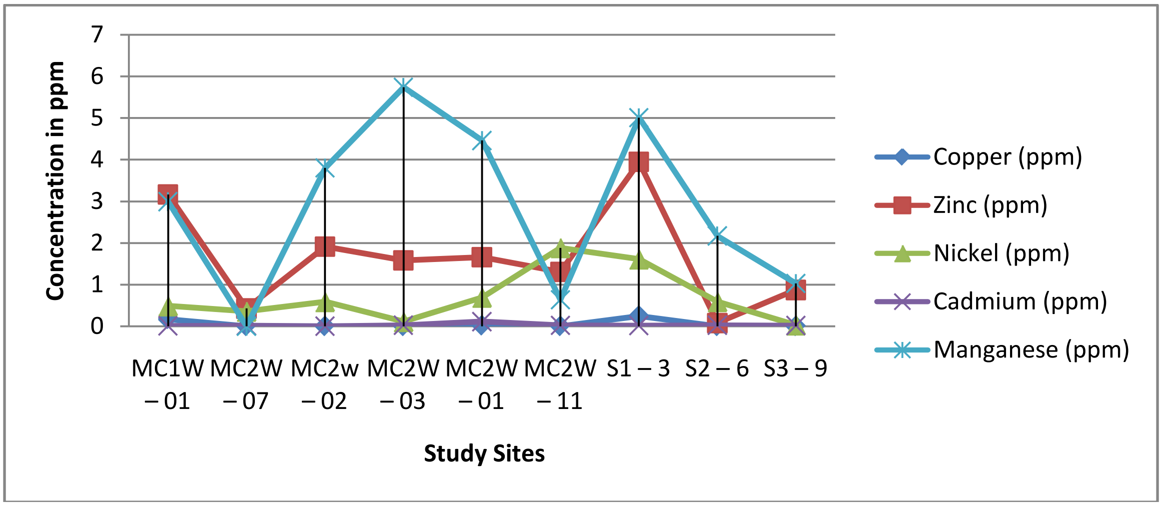
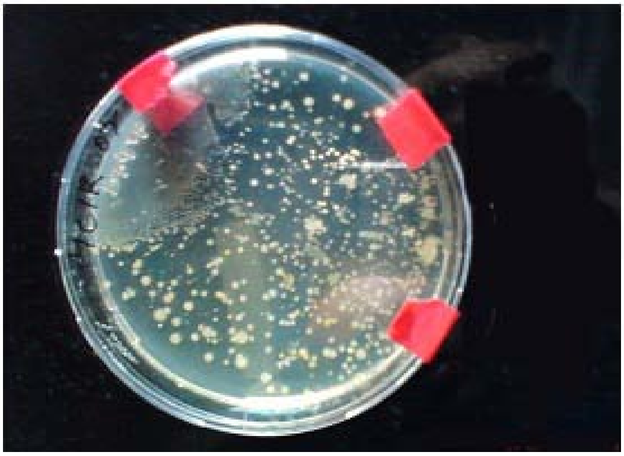


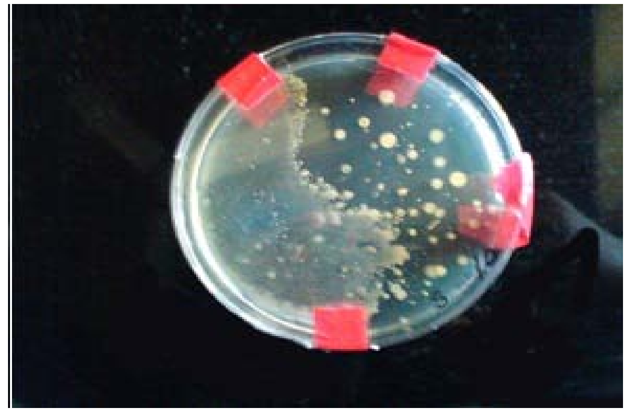
Figure 1 : Showing variation in concentration of heavy metals at different sites.

Table 2 : Geochemistry of Caves water

S. No.	Sample	Ph	Temp. (^{0}C)	Acidity (ppm)	Alkanity (ppm)	Conductivity (μS)	Hardness (ppm)	Turbidity (ppm)	Phosphate (ppm)	Sulphate (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



MC2R-14
PLATE-1



S-10
PLATE-2

Table 3 : MICROBIAL COLONY CHARACTERISTIC.

Sample No.	Viable Count	CFU/g	Colour	Forrm	Elevation	Margin	Texture	Gram stain	Type
MCIR-01	15	15×10^2	Pale yellow, Creamy white.	Circular, Irregular,	Flat, Convex.	Entire, undulate, erose.	Smooth, Slimy	+ve +ve	*Cocci, Cocci,*
MCIR-02	34	34×10^2	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	32×10^2	Creamy white, Pale yellow.	Circular, Punctiform	Convex, Raised.	Entire, Undulate,	Smooth, Slimy.	-ve -ve	*Cocci. Cocci*
S-15	19	32×10^2	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	45×10^2	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	14×10^2	Orange, Translucent.	Circular, Irregular	Convex, Pulvinate.	Entire, Undulate,	Smooth, Powdery,	-ve +ve	*Cocci cocci*
S-7	17	17×10^2	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	14×10^2	Red, Pale yellow,	Circular, Irregular.	Raised, Convex.	Undulate, Filamentous.	Rough, Slimy.	-ve -ve	*Cocci Cocci*
MCIR-13	17	17×10^2	Pale yellow, grayish white,	Circular, Irregular.	Raised, Convex,	Entire, Undulate,	Powdery, Smooth.	+ve +ve	*Cocci Cocci*
MCIR-24	Uncountable	Uncountable	White, Creamy white.	Punctiform, Circular.	Convex, Pulvinate.	Entire , Erose.	Smooth, Slimy ,	-ve +ve	*Cocci, Cocci*

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= Alkanity, E= Conductivity, F= Hardness, G= Turbidity, H= Phosphate, I= Sulphate*