Iraqi Bulletin of Geology and Mining Vol.7, No.3, 2011 p 63 – 79

HYDROGEOCHEMISTRY AND GEOMICROBIOLOGY OF DARZILA SPRING IN SANGAW, SULAIMANIYAH, NE IRAQ

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Received: 22/ 03/ 2011, Accepted: 06/ 10/ 2011
Key words: Sulphide spring, Geomicrobiology, Hypogenic cave, Karst

ABSTRACT

Darzila is a hypogenic cave located in Sangaw near Sulaimaniyah; Northeast of Iraq, offers a remarkable opportunity to observe microbial interactions within environment, which is related to the karst aquifers. Both water and atmosphere in the cave are rich in hydrogen sulphide. The predominant cation in the cave's water sample is Ca^{2+} and anion is SO_4^{2-}. The chemical speciation shows that samples 1 and 3 are supersaturated with gypsum. The high ionic ratio values of Ca/ Mg and Ca/ SO_4 indicates further dissolution of anhydrite and calcite. While the high ratio value of Na/ Cl, in sample 4 is attributed to ionic exchange inside the cave. Redox reactions in the cave appear to be microbially mediated. Bacteriological analysis of water samples shows that there are three *Thiobacilli* species, a genus that often obtains its energy from the oxidation of sulphur compounds. Oxidation of hydrogen sulphide to sulphuric acid, and hence the cave enlargement, is probably enhanced by these bacteria. Two cave-enlargement processes were identified: **i)** Sulphuric acid derived from oxidation of the hydrogen sulphide converts subaerial limestone surfaces to gypsum. The gypsum falls into the cave stream and dissolves. **ii)** Strongly acidic droplets form on the gypsum and on microbial filaments, dissolving the limestone, where they drip onto the cave floors. The possible source of organic matter is from deep oil-rich reservoirs (or bitumen occurrences within the limestone), the presence of organic matter at the bank of Awa Spi stream support this idea.

هايدروجيوكيميائية وجيومايكروبايولوجية عين درزيلة في سنگاو، سليمانية، شمال شرق العراق

پولا آزاد خانقاه و دياري علي محمد أمين المنمي

المستخلص

كهف درزيلة هو "هايبوجيني" يقع في منطقة سنگاو قرب السليمانية، شمال شرق العراق. الكهف يتيح فرصة رائعة لمراقبة التفاعلات الميكروبية داخل بيئة الخزانات الخسفية. المياه والغلاف الجوي في الكهف غني بغاز كبريتيد الهيدروجين. الأيون الموجب السائد في نماذج مياه الكهف هو Ca^{2+} والأيون السالب السائد هو SO_4^{2-} وتبين من حساب السحنات الكيميائية بان النموذجين 1 و 3 هما فوق الإشباع بمعدن الجبس. إن ارتفاع قيم النسب الأيونية لنسب من الكالسيوم Ca/ Mg و Ca/ SO_4 هي إشارة إلى المزيد من الذوبان لمعدني الانهيدرايت والكالسيت. في حين تعتبر القيمة العالية Na/ Cl في العينة 4 دليلاً على التبادل الأيوني داخل الكهف. تتم تفاعلات الأكسدة والاختزال في الكهف بواسطة البكتريا. تبين من التحليل البكتريولوجي لعينات المياه أن هناك ثلاثة أنواع من بكتريا *Thiobacilli*، وهذا الجنس يحصل في أكثر الأحيان على احتياجاته من الطاقة من خلال أكسدة مركبات الكبريت. أكسدة كبريتيد الهيدروجين لحامض الكبريتيك، يؤدي إلى توسيع الكهف معززاً بوجود هذه البكتريا. وقد تم تحديد اثنتين من عمليات توسيع الكهف

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1) حامض الكبريتيك المشتق من أكسدة كبريتيد الهيدروجين وتحويل أسطح الحجر الجيري الشبه هوائي إلى الجبس، ويتم إذابة وإزالة الجبس الساقط بواسطة مياه الكهف. **2)** تشكل قطيرات ذات حامضية عالية على الجبس وعلى الخيوط الميكروبية، حيث يذوب الحجر الجيري بالتنقيط على طوابق الكهف. إن المصدر المحتمل للمادة العضوية هو من الخزانات العميقة الغنية بالنفط (أو وجود القار داخل جسم الحجر الجيري)، وإن وجود المواد العضوية في أطراف نهر آوة سپي يدعم هذه الفكرة.

INTRODUCTION

Although there are some studies about the hydrochemistry and speleogenesis process of some springs in Sangaw area, like the studies of Babasheikh (2000); Stevanovic *et al.* (2009), Iurkiewicz and Stevanovic (2010), still this area is poorly inspected and needs both hydrogeochemical and geomicrobiological studies in detail. Many specific karst systems are formed by sulfuric acid dissolution of evaporitic and carbonate rocks. It also has been preliminary studied in the Darzila cave, which belongs to Sangaw District, commonly called Garmian (“Garmian”, in Kurdish language means very hot). Some of the world's most notable caves, including Carlsbad Cavern Cave, de Villa Luz and Lechuguilla Cave in New Mexico, USA, and Cupp Coutunn Cave system in Turkmenia, formed by rising acidic water of deep-seated origin (Hose *et al.*, 2000).This type of caves is called “Hypogenic”, which are formed by water when it is rising up from below and dissolving the rock. Obviously, as a result of mixing two different types of water together, it results from a source of aggressiveness produced at depth (CO_2 or H_2S) and is linked to confined or rising flow, without the direct influence of surface recharge (Ford and Williams, 2007).

Egemeier (1981) proposed that many such caves are developed, at least in part; through a process called "replacement solution". He mentioned that where H_2S-charged water enters air-filled caves through water-filled conduits, releasing dissolved H_2S into the cave atmosphere, the H_2S gas reacts with O_2, $CaCO_3$, and H_2O in a variety of reactions that lead to the deposition of elemental sulphur and gypsum on subaerial walls and ceilings. The coatings become so heavy that they cannot sustain their weight and fall to the floor, and the streams rapidly dissolve the gypsum and remove it from the cave. Egemeier (1981) noted the possible role of microbes in reducing the sulphate to H_2S in the waters before they enter the cave, but did not suggest microbial participation in oxidation reactions within the cave. It is recognized that some hypogenic caves are formed, at least in part, by rising H_2S in the water table have extended many caves throughout the world (Davis, 1980; Korshunov and Semikolennyh, 1994; Galdenzi and Menichetti, 1995 and Hose *et al.*, 2000). Several workers have suggested that microorganisms participate in certain redox reactions in hypogenic caves, producing H_2SO_4 and elemental sulphur (Spirakis and Cunningham, 1992; Sarbu *et al.*, 1996).

LOCATION OF THE SITE

Darzila Spring is located about 1 Km north of Darzila village, defined by latitude 35^{o} 08$^{'}$ 47$^{''}$ and longitude 45^{o} 16$^{'}$ 44$^{''}$, and about 18 Km northwest of Sangaw town, southwest of Sulaimaniyah city. It is located in the southern limb of Ag Dagh double plunging anticline (Fig.1). The altitude of the cave is approximately 686 m above sea level; it is formed in a limestone rock unit, which belongs to Pila Spi Formation (Eocene) within the southern limb of Ag Dagh anticline. A significant NE – SW striking lineament inferred also from satellite imagery (Numan 2002, unpublished data), crosses the southeastern plunge of the Ag Dagh anticline and the associated weakness zone is likely to have controlled both the surface and the underground water flow. Most observed crevasses and cracks in the area display similar strikes in the spring; probably rise along a fault. The diameter of the cave on the top is about 30 m and there is one passage that leads to the inside of the cave, about 1.5 m in diameter (Fig.2 A and B). Total explored passage length is about 200 m.

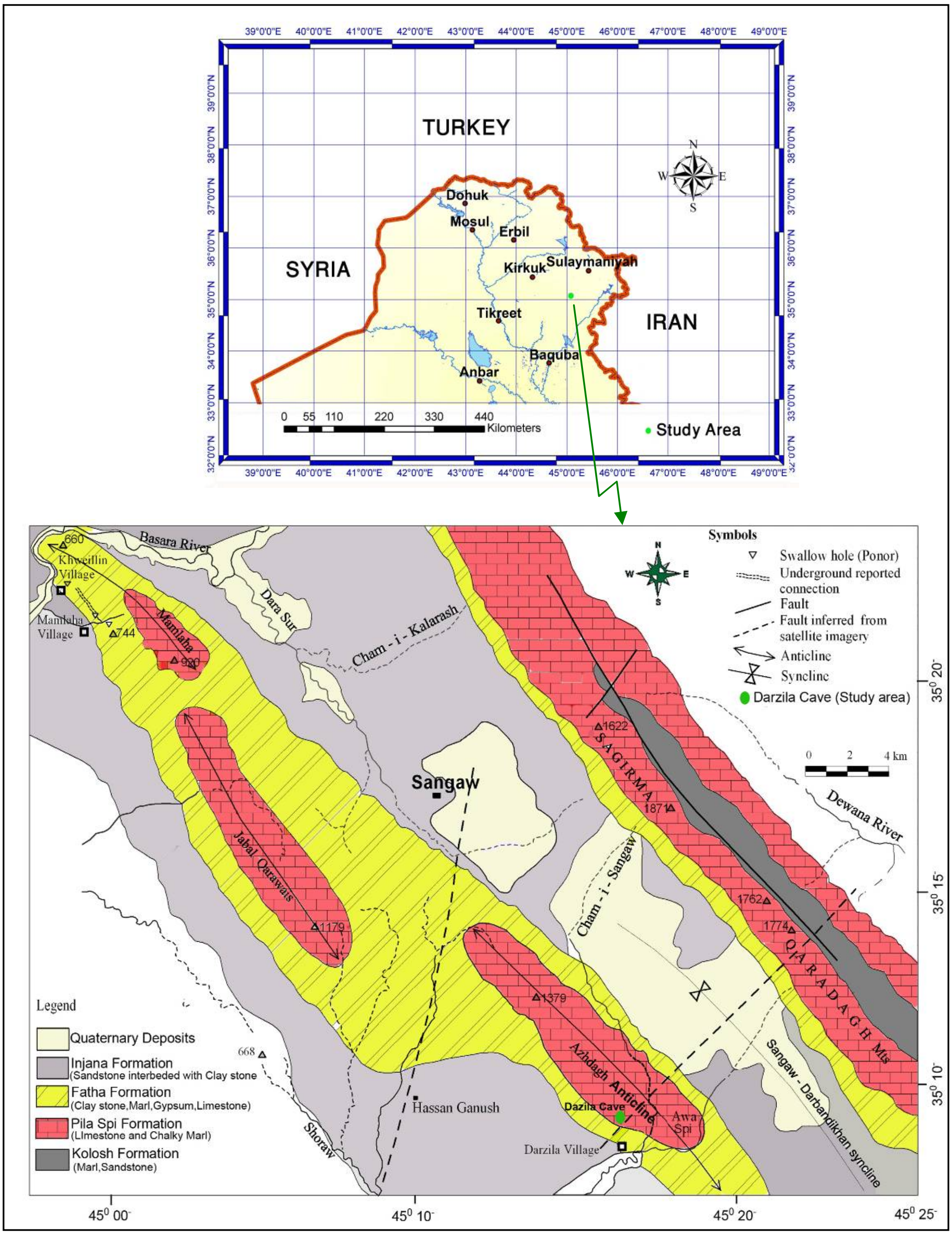


Fig.1: Location and geological map of the studied area
(after Ma'ala, 2007)

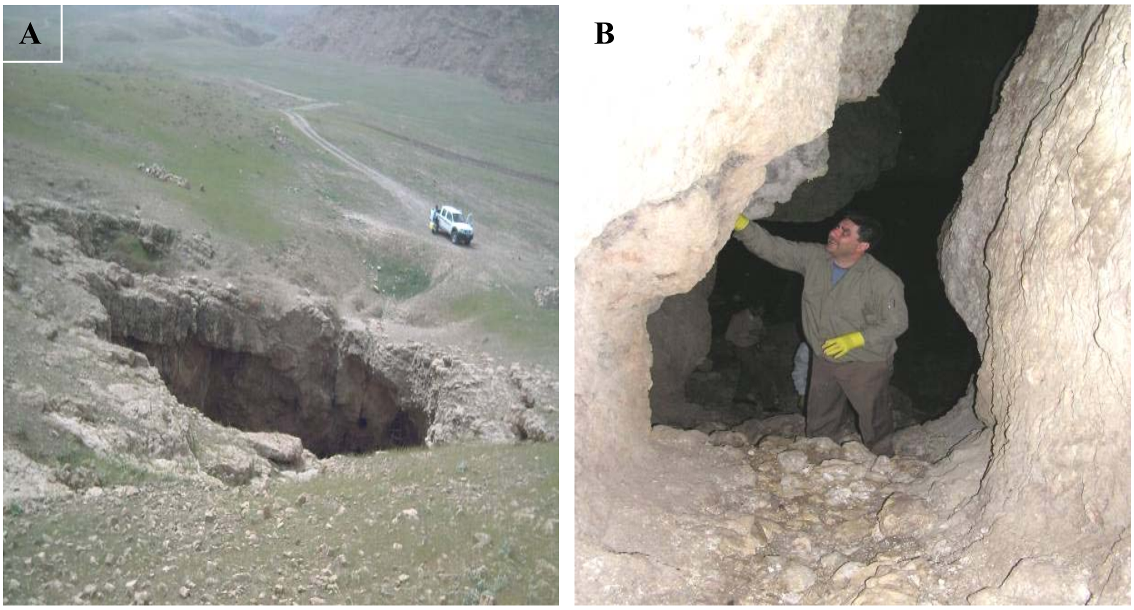


Fig.2: **A)** Top of the Darzila Cave; **B)** Entrance Hole to the cave

TECTONIC AND GEOLOGY

The studied area is located within the Foothill Zone; Cham Chamal – Butma Subzone (Jassim and Goff, 2006). The prominent features of this sub-zone are long anticlines trending NW – SE, often not associated with longitudinal faults, broad and deep synclines are a feature of the sub-zone.

Geologically, the studied area includes different geological rock units, which are; Pila Spi, Fatha, and Injana formations (Fig.1). They are described hereinafter.

Pila Spi Formation (Middle – Late Eocene) comprises two units: The upper part, which comprises well bedded, bituminous, chalky, and crystalline limestones, with bands of white chalky marl and with chert nodules toward the top. The lower part comprises well bedded, hard, porous or vitreous, bituminous, white, poorly fossiliferous limestone, with algal or shell sections. Darzila spring is located within the Pila Spi Formation.

The Fatha Formation (Middle Miocene) comprises of four informal units (Jassim and Goff, 2006) from the bottom to the top are: The Transition Beds consist mainly of anhydrite separated by the limestone and mudstone. The Saliferous Beds, of rock salt and anhydrite with siltstones, mudstone and some limestone intercalation. The Seepage Beds consist of anhydrite, with some siltstones and with some limestone beds. The Upper Red Beds consist of reddish mudstones and siltstones, with relatively limestone and anhydrites. In the studied area, the formation consists of cyclic repetition of red claystone, marl, gypsum and occasional interbeds of limestone and sandstone. The upper contact is gradational with the Injana Formation, while its lower contact is mostly unconformable with Pila Spi Formation (Buday, 1980 and Jassim and Goff, 2006).

Injana Formation (Late Miocene) consists of fine grained pre-molasse sediments, the basal units comprises thin bedded calcareous sandstone, red and green mudstones, with one thin gypsum bed and a purple siltstone horizon with glass shards. Its lower contact is gradational with Fatha Formation and the upper contact is gradational with Mukdadiya Formation; marked by appearance of gravely sandstone. Fatha and Injana formations form the flank of Ag Dagh anticline.

HYDROLOGY AND HYDROGEOLOGY

The climate of the studied area is continental; arid to semi arid climate, so it is known locally as "Garmian" (hot) area, cold winters and long hot summers, with annual rainfall rate amounting only to about 580 mm (Sangaw station) for the period of 2000 – 2010. Hydrologically, the studied area is located in Chamchamal – Sangau Basin. The main aquifer system is the Pila Spi aquifer, which is highly fissured, well-karstified with many features, like channels and caves that can be observed on the surface, it is confined aquifer, because it is overlain by Fatha Formation. The effective infiltration capacity of the Pila Spi karstic aquifer is high and a large part of the rainfall contributes to the groundwater recharge. The assessment of this infiltration rate relies on the occurrence of highly fractured, cavern depressions, sinkholes and karstified rocks, lack of vegetation and a poorly developed hydrographic system. Technical hydrogeological investigations recently performed in Northern Iraq (Stevanovic and Iurkiewicz, 2004a), have indicated the Pila Spi aquifer recharge coefficients amounting to (30 – 50) % of the total rainfall values (Stevanovic and Markovic, 2004b). During the wet seasons, the reduced evaporation rate, rainfall and even the layer slope contribute to the above mentioned rate on outcropping surface of the “Pila Spi” aquifer. Meteoric water infiltrating through layers of Pila Spi and Fatha formations are enriched in sulfate due to less soluble nature, therefore the Pila Spi aquifer is gradually enriched of more sulfate along the flow path of the groundwater.

A low gradient stream flows through most of the cave. Five groundwater inlets feed the cave, which rise through discrete conduits and others, two of them are acidic; two are milky colored rich in hydrogen sulphide, whereas the fifth is oxygenated water. The milky color of the stream (Fig.3) results from suspended elemental sulphur. Prolonged exposure of skin to the acidic water causes a mild burning sensation. The stream discharge does not vary significantly. The main resurgence in June 2009 was measured at 40 l/s in the dry season and 60 l/s in the wet season of April 2009.

Soluble gypsum covers the walls of the cave, Elemental sulphur forms on walls, whereas the limestone surfaces are coated with gypsum. Sulphur is most stable at low pH. The films and droplets of the acid are quickly neutralized by contacting with carbonate rocks (Fig.4A and B).

The source of the cave water has not yet been conclusively identified but it may be from the infiltration of meteoric water to the Pila Spi Formation. Petroleum fields near the area and the gypsum of Fatha Formation are a probable source of the H_2S-rich water.

Fig.3: Milky color stream inside Darzila Cave

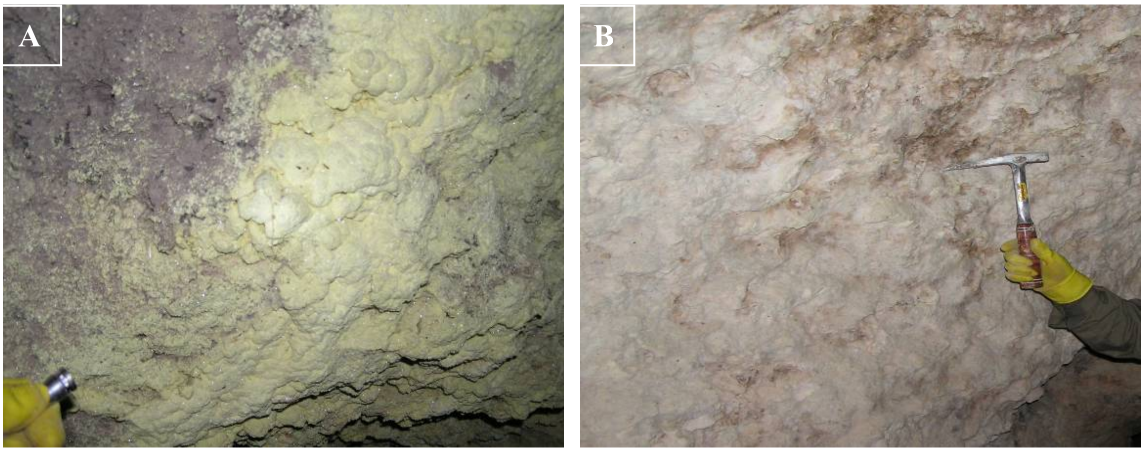


Fig.4: Deposition of **A)** Elemental Sulfur
B) Gypsum on subaerial walls and ceilings of the Darzila Cave

METHODOLOGY

▪ Hydrochemical Measurements

During the visit to the studied area, which took place in June 2009, five water samples were collected from 5 points along the cave. During the investigation, analysis was carried out for temperature, pH, and EC; directly in the site by using portable multiparameter analyzer model TPS/90FL-T Field Lab. Analyzer. Hydrogen sulphide is stabilized in the field by pouring a portion of the sample to 100 ml volumetric flask, then adding 4 ml 20% zinc acetate [Zn $(CH_3COO)_2$] and 1 ml (1N) NaOH, then the volume is completed with water sample. Back at the laboratory further analysis were made, 4 ml HCl was added to the samples to release H_2S gas, then 5 ml of 0.01 N iodine solution ($ZnCl_2 + H_2S = ZnS + 2HCl$) was added. The formed ZnS reacted with iodine ($ZnS + I_2 = ZnI_2 + S$), which is resulted in the formation of sulphur. An excess of iodine was titrated by sodium thiosulphate using starch indicator (Al-Janabi *et al*., 1992). Major ions were analyzed within one day of collection. Water samples for major dissolved ion analysis were collected in 500 ml polyethylene bottles. Water samples were filtered with – 0.4 μm filter paper for cations and anions analyses; upon return from the field. Subsequently, for the purpose to keep samples away from the chemical and biological reactions, we have kept all the water samples in the fridge (4° C) and dark location following Schoenleber (2005) and APHA (1998).

All samples were analyzed for the concentrations of major dissolved cations (Ca^{2+} and Mg^{2+}), dissolved anions (Cl^- and SO_4^{2-}), and Fe, Zn, Cu, Ni, Pb, F, Cd, Be, Mn, NH_3, NO_2, and NO_3 in water with a Dionex DX500 ion chromatography system and Atomic Absorption (AA200) Perkin Elmer at the Kurdistan Institution for Strategic Studies and Scientific Research. Na^+ and K^+ were measured using a flame photometer (Genway PFP7). Bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) were analyzed by titrating with 0.04N HCl, using Methyl orange and Phenolnephthalene indicator. Repeated measurements and charge balance calculations are within the acceptable limit (1 – 5 %), except in the case of samples 1 and 3 for cations and anions. Charge balances (CB) were calculated using the equation (Dominico and Schwartz, 1998):

$$CB\ (as\%) = [(\sum cations - \sum anions)/(\sum cations + \sum anions)] \times 100$$

Chemical equilibrium, ion pairing, carbon dioxide partial pressure, and saturation indices for significant minerals were calculated by the software package WATEQ4F following (Ball and Nordstrom (2003a and b). The saturation index is defined here as (Drever, 1997):

$$SI_x = \log \frac{IAP\ (T)}{Ksp\ (T)} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

when

SIx: is the saturation index of mineral x
IAP (T): is the ion activity products at specified temperature (°C)
and *Ksp*: is the equilibrium solubility product costant of mineral x

- **Microbiological Identification**

In order to identify the types of bacteria, which are responsible for some processes inside the Darzila Cave, five water samples were analyzed bactereologically at the University of Sulaimaniy, Department of Biology; using Glucose broth, 9k medium for *Thiobacillus acidophilus* Bactria, Starkey medium (broth) (Thiosulphate agar medium) for *Thiobacillus thiooxidans* Bacteria, and iron oxidizing medium for *Thiobacillus Ferroxidans* Bactria. The media compositions ranged from mineral salts to various organic compounds and contained sulphur compounds of various valences. Some media included thiosulphate, sulphate, elemental sulphur, or sodium sulphate (Kuske *et al.*, 1997).

NATURAL SULPHURIC ACID GENERATION BY REDOX REACTIONS

Sulphur-oxidizing reactions are dominant in the sub-aerial environment in the Darzila Cave. Water samples (1 and 3), which are rich in sulfide generate natural sulphuric acid by oxidation *(eq.2 and 3)*. Oxygen, mainly from exchange of air through skylights, is also absorbed by the water.

Oxidation of H_2S by the dissolved oxygen to sulphuric acid takes place in the water, reducing the activities of both dissolved gases and allowing more of them to be absorbed (Ehrlich, 2002; Chapelle, 2001; Konhauser, 2007; Culver and White, 2005).

$$2H_2S + O_2 \xrightarrow{\text{Oxidizing Bacteria (Thiobacillus)}} 2H_2O + 2S \ldots\ldots\ldots\ldots\ldots (2)$$

$$2S + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Because of the high solubility of sulphuric acid, the process can proceed almost indefinitely. The water pH for samples 1 and 3 was 2.19 and 2.3, respectively. Slow drips, in which the reaction has persisted for a long time, burn skin and disintegrate clothing. At these pH values, the dominant sulphur species is H_2SO_4, rather than SO_4^{2-}. Production of sulphuric acid is limited in the sulphide-rich caves and streams, because the uptake of oxygen is rather slow in comparison with that in samples 1 and 3, which have a far greater ratio of surface area to volume.

Under ordinary conditions, sulphuric acid would be almost instantly neutralized by contact with carbonate rock. However, acidic drips dissolving the carbonate have produced calcium and sulphate with activities high enough to precipitate a rind of crystalline gypsum up to 20 cm thick, which shields the carbonate walls from the aggressive water *(eq.4)*.

$$CaCO_3 + 2H^+ + SO_4^{2-} \longrightarrow Ca^{2+} + SO_4^{2-} + H^+ + HCO_3^- \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

MICROBIOLOGY

▪ Microbial Mediation of Sulphur Redox Reactions

Microbial activity commonly mediates sulphur redox reactions. Complete oxidation of hydrogen sulphide to sulphuric acid can also be achieved through the mediation of a number of sulphur-oxidizing bacteria in the genus *Thiobacillus* and others (Ehrlich, 2002). One of the most thoroughly studied, *Thiobacillus thiooxidans* isolated by Waksman and Jaffe (1922) is autotrophic, capable of fixing carbon dioxide into biomass without any organic carbon source. It produces metabolically useful energy from the oxidation of sulphur or sulphide with resulting production of sulphuric acid. The process is strongly exothermic, *H*° = – 46 kJ/mol and G° = – 43 kJ/mol, providing a ready source of energy to the organisms. Under anaerobic or low oxygen conditions, *T. thiooxidans* produces a local environmental pH some what less than 2. In the presence of air, the sulphide oxidation accelerates and pH value can drop below 1. This may be the case in the acidic water measured inside the cave.

Another well-known chemolithotrophic, acidophilic (rock-eating, acid-loving) bacterium, *T. ferrooxidans,* was isolated from an acid drainage source by Colmer and Hinkle (1947). These species are only a small sample of the acidophilic bacteria that contribute to the leaching of sulphides and heavy metal solubilizations in mineral environments (Johnson and Roberto, 1997).

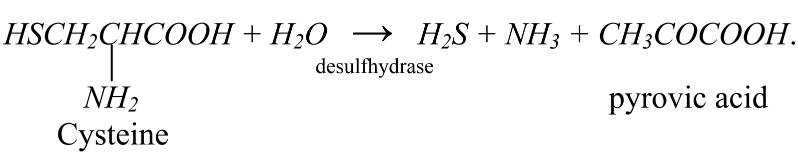
INTERPRETATION AND DISCUSSION

Darzila Cave, in Sangaw area, is identified as a sulphide-rich, hypogenic cave, with extremely acidic microenvironments (pH ranges between 2.19 – 2.3) probably attributable partly to biogenic oxidation reactions. Acidophilic and even hyper acidophilic bacteria are growing in environment when pH is less than 2 and have been described from many other habitats, including hot springs and acid mine drainage water. The authors suggest a microbial role in the development of acidic environments and cave enlargement in Darzila spring.

The following equations could be summarized for the formation of H_2S and H_2SO_4 in the Darzila Cave system: H_2S may generate by two ways: First by oxidizing organic matter by *Dissolfovibrio disolfuricans* bacteria with presence of gypsum *(eqs.5 and 6)*, and the second by Deisulfurization of amino acid cysteine by bacteria may occur anaerobically by the reaction *(eq.7)* (Chapelle, 2001; Ehrlich, 2002; Culver and White, 2005; Konhauser, 2007). The possible source of organic matter is from deep oil-rich reservoirs (or bitumen occurrences within the limestone body), the presence of organic matter at the bank of Awa Spi stream supports this idea (Fig.5).

$$CaSO_4 + 2(CH_2O) \rightarrow CaS + 2CO_2 + 2H_2O \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

$$CaS + CO_2 + H_2O \rightarrow CaCO_3 + H_2S \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (6)$$



$$\underset{\text{Cysteine}}{HSCH_2\underset{\underset{NH_2}{|}}{C}HCOOH} + H_2O \xrightarrow[\text{desulfhydrase}]{} H_2S + NH_3 + \underset{\text{pyrovic acid}}{CH_3COCOOH} \ldots\ldots\ldots\ldots\ (7)$$

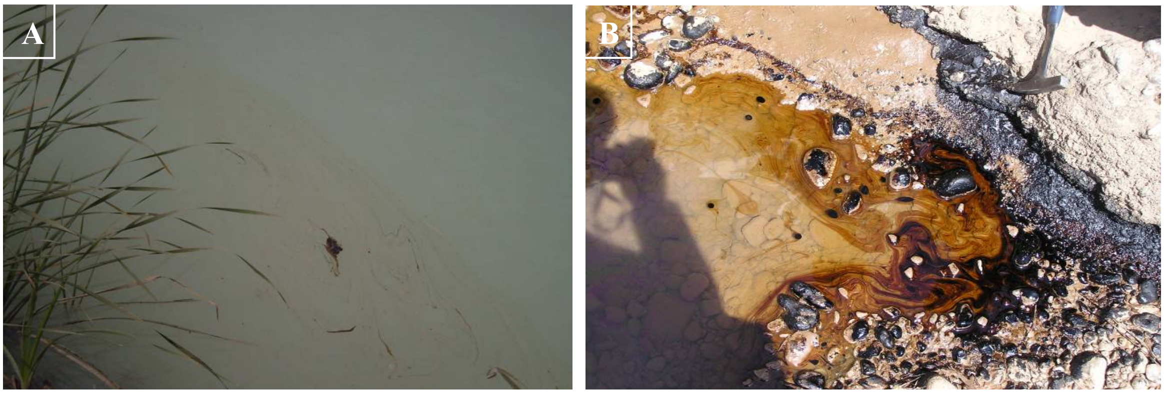


Fig.5: **A)** Organic Plume (Asphalt) in Awa Spi stream
B) Organic matter at the bank of the stream

It is obvious from the equations 5 and 6 that there is generation of $CaCO_3$ inside the cave, this process can be seen inside the cave, the CaS is like a thin film floating above the surface of the water (Fig.6).

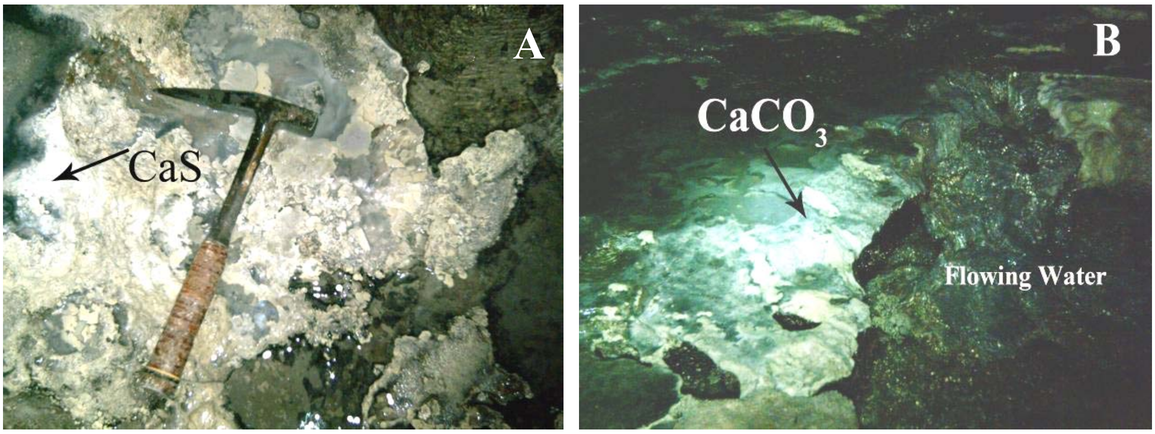


Fig.6: **A)** CaS above the water surface
B) $CaCO_3$ precipitated recently

GENERAL EVALUATION OF THE WATER ANALYSIS

▪ Major Ions

The results of chemical analysis of water samples are tabulated in Table (1). Ca^{2+} and SO_4^{2-} are the dominant ions, the second species in abundance are Mg^{2+} for cations and Cl^- for anions, Ca^{2+} concentration is in range of (195 – 542.7) mg/l, also Mg^{2+} ranges is (10.5 – 48) mg/l, Na^+ ranges (11.8 – 22.4) mg/l, K^+ (1.65 – 41.8) mg/l, Cl^- (20.18 – 330.7) mg/l, HCO_3^- (0 – 145) mg/l, SO_4^{2-} (585 – 10954) mg/l. The source of Ca^{2+}, SO_4^{2-} and Cl^- is from the lithology of Fatha Formation. The abnormal concentrations of whole elements in the water samples 1 and 3 are attributed to the high value of pH, which is considered as highly acidic water and dissolves many minerals when passes over them.

Groundwater inlets into the Darzila Cave fall into two clear categories (type A and B). Type A has a high TDS content (11904 – 12083 mg/1) , milky color represented by water samples 1 and 3, and type B has a modest TDS content (1032 – 1073 mg/l) represented by water samples 2, 4, and 5, and it is oxygenated water. Only one sample of pure type A groundwater was accessible for reliable sampling. Cave streams fed by the two water types have conspicuously different appearances. Elemental sulphur coats the walls above the sulfate-rich inlets, and white bacterial filaments cover the wetted rock surfaces.

The sulfate-rich water is supersaturated with gypsum and under saturated with other minerals. When the data of the water samples are plotted on the Piper Diagram (Fig.7), all samples are located in the field number six, which means that the secondary salinity (non-carbonate hardness) exceeds 50%, and the water type is Ca – SO_4.

Table 1: Physical parameters and major ions concentrations in water samples inside the cave

Parameter	**Units**	**S1**	**S2**	**S3**	**S4**	**S5**
T	°C	19	25	19	23	22
pH	–	2.19	7.1	2.3	7.08	6.44
EC	µs/cm	18600	1623	18880	1678	1629
TDS	mg/l	11904	1038.7	12083	1074	1042.6
Ca^{2+}	ppm	542.7	203	570	212.7	195
	epm	27.13	10.16	28.5	10.63	9.76
	% epm	91.85	68.25	89.88	69.78	66.6
Mg^{2+}	ppm	10.5	50	14	48	52
	epm	0.88	4.17	1.17	4.0	4.3
	% epm	2.96	28	3.68	26.3	29.6
Na^{+}	ppm	18.4	11.8	22.4	12.5	11.8
	epm	0.8	0.51	0.97	0.54	0.51
	% epm	2.71	3.46	3.07	3.57	3.51
K^{+}	ppm	28.6	1.65	41.8	2.42	1.65
	epm	0.73	0.04	1.07	0.06	0.04
	% epm	2.48	0.28	3.38	0.4	0.29
Sum of cations	ppm	600.2	266.6	648.1	275.6	260.6
	epm	29.5	14.9	31.7	15.2	14.7
	% epm	100	100	100	100	100
SO_4^{2-}	ppm	10850	585	10954	604.6	599.4
	epm	226	12.2	228.2	12.6	12.5
	% epm	96.1	81.74	95.14	81.75	80.34
Cl^{-}	ppm	330.7	22.4	420	20.2	24.5
	epm	9.2	0.62	11.7	0.56	0.68
	% epm	3.9	4.2	4.9	3.6	4.4
HCO_3^{-}	ppm	0	128.14	0	137.3	144.9
	epm	0	2.1	0	2.25	2.38
	% epm	0	14.1	0	14.6	15.3
CO_3^{2-}	0	0	0	0	0	0
Sum of anions	ppm	11180.7	735.54	11374.2	762	768.8
	epm	235.2	14.9	239.9	15.4	15.54
	% epm	100	100	100	100	100

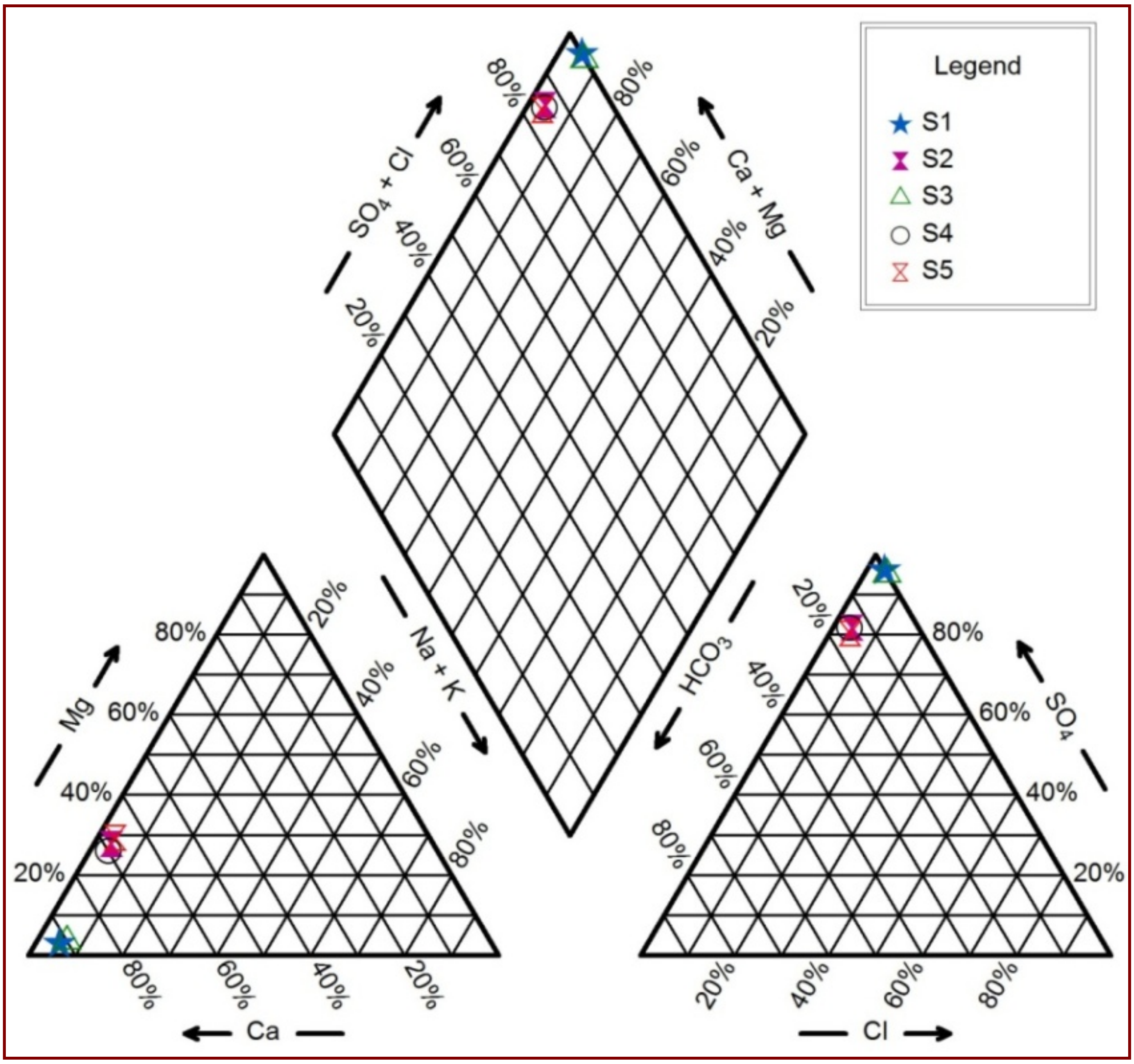


Fig.7: Piper Plot of hydrochemical data

▪ Minor Ions and Trace Elements

The concentrations of Fe, Zn, Cu, Ni, Pb, F, Cd, Be, Mn, NH_3, NO_2, and NO_3 are given in Table (2). It is clear that the concentration of NO_3^- is high in samples 1 and 3, the source of the nitrate is from biogenic sediments in caves, which can be divided into three major types: Detrital (external material directly transported from the surface), internal (excrement or guano produced by animals inside the cave), and secondary (material formed inside the cave).

Decomposition of bat guano associated with bat urine produces free ammonia; some of this ammonia may be converted to nitric acid by bacterial action. The source of ammonia may be from the reduction of nitrate with existing of organic matter *(eq.8)*:

$$2C_{organic} + NO_3^- + H_2O + H^+ \xrightarrow{\quad} 2CO_2 + NH_3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

The concentration of Fe ion in water samples 1 and 3 is higher than other samples, due to low pH of the water. According to Mason (1966) decreasing of every two units in pH results in increasing of Fe concentration of about 100000 times.

Table 2: Minor ions concentrations, trace elements, TU and H_2S of water samples inside the cave

Parameter	**Units**	**S1**	**S2**	**S3**	**S4**	**S5**
PO_4^{3-}	mg/l	n.d	n.d	n.d	n.d	n.d
NO_3^-	mg/l	44.6	n.d	79.5	n.d	n.d
NO_2	mg/l	n.d	n.d	n.d	n.d	n.d
NH_3	mg/l	n.d	0.04	n.d	0.2	0.36
H_2S	mg/l	5	56	7	68	95
Fe	mg/l	9.07	0.25	17.78	0.34	0.28
Ni	mg/l	0.536	0.393	0.353	0.414	0.472
Cd	mg/l	0.061	0.047	0.06	0.047	0.05
Pb	mg/l	0.45	0.254	0.485	0.355	0.270
Zn	mg/l	0.404	0.031	0.333	0.032	0.012
Cu	mg/l	0.039	0.032	0.053	0.03	0.025
Be	mg/l	46.22	2	1.682	n.d	n.d
F	mg/l	25.5	0.089	0.24	0.084	0.104
Mn	mg/l	2.86	0.642	0.021	0.066	0.584
Turbidity	NTU	14.5	99.2	107	19.91	208

GEOCHEMICAL MODELING OF DARZILA CAVE WATER

To clarify the nature of the two disparate stream types entering the cave, the reaction path routine of the program SI was used to model their chemistry. Ionic strength, Log P_{CO2}, and saturation indices, of the minerals calcite, aragonite, dolomite, gypsum, anhydrite, fluorite, and halite were calculated (Table 3). The results show the dissolution of halite and anhydrite and precipitation of gypsum in water samples 1 and 3, while in samples 2, 4, and 5 there is a dissolution of anhydrite, aragonite, calcite, dolomite, gypsum, and halite. These chemical characteristics and mineral interactions are common in many deep karst waters that pass through evaporative sedimentary sequences (Langmuir, 1997; Palmer and Palmer, 1998). The simplicity of the geochemical models suggests that the water derives from a sedimentary environment, probably the Fatha Formation. However, the complexity within the cave may indicate a role of bacteria.

Table 3: Ionic Strength, Log P_{CO2}, and mineral saturation indices for water samples of Darzila Spring

Sample No.	**Ionic Strength**	**Log P_{CO2}**	**SI Anhydrite**	**SI Aragonite**	**SI Calcite**	**SI Dolomite**	**SI Gypsum**	**SI Halite**	**SI Fluorite**
S1	0.20215	0	– 0.017	–	–	–	0.209	– 7.02	0.092
S2	0.02144	0.774	– 0.886	–3.142	– 2.997	– 6.847	– 0.662	– 8.195	– 2.919
S3	0.2052	0	0	–	–	–	0.226	– 6.854	– 3.779
S4	0.0219	– 2.0	– 0.858	– 0.303	– 0.158	–	– 0.631	– 8.217	– 2.902
S5	0.0283	– 1.334	– 0.894	– 0.952	– 0.807	–2.433	– 0.666	– 8.156	– 2.755

Table (4) shows the ionic ratios and amount of dissolved minerals, the high ratio values of Ca/ Mg and Ca/ SO_4 indicates that further dissolution of anhydrite and calcite, while the high ratio value of Na/ Cl in sample 4 is attributed to ionic substitution inside the cave.

Table 4: Ionic ratios and amount of dissolved minerals
for water samples of Darzila Spring

Ionic ratios and dissolved minerals	Water sample No.					Seawater
	S1	S2	S3	S4	S5	
Ca/ Mg (mg/l)	51.7	19.3	40.7	4.43	3.75	0.319
Ca/ SO_4 (mg/l)	0.05	0.35	0.052	0.352	0.325	0.152
Na/ Cl (mg/l)	0.056	0.53	0.053	0.619	0.48	0.556
Halite (mg/l)	46.8	30.03	57	31.81	30.03	–
Anhydrite (mg/l)	15384.5	829.5	15532	857.2	849.85	–

MICROBIAL INFLUENCE ON DARZILA CAVE PROCESSES

The initial investigations of the authors focused on sulphide-oxidizing bacteria, like *Thiobacillus* because of the importance of sulphuric acid in speleogenetic processes. *Thiobacilli* Gram-negative rods, classified in the gamma subdivision of the Proteobacteria. This bacteria often obtain their energy from the oxidation of sulphur compounds such as elemental sulphur, sulphides, and thiosulphate. *Thiobacillus ferrooxidans* can oxidize both iron and sulphur compounds, while *Thiobacillus thiooxidans* oxidizes only sulphur compounds. Several *Thiobacilli* generate sulphuric acid and grow at low pH values. The characteristics of *Thiobacilli,* the genus may generate sulphuric acid and at least it is partly responsible for the low pH values.

The preliminary sequence analysis by the authors suggests that the dominant clone may be of one species, or a group of very closely related microspecies. More complete sequencing of clones and comparison of these completed sequences along with further work on pure cultures of microbes isolated from Darzila habitats will shed light on this hypothesis. Sulphate reducing bacteria (SRB) are complex organisms, many of them can carry out numerous different sulphur transformations (Fuseler *et al*., 1996). They reduce sulphate to sulphide with concomitant oxidation of an organic substrate. Additionally, many of them can also oxidize sulphide, thiosulphate, sulphate, and elemental sulphur using nitrate, nitrite, or oxygen as electron acceptors.

Preliminary identification of green sulphur bacteria growing in the apparent total absence of light and the possible contribution to elemental sulphur deposits by their action is another potentially important link between microbial action and the deposition of cave minerals. From Fig. (8) and Table (5) it is obvious that the low pH values of samples 1 and 3 are attributed to presence of *Thiobacillus acidophilas* bacteria.

Table 5: Existing bacteria types in water samples

Bacteria Sample No.	***Thiobacillus thiooxidants***	***Thiobacillus ferroxidans***	***Thiobacillus acidophilas***
S1	–	+	+
S2	+	+	–
S3	–	+	+
S4	+	+	–
S5	+	+	–

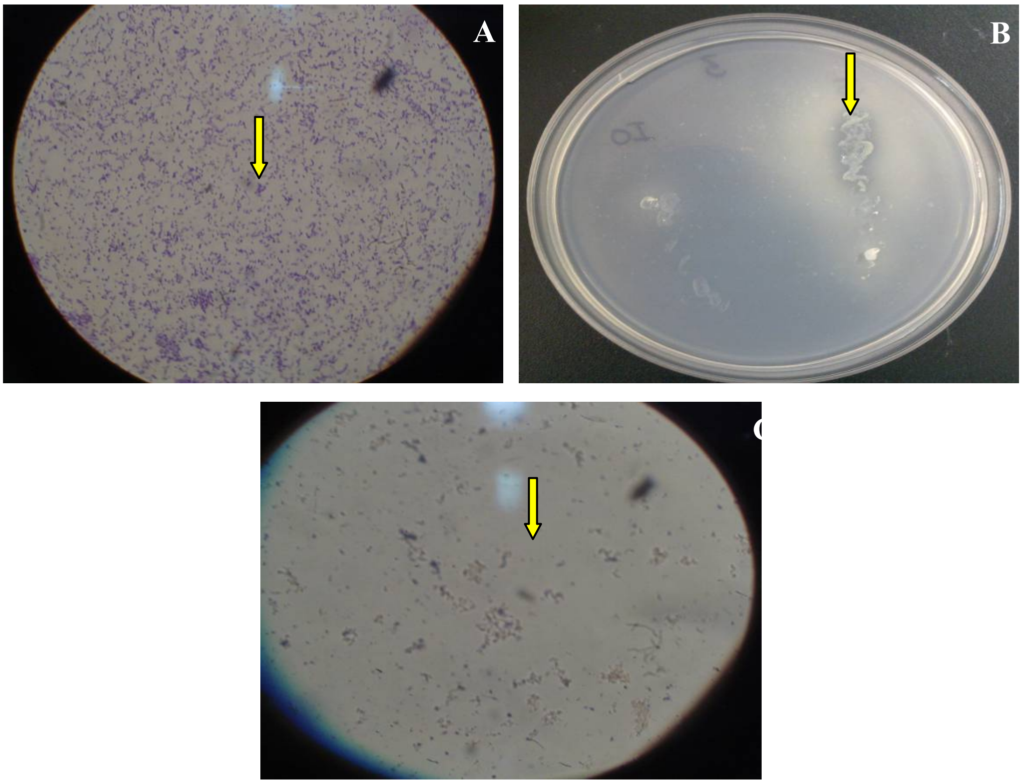


Fig.8: **A)** *Thiobacillus acidophilus* bacteria
B) *Thiobacillus thiooxidans* (Thiosulphate Agar Medium)
C) *Thiobacillus ferroxidans*

ORIGIN OF DARZILA CAVE MECHANISMS, AND ENLARGEMENT

Several mechanisms for the cave enlargement could be observed. The first scenario is that the water, which is rich in sulphuric acid due to oxidation of H_2S, therefore limestone is replaced by gypsum. However, all other waters in the cave are able to remove the gypsum by dissolution, enlarging the cave. Gypsum that falls to the floor as blocks or paste is dissolved by the cave stream and by freshwater drips from the surface beneath skylights (Fig.9). The second scenario is that in places, acid droplets fall directly on limestone, forming rills. They are common especially where the sulphuric acid solutions seep through the outer parts of the gypsum crust and concentrate in drips along the shores of the cave streams. During periods of high infiltration, the production of acidic drips may be accelerated, causing the main cave stream to become aggressive with respect to calcite. Enhanced oxidation of colloidal sulphur and hydrogen sulphide in the vicinity of skylights can aid in producing aggressiveness.

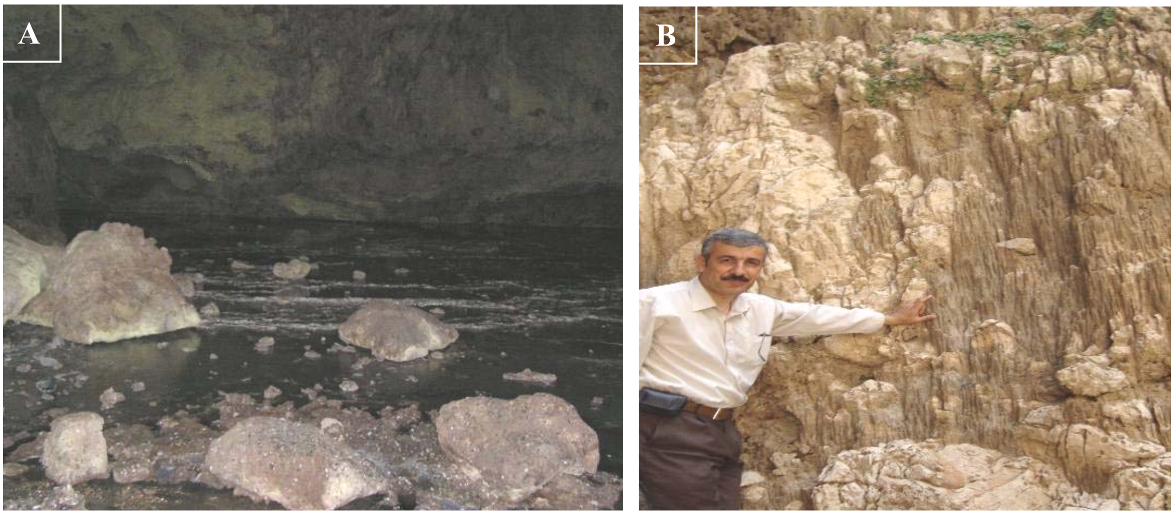


Fig.9: **A)** Fallen gypsum blocks; **B)** Rills formed by fallen droplets

CONCLUSIONS

- Darzila Cave is a hypogenic cave and rich in hydrogen sulphide.
- The results of hydrochemical analysis showed that Ca^{2+} and SO_4^{2-} are the dominant ions; the second species in abundance are Mg^{2+} for cations and Cl^- for anions.
- Groundwater inlets into the cave fall into two clear categories (type A and B). Type A has a high TDS content (11904 – 12083 mg/1), with milky color represented by samples 1 and 3, and type B has a modest TDS (1032 – 1073 mg/l) content represented by samples 2, 4, and 5, and it is oxygenated water.
- When the water samples plotted on the piper diagram, all samples are located in the field number six, which means that the secondary salinity (non-carbonate hardness) exceeds 50%, and the water type is $Ca – SO_4$.
- The results of chemical speciation show the dissolution of halite and anhydrite and precipitation of gypsum in water samples 1 and 3, while in samples 2, 4, and 5 there is dissolution of anhydrite, aragonite, calcite, dolomite, gypsum, and halite.
- The high ratio values of Ca/ Mg and Ca/ SO_4 indicates further dissolution of anhydrite and calcite, while the high ratio value of Na/ Cl, in sample 4 is attributed to the presence of ionic substitution inside the cave.
- Bacteriological analysis of water samples shows the presence of three *Thiobacilli* species.
- Two cave-enlargement processes were identified: **i)** Sulphuric acid derived from oxidation of the hydrogen sulphide converts subaerial limestone surfaces to gypsum. The gypsum falls into the cave stream and is dissolved. **ii)** Strongly acidic droplets form on the gypsum and on microbial filaments, dissolving limestone where they drip onto the cave floor.

ACKNOWLEDGMENT

The authors express their sincere thanks to Dr. Hunar H. Arif for analyzing water samples for microbiological identification.

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