COMU J Mar Sci Fish 2022 5 (1): 26-38
https://doi.org/10.46384/jmsf.1032339
e-ISSN: 2651-5326

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: http://jmsf.dergi.comu.edu.tr Online Submission: http://dergipark.org.tr/jmsf

RESEARCH ARTICLE

Hydrochemical and Bacteriological Status of a High Altitude Karstic Cave Stream (Güvercinkaya Cave: Çanakkale, Türkiye) with Aquatic Macroinvertebrates Findings

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Received: 04.12.2021 /Accepted: 15.02.2022 / Published online: 20.07.2022

Key words:

Karstic Cave
Groundwater
Hydrochemistry
Bacterial Contamination
Aquatic Macroinvertebrates

Abstract: Caves are laboratories for many disciplines that work in natural sciences including mineralogy, biology, hydrogeology, and archaeology. In this study, bi-monthly samplings were carried out from three smapling locations within and around the Güvercinkaya Cave, a high-altitude cave located in nortwestern Turkey, to evaluate the hydrochemical and microbiological properties and the aquatic macroinvertebrates of the cave stream. Some parameters of the water including pH, electrical conductivity, temperature, oxidation-reduction potential, dissolved oxygen were measured in-situ, while elemental (70 in total) and ionic composition of water were analyzed in the laboratory. Microbiological analyses of the cave stream were examined through analyses of total bacteria, total coliforms, fecal coliforms, fecal Streptococcus, and Escherichia coli. According to the Piper diagram of hydrochemical data, the cave stream had mainly Ca-Mg-HCO3 character, on the other hand, the Schoeller diagram indicated a common water source in Güvercinkaya cave due to the similar components of the main ionic components of the water. As a result of microbiological analysis, fecal contamination was determined, indicating an active wildlife in the cave. Additionally, several aquatic macroinvertebrates taxa, *Rhynchelmis limosella*, Dugesia sp., *Gammarus uludagi* which have non-troglobiont character were found in the cave stream. *Rhynchelmis limosella* detected in this study is the first record for the Turkish fauna.

Anahtar kelimeler:

Karstik Mağara
Yeraltı Suyu
Hidrokimya
Bakteriyel Bulaşma
Sucul Makroomurgasızlar

Yüksek Rakımlı Karstik Bir Mağara Deresinin (Güvercinkaya Mağarası: Çanakkale, Türkiye) Hidrokimyasal ve Bakteriyolojik Durumu ile Sucul Makroomurgasız Bulguları

Öz: Mağaralar maden bilimi, biyoloji, hidrojeoloji ve arkeoloji dahil olmak üzere doğa bilimlerinin pek çok disiplini için bir laboratuvar niteliğindedir. Bu çalışmada, Türkiye'nin kuzeybatısında yer alan yüksek rakımlı bir mağara olan Güvercinkaya Mağarası'nın seçilen bölümlerinden, hidrokimyasal ve mikrobiyolojik özellikleri ile sucul makroomurgasızlarının değerlendirilmesi için iki aylık periyotlarda bir yıl örneklemeler yapılmıştır. Suyun pH, elektriksel iletkenlik, sıcaklık, oksidasyon-redüksiyon potansiyeli, çözünmüş oksijen gibi bazı parametreleri yerinde ölçülürken, elementler (toplam 70 adet) ve suyun bazı iyonları laboratuvarda analiz edilmiştir. Mikrobiyolojik analizlerde toplam bakteri, toplam koliform, fekal koliform, fekal Streptococcus ve *Escherichia coli* analizleri yapılmıştır. Hidrokimyasal verilerin Piper diyagramı değerlendirildiğinde, mağara deresinin esas olarak Ca-Mg-HCO3 karakterine sahip olduğunu, Schoeller diyagramının ise ana iyonik bileşenlerin, aynı modeki takip etmesinden dolayı ortak bir su kaynağını işaret ettiği görülmüştür. Mikrobiyolojik analizler sonucunda, mağarada aktif bir yaban hayatı olduğunu gösteren dışkı kaynaklı bir kontaminasyon belirlenmiştir. Ayrıca mağara deresinde, troglobiont olmayan sucul omurgasızlardan *Rhynchelmis limosella*, Dugesia sp. ve *Gammarus uludagi* tespit edilmiştir. Bu çalışmada tespit edilen *Rhynchelmis limosella* Türkiye faunası için ilk kayıttır.

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How to cite this article: Odabaşı, D. A., Odabaşı, S., Deniz, O., Çakır, F., Elipek, B., Arslan, N., Özbek, O., Özalp, H.B. (2022). Hydrochemical and bacteriological status of a high altitude karstic cave stream (Güvercinkaya cave: Çanakkale, Turkey) with aquatic macroinvertebrates findings. COMU J. Mar. Sci. Fish, 5(1): 26-38. doi:10.46384/jmsf.1032339

Introduction

Caves are common geological formations of karst regions, and is commonly assocated with limestone which is found in one-fourth of the world (LaMoreaux et al., 1997). They play an important role in human life since the earliest times in history. Today, caves could be accepted as laboratories for scientists working in the fields of natural and life sciences such as mineralogy, biology, hydrogeology and archaeology. Regarding their natural characteristics, the principal difference of the cave ecosystems from the surface ecosystems is the lacking of light (Simon, 2019) which drives autochthonous food webs through primary producers (Azad and Borchardt, 1969; Biswas, 2010). Thus, cave-dwelling organisms mostly rely upon allochthonous and detrital energy sources making them susceptible to changes in environmental parameters when compared to surface fauna (Mammola et al., 2019).

Karst aquifers (like springs and caves streams) are of importance as a groundwater source for drinking and irrigation especially in Mediteraanean countries (Ford and Williams, 2007; Bakalowicz, 2015). Since majority of basins are located in unpolluted, low-populated areas, they can provide large amounts of high quality water for human consumption (D'Angeli et al., 2017). Therefore, research efforts have mostly focused on hydrochemistry (Stevanović, 2015; Mukherjee and Singh 2020) and microbiology of karst aquifers (Savio et al., 2019; Hershey et al., 2019). On the other hand, assessment of karst regions in terms of biological aspects has been one of the most studied topics including troglobiont or stygofauna which often exhibit specialized physiological adaptations, behavioral adjustments, and morphological changes (Barr, 1968; Biswas, 1992; Sket, 2008; Brancelj et al., 2020; Boyd et al., 2020).

Biospeleological researches dated back to early XIX. century (1830) in Europe, mainly Slovenia, followed by other countries onwards (Sket, 2008). In Turkey, studies on cave ecosystems started with Dr. Abdullah Bey in Yarımburgaz Cave in İstanbul in 1865 (Kunt et al., 2010). These studies focused on areas such as geology (Alagöz, 1944; Aygen, 1959; İzbırak, 1979; Şengör, 1986; Nazik, 1989) and biology (Balık et al., 2002; Taşdemir and Ustaoğlu, 2005; Özkan, 2009; Danyer et al., 2013; Erkakan and Özdemir, 2014). However, data on karst aquifers, caves and groundwaters in Turkey are still limited.

In the last two decades, efforts on cave research has increased significantly in Turkey (Kunt et al., 2010). Turkey is characterized by a very complex geology, whose main features are still poorly understood despite an increasing amount of geological data (Okay, 2008). Due to its geological evolution, Turkey has a variety of cave types including sea caves and caves of soluble rock. The latter is the most common type that generally forms within the limestone, in other words, carbonate rocks. Carbonate and sulfate rocks that are prone to dissolution are made up of 40% of Turkish territory (Nazik et al., 2003).

Güvercinkaya Cave (GC) is located near the Kazdağı National Park, in the northwest of Turkey in Çanakkale, (Figure 1). The cave has a year-round hydrologic regime considered as a cave stream opening with a waterfall to the surface (Figure 2). The major sources of water are groundwater vents, meltwater and seasonal precipitation that reaches the cave through cracks; therefore it has a very variable flowing regime according to the seasons. The only study about the cave was conducted by a group of French speleologists in 2001, however, the cave has frequently been visited by many European explorers since 1809 (Wolozan, 2003).

In this study, we aimed to asses the hydrochemical and bacterial structure, and macroinvertebrate fauna of the GC karstic stream. This study is also the first interdisciplinary study in Turkey's high altitude water cave ecosystem, which can fill a knowledge gap.

Material and Methods

Study area

Kazdağı Mountain range with its highest peak of 1770 m is located in the Biga Peninsula and separates the Aegean and Marmara regions of Turkey. Part of Kazdağı Mountain has been declared a national park due to its rich diversity of flora and fauna in 1994 (Odabaşı and Georgiev, 2014). The study area, GC, is located at Kazdağı Mountain within the city borders of Çanakkale, northwest of Turkey. The cave is located on the north-facing slope of Kazdağı Mountain range at an altitude of 938 m above sea level. The nearest settlement to the cave is the Evciler village, which is located at a lower altitude 12 km further. Access to the region is very difficult as it is surrounded by high hills (Figure 1). The coordinates of the sampling sites were given in Table 1.

Sampling

In this study, a bi-monthly (6 times in total) sampling was carried out to obtain the chemical and microbiological water quality parameters between November 2015 and October 2016. Benthos sampling was carried out twice, during the lower flow rate periods in November 2015 and October 2016. For field studies, three sampling sites were chosen within and around the cave. The first sampling site (GC1) was located under the the natural entrance of the cave that was receiving very limited sunlight indirectly. The second sampling site (GC2) was located at the siphon, mouth of the main water source of the cave stream, approximately 60 meters away from the cave entrance. The depth of GC2 was 8 m and thus, samples were collected by diving. The third sampling site (GC3) was a pool formed by cascading water located at the outlet of the cave (Figure 2). Benthic macroinvertebrate samples were taken using a standardized multi-habitat sampling procedure (Hering et al., 2004) from available habitats by D-frame hand-net only if suitable environmental conditions were provided. A cave diving was performed during the benthos sampling in the second sampling site (CG2).

Figure 1. Study area on the map

Table 1. Coordinates (UTM ED50) and altitudes (above sea level) of the sampling sites

Coordinates (Decimal degree - WGS84)		Altitude (m)	Water Type	Name of the Location	Code of the Location
39.718182 N	26.806879 E	911	Groundwater	Entrance of the cave	GC1
39.718114 N	26.805171 E	948	Groundwater	Sump of the cave	GC2
39.718353 N	26.806494 E	906	Surface water	The waterfall (outlet)	GC3

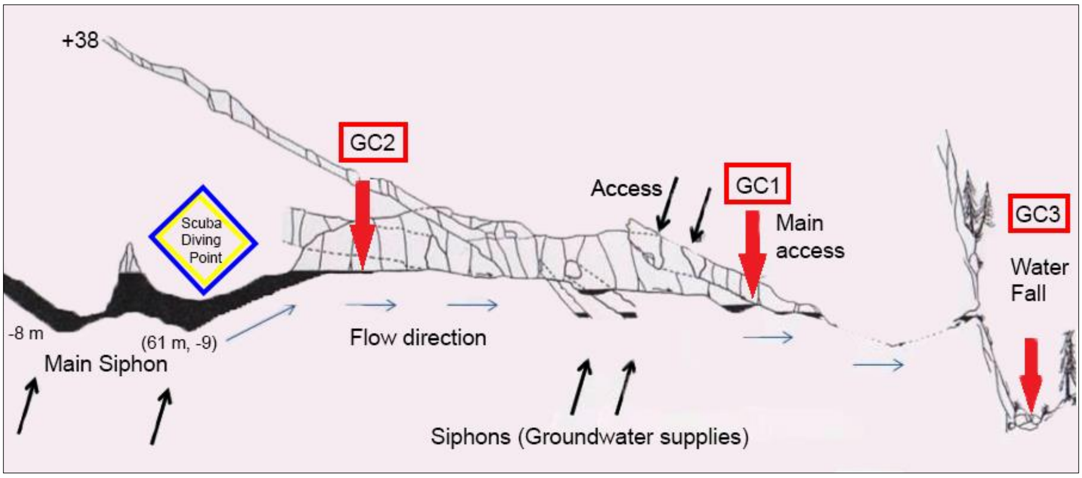

Figure 2. Location of sampling sites in the Güvercinkaya Cave (Modified from Wolozan, 2003)

Hydrochemical analysis

Bi-monthly samplings were carried out to obtain data about hydrochemical conditions of the study site. Some of the parameters such as temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO) were measured in-*situ* using portable multi-parameter equipment (Hach-Lange 40d). The water samples were filtered using a manual vacuum pump with a filter paper (0.42µm) and transferred from the field to the laboratory within insulated coolers for analysis of sulfate (SO_4), bicarbonate (HCO_3), and carbonate (CO_3) following the standart methods of APHA (1999) (Table 2). Aliquots were acidified to pH<2 and placed into 50 mL polypropylene centrifuge tubes to analyze 70 elements comprising; Ag, Al, As, Au, B, Ba, Be, Bi, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, Ln, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, Y, Yb, Zn, and Zr.

Table 2. Summary of analytical methods used for water analysis and benthos sampling in this study

Sampling/Analysis	Abbreviation	Unit	Method	Analytical Method	Device	Reference
Benthos sampling	-	m^2	BS EN 16150:2012	-	D-Frame Handnet	AQEM Consortium, 2002
Bicarbonate	HCO_3^-	mg L^{-1}	APHA 2320 B.	Titration Method		APHA, 1999
Carbonate	CO_3^{2-}	mg L^{-1}	APHA 2320 B.	Titration Method		APHA, 1999
Ammonium Nitrogen	NH_4^+	mg L^{-1}	APHA 4500-NH_3 F.	Spectrophotometric	Spectrophotometer	APHA, 1999
Nitrate Nitrogen	NO_3^-	mg L^{-1}	APHA 4500-NO_3 E.	Cadmium reduction method	Spectrophotometer	APHA, 1999
Nitrite Nitrogen	NO_2^-	mg L^{-1}	APHA 4500-NO_2^- B.	Colorimetric method	Spectrophotometer	APHA, 1999
Sulphide	S^{2-}	mg L^{-1}	APHA 4500-S_2^- A.	Turbidimetric Method	Spectrophotometer	APHA, 1999
Sulphate	SO_4^{2-}	mg L^{-1}	APHA 4500-$SO4^{2-}$E.	Turbidimetric Method	Spectrophotometer	APHA, 1999
Elements	See the text	µg L^{-1}		Spectrometry	ICP-MS	APHA, 1999
Total Bacteria (37°C)	TB	cfu/mL	APHA 9215C	Spread Plate Method	Incubator	APHA, 1999
Fecal *Streptococcus*	FS	cfu/mL	APHA 9230	Spread Plate Method	Incubator	APHA, 1999
Total Coliforms	TC	mpn/100 mL	APHA 9221	Most Probable Number	Incubator	APHA, 1999
Fecal Coliforms	FC	mpn/100 mL	APHA 9221	Most Probable Number	Incubator	APHA, 1999
Escherichia coli	*E. coli*	mpn/100 mL	APHA 9221	Most Probable Number	Incubator	APHA, 1999

Bacterial analysis

Several bacteriological analyses including Total Bacteria (TB), Total Coliforms (TC), Fecal Coliforms (FC), Fecal *Streptococcus* (FS), and *Escherichia coli* were performed following the standart methods on the water samples from the sampling sites (Table 2). For TB, the standard "spread-plate" method was employed on plate count agar with an incubation temperature of 37 °C for 24-48 hours in aerobic conditions. The Most-Probable-Number technique was used with a single bottle containing a 100-mL sample portion for the determination of coliforms (TC and FC). Enriched LST broth and confirmation test was carried out in BGLB broth for TC (37 °C for 24-48 h) and in EC broth for FC (44 °C for 24-48 h). Indol production was tested for *E. coli*. Results were expressed as Colony Forming Unit (cfu/mL) and Most Probable Number (mpn/100 mL).

Statistical analysis

Some parameters of hydrochemical data including HCO_3, CO_3, SO_4^{2-}, Ca, Mg, Na, Cl, and K were subjected to AquaChem software (Waterloo Hydrogeologic, version 2014.2) that yielded the Piper plot, a Trilinear Diagram, to visualize the ions in the water based on their abundances. The Shoeller diagram was drawn to show the hydrochemical differences of water from different sources (sites) using AquaChem software. The in-*situ* measured parameters and some chemical values of water in the sampling sites were presented in the tables (3-5) with descriptive statistics e.g. mean, standart deviation (STD), minimum (Min.), and maximum (Max.). Parameters that appear to be clearly different from each other were subjected to the Student-t test using Microsoft Excel 97-2003.

Results and Discussion

Hydrochemical parameters

The hydrochemical data along with some of the descriptive statistics i.e. mean, standart deviation (STD), minimum (Min), and maximum values (max) are presented in Table 3, 4, and 5. Among all data, only temperature showed seasonal fluctuations between 8.1 and 10.8 °C. The water temperature was between 8.1 and 8.7 °C in GC1 and GC2 inside the cave, whereas 8.4 and 10.8 °C were recorded from in GC3, located just outside the cave. The difference in temperature values between GC1 and GC3 was significant ($p<0.05$) (Table 6). The pH values of the water samples ranged from 7.42 to 8.64 indicating alkaline conditions. The pH values of the samples from all the sampling sites are in the permissible limits according to the Turkish Water Pollution Control Regulation (TWPCR, 2004). The results indicated that bicarbonate ($HCO3^-$) was the dominant parameter over the ionic parameters ($HCO_3^- > SO_4^{2-} > NO_3^- > CO_3^{2-}$). The EC values varied between 223 and 498 µS/cm, however, the mean EC value was below 300 µS/cm. According to the EPA of United States, the conductivity of freshwater outside the ranges of 150 – 500 µmhos/cm (=µS/cm) may not support suitable conditions for certain species of aquatic organisms (https://archive.epa.gov/water/archive/web/html/vms59.html). The EC values of the present study indicated that the cave stream showed a lower level of ionic activity. The mean EC value in the present studywas lower when compared to those in other karst water studies of Wang et al., (2019) and Vardanjani et al., (2018), who found EC values in higher ranges (340 to 757 µS/cm).

The equilibrium states of ions in the water can be understood from Eh and pH measurements that give an idea about the processes controlling the formation and movement of many minor and trace elements in groundwater quality investigations (Freeze and Cherry, 1979). Eh values in our data (except for July 2016), showed that oxidation (cations predominate) conditions are dominant in the water.

The dissolved oxygen values were varied between 1.09 and 9.93 mg/L in the sampling sites of GC. Since surface waters are in contact with the atmosphere, DO balance can be maintained. However, in groundwater, DO might be consumed by the oxidation of rocks and biological activities (Mazor, 2004). In the present study, we determined that the DO content varies depending on the flow rate in the cave system. The highest DO level in the sampling sites was obtained during higher flow rate periods (from February to May), while the lowest DO levels coincided with lower flow rate periods (from July to November) (Table 3 and 4). Similar results regarding the dissolved oxygen level of groundwater were also reported from the study of Stroj et al., (2020).

The presence of ammonium nitrogen (NH_4^+), which indicates wastewater contamination, poses a risk for aquatic organisms. In the study area, NH_4^+ values were lower than 0.015 mg/L in February, March, July, and October 2016, while higher values (0.031 and 0.061 mg/L, respectively) were measured in November 2015 and May 2016 Caves are typically used by bats as permanent shelters (Zukal et al., 2017). According to Berková and Zukal (2006) and Zukal et al., (2017), bats in temperate regions tend to hibernate in November and departure period (flight activity) is between April and June. In this study, during November 2015 and May 2016 higher NH_4^+ levels were detected due possibly to lower flow rates in the cave stream. However, it was determined that NO_2^- and NO_3^- values were below the measurement limits ($NO_2^- <0.005$ mg/L and $NO_3^-<0.23$ mg/L) in the gauging sites throughout the course of the study. Ammonium nitrogen in groundwater is converted to nitrate under aerobic conditions (Chen and Liu, 2003) and low NO_3^- and NO_2^- levels may be due to the running water in the cave stream.Sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) were very low in the study area, while sulfide (S^{2-}) was not detected (Table 3, 4, 5).

According to the *Piper Diagram* (Figure 3) produced by AquaChem (Calmbach, 1997), the water of the sampling area is rich in Ca- HCO_3^- or Ca-Mg- HCO_3^-. Considering the element analysis data (Appendix 1), all the parameters included in TWPCR (2004) and Turkish standards (TS 266, 2005) are between acceptable levels for surface waters.

The Schoeller Diagram (Schoeller, 1962) is used to determine the source of groundwater by evaluating the composition of the water in terms of milliequivalent (mEq) liter. Due to several water sources in the cave, water samples from different sampling sites were subjected to Schoeller analysis (Figure 4). The parallel lines of the sampling sites in the Schoeller Diagram indicate that the groundwater sources entering the cave come from the same aquifer.

Table 3. Hydrochemical parameters of sampling site 1 (GC1)

	Nov.15	Feb.16	Mar.16	May.16	Jul.16	Oct.16	Mean	STD	Min.	Max.
pH	7.42	7.94	8.06	7.46	7.81	7.83	7.75	0.26	7.42	8.06
EC (µS/cm)	284.00	223.00	249.00	249.00	265.00	261.00	255.17	20.36	223.00	284.00
T (°C)	8.20	8.20	8.30	8.71	8.10	8.10	8.27	0.23	8.10	8.71
Eh (mV)	258.00	6.20	122.00	266.00	-144.00	-10.00	83.03	162.30	-144.00	266.00
DO (mg/L)	-	-	-	6.28	4.27	1.12	3.89	2.60	1.12	6.28
SO_4 (mg/L)	5.00	4.00	2.00	5.00	8.00	5.00	4.83	1.94	2.00	8.00
HCO_3 (mg/L)	110.00	159.00	170.00	170.00	171.00	167.00	157.83	23.84	110.00	171.00
CO_3 (mg/L)	0	0	0	0	0	0	0	0	0	0
NH_4-N (mg/L)	0.031	<0.015	<0.015	0.056	<0.015	<0.015	0.0435	0.0177	0.031	0.056
NO_3)mg/L)	0	<0.23	<0.23	<0.23	<0.23	<0.23	0			
NO_2 (mg/L)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005				
S_2 (mg/L)	0	0	0	0	0	0	0	0	0	0
Flow (m^3/s)	0.25	3.50	3.50	2.50	0.70	0.70	1.86	1.49	0.25	3.50

Table 4. Hydrochemical parameters of sampling site 2 (GC2)

	Nov.15	Feb.16	Mar.16	May.16	Jul.16	Oct.16	Mean	STD	Min.	Max.
pH	7.94	-	8.07	7.57	7.73	8.07	7.88	0.20	7.57	8.07
EC (µS/cm)	282.00	-	251.00	251.00	265.00	273.00	264.40	12.19	251.00	282.00
T (°C)	8.10	-	8.10	8.10	8.20	8.40	8.18	0.12	8.10	8.40
Eh (mV)	220.00	-	121.00	290.00	-128.30	156.00	131.74	142.24	-128.30	290.00
DO (mg/L)	-	-	-	4.25	2.73	1.09	2.69	1.29	1.09	4.25
SO_4 (mg/L)	4.00	-	1.00	3.00	6.00	6.00	4.00	1.90	1.00	6.00
HCO_3 (mg/L)	210.00	-	168.00	178.00	176.00	192.00	184.80	14.78	168.00	210.00
CO_3 (mg/L)	0	-	0	0	0	0	0	0	0	0.00
NH_4-N (mg/L)	0.04	-	<0.015	0.06	<0.015	<0.015	0.05	0.01	0.04	0.06
NO_3)mg/L)	<0.23	-	<0.23	<0.23	<0.23	<0.23				
NO_2 (mg/L)		-	<0.005	<0.005	<0.005	<0.005				
S_2 (mg/L)	0	-	0	0	0	0	0	0	0	0.00
Flow (m^3/s)	0.50	-	2.50	2.50	0.70	0.60	1.36	0.93	0.50	2.50

Table 5. Hydrochemical parameters of sampling site 3 (GC3)

	Nov.15	Feb.16	Mar.16	May.16	Jul.16	Oct.16	Mean	STD	Min.	Max.
pH	8.04	7.86	8.10	8.07	8.64	8.23	8.16	0.27	7.86	8.64
EC (µS/cm)	281.00	230.00	248.00	498.00	241.00	246.00	290.67	103.00	230.00	498.00
T (°C)	8.40	8.40	8.50	9.20	10.80	8.60	8.98	0.94	8.40	10.80
Eh (mV)	230.00	6.30	122.00	196.00	-61.00	44.00	89.55	112.94	-61.00	230.00
DO (mg/L)	-	-	-	9.93	2.72	1.11	4.59	4.70	1.11	9.93
SO_4 (mg/L)	4.00	4.00	3.00	5.00	7.00	6.00	4.83	1.47	3.00	7.00
HCO_3 (mg/L)	177.00	159.00	168.00	168.00	160.00	162.00	165.67	6.77	159.00	177.00
CO_3 (mg/L)	0	0	0	0	8.00	6.00	2.33	3.67	0	8.00
NH_4-N (mg/L)	0.04	<0.015	<0.015	0.06	<0.015	<0.015	0.05	0.02	0.04	0.06
NO_3)mg/L)	<0.23	<0.23	<0.23	<0.23	<0.23	<0.23				
NO_2 (mg/L)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005				
S_2 (mg/L)	0	0	0	0	0	0	0	0	0	0.00
Flow (m^3/s)	0.30	3.50	3.50	2.50	0.70	0.60	1.85	1.49	0.30	3.50

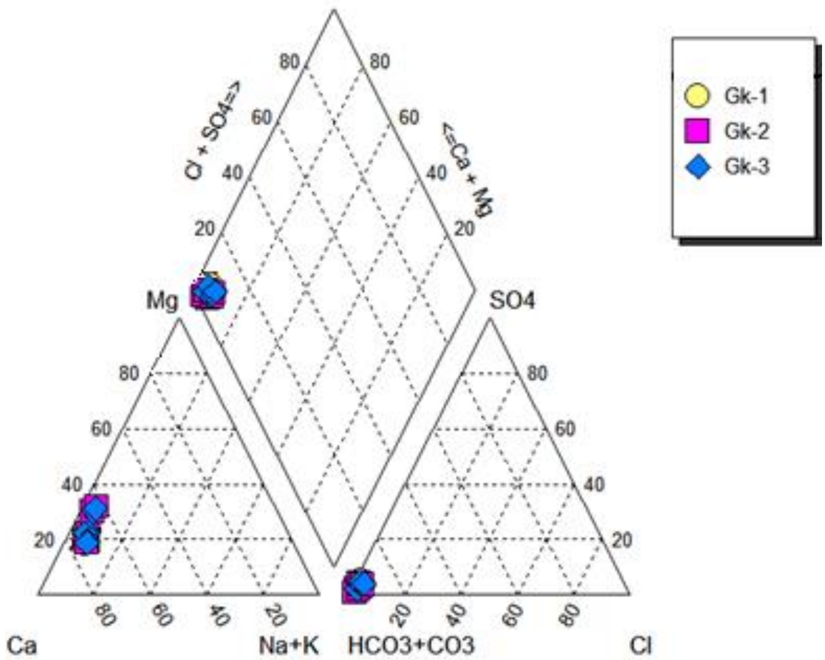

Figure 3. Piper Diagram of water samples from the study area

The histogram plot showing the course of major ions pointed out that the main ions remained homogeneous throughout the study (Figure 5). This is because the study area is a groundwater-fed waterbody to a large extent.

Table 6. Comparison the mean values of hydrochemical data between sampling sites by Student-t-Test

Groups compared	pH	EC (µS/cm)	T (°C)	DO (mg/L)	SO_4 (mg/L)	HCO_3 (mg/L)	Flow (m^3/s)
	p-value	p-value	p-value	p-value	p-value	p-value	p-value
GC1 vs GC2	0.201	0.542	0.311	0.184	0.142	0.220	0.474
GC1 vs GC3	0.039*	0.135	0.446	0.696	1.000	0.541	0.695
GC2 vs GC3	0.107	0.110	0.504	0.422	0.089	0.045	0.495

*Significantly different due to the p-value<0.05.

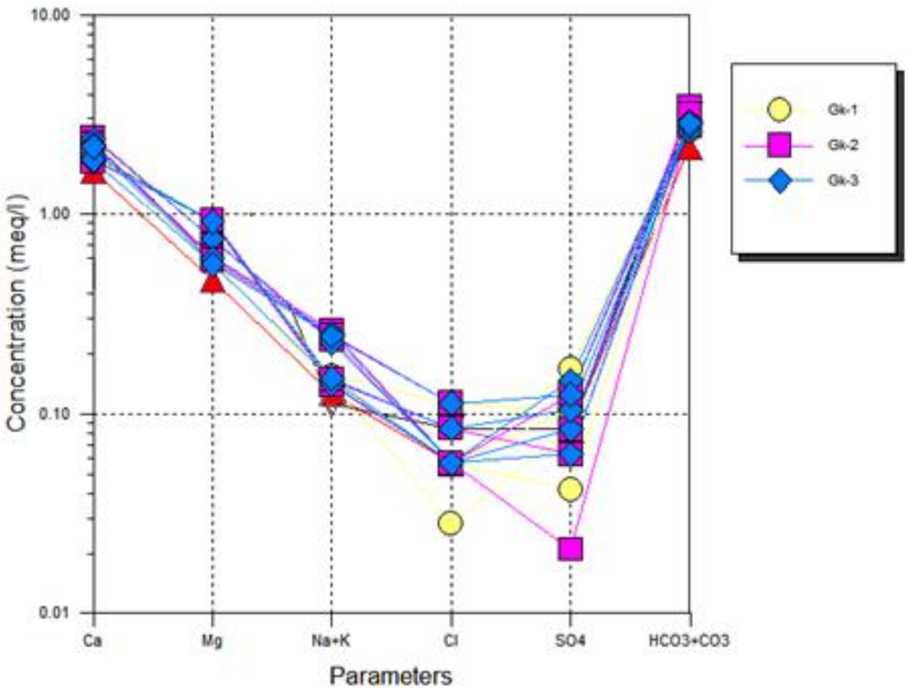

Figure 4. Schoeller Diagram of water samples from the study area

Bacteriological conditions of water

Until recently, many people thought that the environments below the surface were perfectly sterile. However, caves have been contaminated by surface-dwelling microorganisms, many of which reach the environment through surface runoff, air currents, animals, and humans. For this reason, it is difficult to know whether the microorganisms belong to the subsurface environments (Gounot, 1994). In the present study, microbiological parameters of water including total bacteria in (TB), total coliform (TC), fecal coliform (FC), *Escherichia coli* (*E. coli*), fecal streptococci (FS) varied at different sampling periods and sampling points (Table 7). According to the data, the FC and *E. coli* were not found in the sampling sites. Besides, the highest values of the remaining parameters were measured in November 2015 (Nov.15), February 2016 (Feb.16), and October 2016 (Oct.16) during lowest flow period. The fecal streptococci belong to the genera *Enterococcus* and *Streptococcus* are gram-positive bacteria that are predominately found in animals (Houssain, 2014), while *E. coli* is usually found in human and animal feces and could reach water sources (Bennett et al., 2018). Since the study site is in a remote area and *E. coli* could not be detected, this contamination could be of animal origin. Coca Moreno et al., (1996) and Cabral and Marques (2006) found positive correlations between NH_4-N and several microbiological indicators such as total and fecal coliforms, fecal streptococci, and enterococci. Besides, Ponnimbaduge-Perera et al., (2019) demonstrated that bat droppings caused major changes in chemical and microbiological water quality parameters. In the present study, we found parallelism correlation between ammonia and fecal streptococci, as in previous studies (Tables 3-5 and 7). Our results indicated that since GC is used as hibernacula by the local bat population, the water quality was affected. According to TWPCR (2004), TC and FC values of the sampling sites were classified as high quality (I), but not drinkable for the criteria both of WHO (2004) and TS 266 (2005).

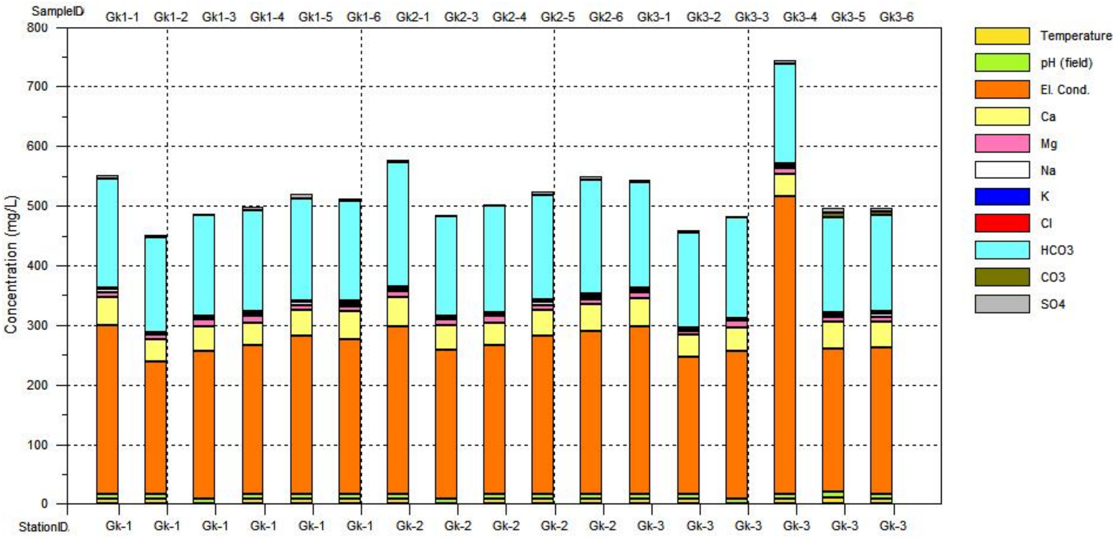

Figure 5. Comparison of major ions and some parameters of water by sampling sites

Table 7. Microbiological parameters of water from the sampling sites

	Parameters/Date	Nov.15	Feb.16	Mar.16	May.16	Jul.16	Oct.16
GC1	TB (cfu/mL)	1110	50	10	50	60	6
	TC (mpn/100mL)	43	7	7	15	15	43
	FC (mpn/100mL)	0	0	0	0	0	0
	E. coli (mpn/100mL)	0	0	0	0	0	0
	FS (cfu/mL)	50	0	0	0	0	0
GC2	TB (cfu/mL)	2480	n.m.	n.m.	50	50	850
	TC (mpn/100mL)	1100	n.m.	n.m.	15	4	75
	FC (mpn/100mL)	0	n.m.	n.m.	0	0	0
	E. coli (mpn/100mL)	0	n.m.	n.m.	0	0	0
	FS (cfu/mL)	50	n.m.	n.m.	0	10	35
GC3	TB (cfu/mL)	2920	550	100	150	160	250
	TC (mpn/100mL)	460	43	43	15	75	15
	FC (mpn/100mL)	0	0	0	0	0	0
	E. coli (mpn/100mL)	0	0	0	0	0	0
	FS (cfu/mL)	70	0	0	0	10	270

***n.m: Not measured.**

Aquatic macroinvertebrates

In total, four benthic macroinvertebrate taxa were found in the sampling sites (Table 8) including *Rhynchelmis limosella* Hoffmeister, 1843 (GC2) and an unidentified enchytraeid (GC1), *Dugesia* sp. (GC1), and *Gammarus uludagi* Karaman, 1975 (GC1 and GC3). Macroinvertebrates were obtained in the first period of sampling (November-2015) during the low flow period.

Table 8. Aquatic macroinvertebrates of the Güvercinkaya Cave

Taxa	Sampling Site	Individual Num.
Rhynchelmis limosella	GC2	1
Enchytraeid*	GC1	1
Dugesia sp.	GC1-GC3	8
Gammarus uludagi	GC1-GC3	16

*Deformated individual

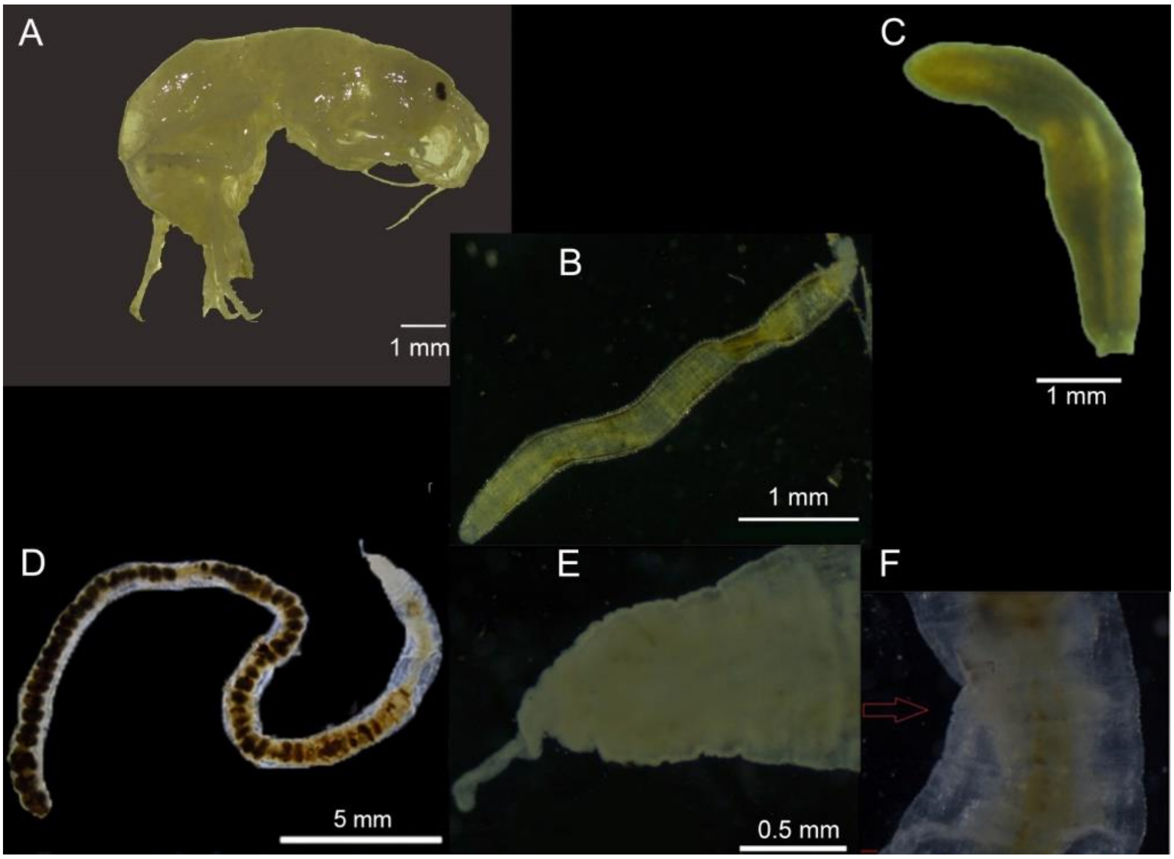

Figure 6. Aquatic macroinvertebrates of the Güvercinkaya Cave: A. *Gammarus uludagi*, B. Enchytraeid, C. *Dugesia* sp., D. *Rhynchelmis limosella*, E. *R. limosella* proboscis, F. *R. limosella* genital opening

Cave-dwelling organisms usually possess specialized physiological and morphological adaptations due to darkness, stable physical and chemical factors and limited energy sources (Barr, 1968; Biswas; 1992; Biswas 2010). A wide variety of adaptations can be seen in cave species. Some organisms are obligate to the cave environment and unable to live in other ecosystems (=troglobiont), while some organisms temporarily use the cave environment (troglophile or trogloxene) (Sket, 2008). In this study, the macroinvertebrate taxa are mainly recorded from surface environments e.g. lakes, streams, springs. For instance, *Rhynchelmis limosella* is a common European species recorded from the Danube River (Mauch, 1989), and *Dugesia* sp. is a widespread genus in the surface waters of the Mediterranean region (de Vries 1985). *Gammarus uludagi* was described from Uludağ (Bursa, Turkey) by Karaman and Pinkster (1977) and then sampled from streams Kazdağı (Çanakkale, Turkey) by Özbek et al., (2017). Since the identified taxa in the present study were sampled from surface waters in previous studies, they can not be considered troglobiont or stygobite. Similarly, no troglobiont fauna were found in the karstic caves of Dupnisa and Yelköprü located in western Turkey (Balık et al., 2002; Özkan, 2009). On the other hand aquatic troglobiont taxa in caves with permanent hydrological regime were reported from Turkey and fromother regions of the world, (Karaman and Ruffo, 1994; Georgiev et al., 2017; Georgiev, 2012; Özbek et al., 2013; Andersen et al., 2016; Sidorov and Samokhin, 2016; and Culver and Hobbs, 2017.

Acknowledgments

This study was financially supported by TUBİTAK (ÇAYDAG, 115Y419). The early data set of this project was presented in the "International Ecology Symposium" (Kayseri, Turkey) held on 11-13 May 2017, Kayseri-Turkey, and the "20th Annual Meeting of Underwater Science and Technology" on 16-17 November 2017, Urla-İzmir. The authors acknowledge the two cave divers who carried the underwater sampling.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

D. A. Odabaşı and S. Odabaşı were designed of study. The data collection and interpretation were made by D. A. Odabaşı, S. Odabaşı, O. Deniz, F. Çakır, B. Elipek, N. Arslan, O. Özbek. H. B. Özalp was planned the diving and underwater sampling. The manuscript was written by D. A. Odabaşı, S. Odabaşı, F. Çakır,and O. Deniz. Language correction made by O. Özbek.

Ethics Approval

The material used in this article is invertebrate species therefore ethics committee approval is not required for this study

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