------ Raf. J. Sci., Vol. 27, No.3, pp. 1-11, 2018------

Isolation and Identification of *Desulfovibrio* spp. from Hammam Al-Alel and Study some of the Environmental Properties of the Water in This Region

Taha A. Mohammed   *May A. Al-Allaf   Ghada A. Mohammad
Environment Research Center/ University of Mosul   College of Science/ University of Mosul
***Mayallaf2@yahoo.com**

(Received 17/ 2 / 2014 ; Accepted 28 / 11 / 2017)

ABSTRACT

The study involved natural sulfur springs and their tributaries on the right bank of Tigris river south Mosul city at Hammam Al- Alel region. Seasonal samples were taken through a year from four locations represent: hot, cold water springs, tributaries formed, in addition to Tigris river water.

Desulfovibrio spp. were isolated from Hammam Al-Alel region, which were responsible of clear variability in water color and the sulfur deposits resulted from sulfate oxidation-reduction reactions. Bacteriological, physical, and chemical properties of this acidic sulfur springs were studied.

The study has improved that concentrations average of sulfate ions were high (255 mg/l) led to oxygen decrease and drop in pH to (5.8) compared with Tigris river pH (8.1), the study also has showed that Mg^{+2}, Ca^{+2}, Na^{+1} and K^{+1} ions concentrations were increased especially during summer, this caused that the environment surrounded this region became toxic, then it is non-hygienic for utilization by man, animals and cultivation.

Keywords: *Desulfovibrio* spp., Sulfur spring, Hammam Al- Alel water quality.

عزل وتشخيص بكتريا .*Desulfovibrio* spp من منطقة حمام العليل ودراسة بعض الخصائص البيئية لمياه هذه المنطقة

الملخص

تناولت الدراسة الينابيع الكبريتية الطبيعية في منطقة حمام العليل الواقعة على الضفة اليمنى لنهر دجلة جنـوب مدينـة الموصل. بأخذ نماذج فصلية لمدة سنة كاملة لدراسة أربعة مناطق شمات الينابيع المعدنية الحارة والباردة وروافدها فضلا عـن مياه نهر دجلة.

تم عزل بكتريا .*Desulfovibrio* spp من منطقة حمام العليل من ملاحظه التغاير الواضح في لون المياه والترسـبات في كل مناطق الدراسة بسبب ترسيب الكبريت بأشكاله المختلفة نتيجة تفاعلات الأكسدة والاختزال الكبريتية، كما تـم دراسـة الخصائص البكتريولوجية والفيزيائية والكيميائية للينابيع الكبريتية الحامضية لهذه المنطقة.

أثبتت الدراسة أن معدل تراكيز ايونات الكبريتات كانت عالية (255 ملغم/لتر) وادت الى نقص في الاوكسجين والـى انخفاض في الدالة الحامضية الى 5.8 مقارنة بالدالة الحامضية للنهر والتي كانت 8.1 . كما اوضحت الدراسة ان هناك زيادة واضحة للايونات ألموجبه وخصوصا الكالسيوم والمغنيسيوم والصوديوم والبوتاسيوم، لاسيما في فصل الصيف جعلت البيئـة المحيطة بهذه المنطقة سمية وغير صالحة للاستخدام البشري والحيواني والزراعي.

الكلمات الدالة: .*Desulfovibrio* spp، الينابيع الكبريتية، نوعية مياه منطقة حمام العليل.

INTRODUCTION

Water is considered a source of various fortunes and the main reason of people's lives (Fadhel, 2013). Ground water is another source of water supply when it is available. Its uses depend on its quantity and quality. Sulfur compounds in water are either as sulphate form (SO_4^{-2}) in aerobic conditions, or present as hydrogen sulfide (H_2S) in anaerobic conditions resulted from *Desulfovibrio* spp. activity (Sacks, 2002). Calcium and magnesium carbonate which are forming Limestone make the ground water passes on it enriched with minerals also. In general ground water at Mosul city is saline with high concentrations of minerals and can be used for irrigation of saline resistance plants (Al-Tayyar *et al*., 1992).

The sulfur reducing bacteria (SRB) play an important role in geochemical processes such as the production of sulfide ores, and as participants in the sulfur cycle where they reduce sulfate in the environment. However, they can also be nuisance organisms because their activities can result in the corrosion of metal pipes due to the formation of iron sulfide (Benson, 2002). This group of bacteria was first described by Beijerinck in 1859 when he cultured a common isolate *Desulfovibrio desulfuricans*, which is enriched in media containing lactate and sulfate. This bacterium is a small, curved, gram-negative rod that is motile by polar flagella (Lodowska *et al*., 2012).

Sulfate reducing bacteria occur in diverse anaerobic environments, especially marine waters, where sulfate is plentiful and active fermentation is being carried out by other groups of bacteria. The sulfate reducers metabolize a limited number of substrate such as lactate and pyruvate, which they use for carbon requirements. ATP is synthesized by anaerobic sulfate respiration in which the sulfate ion serves as a terminal electron acceptor and is reduced to H_2S, in much the same way that oxygen is converted to water in aerobic respiration (Madigan *et al*., 2012).

When the ground water passes on the rocks rich in sulfur compounds and the ability of sulfur dissolution is great, the water will contain high concentration of sulfur compounds as sulphate, sulphite or sulfur, which is combined with other cations as sodium, calcium and magnesium (Al-Rawi *et al*., 1990).The variability of sulfate-ion concentrations and water quality were correlated with biochemical activity and gases movement through the aquifers contained (Al-Sawaf,1997).

The research aims are isolation and identification species of SRB especially the bacterium *Desulfovibrio desulfuricans* from springs water –hot and cold in Hammam Al-Alel region near Tigris river, south Mosul city which is consider enriched in sulfur compounds, and from another site to study the quality of this water.

MATERIALS AND METHODS

1- Studied Area: The studied area was at Hammam Al-Alel region, 20 Kms south east of Mosul city on the right bank of Tigris river. The region was well-known from the past with the enrichment with many springs of hot and cold mineral waters.

The field work and the seasonal duplicate samples were extended through one year started on March 2011 till January 2012. Sampling procedure with both field and laboratory tests were performed according to (APHA, 2007).

The study has involved field observation and sampling. Samples, including water and sediments, were collected from four locations, including hot and cold springs, tributaries and Tigris river. Sterilize containers were used to collect samples for bacteriological tests. Fig. (1) and Fig. (2) shows the studied area and the sampling locations.

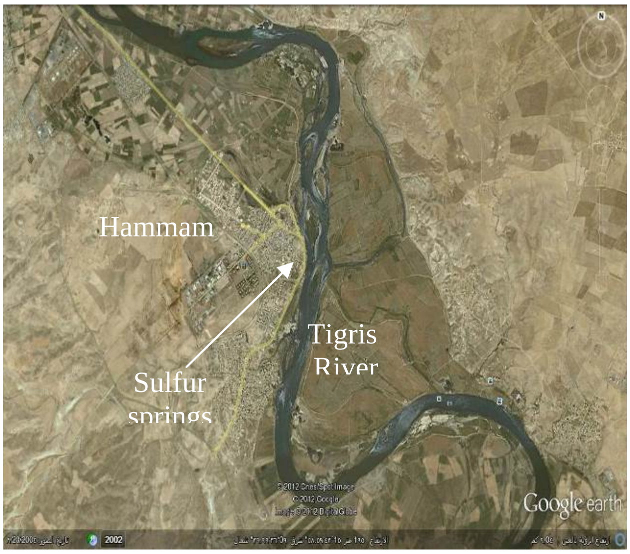

Fig.1: GPS map showing Location of Hammam Al-Alel

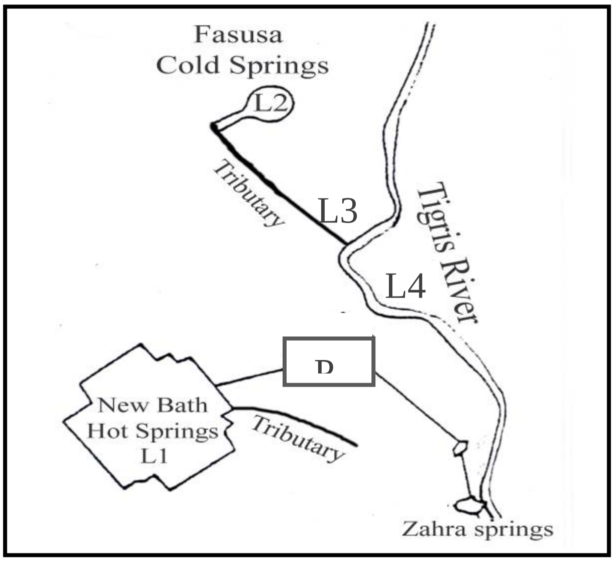

Fig. 2: Sampling locations map sketch

2- Detection of Sulfur Reducing Bacteria (SRB)

A. Media

The basal medium used for cultivation SRB was;

- **API (American Petroleum Institute)**

It contains of (in gram/liter of distilled water) **:**
Yeast extract 1; Dipotassium phosphate 0.01; Ascorbic acid 0.1; Sodium chloride 1; Magnesium sulfate 0.2; Ferrous ammonium sulfate 0.1; Agar14.0; PH 7.4 (Vester and Ingvorsen, 2014).

- **Tryptic Soy Agar (TSA)** was prepared according to the company (Hi-media) instructions, sterilized by autoclave and cooled, then powered into sterilized Petri plates.

B. Preparation of Winogradsky column

Winogradsky column was prepared as follows :

Graduated cylinder (250 ml size), cellulose (filter paper, or newspaper), calcium sulfate, calcium carbonate, dipotassium phosphate, fresh mud and water collected from various sources, beakers (250 ml), glass stirring rods, aluminum foil, incandescent lamps.

1. Using some sources of cellulose, a thick slurry was prepared in a beaker by adding water. Paper was tear in small pieces and macerated in a small volume of water.
2. Graduated cylinder was filled with the cellulose slurry until one-third of the cylinder.
3. To 200g of mud, 1.64 g of calcium sulfate and 1.3 g each of calcium carbonate and dipotasium phosphate were added.
4. Some water samples were collected with mud and mixed the ingredients well with a stirring rod.
5. Mud was poured into the column on top of the cellulose slurry.
6. The slurry were mixed gently (mud and water) by using a glass rod two-third of the column.
7. Column was filled with water until it is 90% full.
8. Column was covered with a piece of aluminum foil to prevent evaporation. Initial appearance of the column in laboratory was recorded.
9. Sides of the column were warped with aluminum foil to exclude light.
10. Column was incubated at room temperature for two weeks. With time dramatic changes occur in the appearance of the column as various bacterial groups began to grow (Benson, 2002).

C. Cultivation Conditions

Sediments and Winogradsky column samples were cultivated by streak-plate method directly and incubating for 10 days at 37^{o}C anaerobically by using gas bag. Culture purity was checked by macroscopic observation of different colonies types and microscopically then biochemical tests were made including Urease, Indol, SIM, Catalase and Nitrate reduction tests (Benson, 2002).

To determine the total count of microorganisms present in water samples, standard plate count (SPC) procedure was universally used. The procedure included diluting the samples with series of sterile water, by using dilution procedure up to 10^{-5}. One ml of 10^{-4} & 10^{-5} was transferred into sterilized Petri plates. Melted nutrient agar was cooled, then poured in each plate. After N.A solidified, the plates were incubated for 24-48 hrs at 37^{o}C. Two plates for each dilution have been achieved. MacConkey agar was used to detect coliform and faecal coliform bacteria and incubated in 37^{o}C and 44^o C respectively (Ahmed, 2009). Two Plates for each dilution have been achieved.

Physical and Chemical Properties:

A. Field tests were performed including: Temperature, pH, dissolved oxygen and turbidity.

B. The laboratory tests; including: water quality parameters such as electrical conductivity, total hardness as $CaCO_3$, calcium hardness as $CaCO_3$. The cations as Calcium, Magnesium, Sodium and Potassium, in additions to the anions as sulphate, total sulfur, alkalinity as $CaCO_3$, and COD were studied.

RESULTS AND DISCUSSION

Bacteriological, Physical and chemical properties have been investigated and discussed in the following:

Bacteriological properties:

Macroscopically, the colonies appeared black and brown color on both media (API,TSA) Fig.(3) and (4). Also Winogradsky column showing the variety of bacteria including SRB Fig.(5). Microscopic examination and gram stain showed gram negative rods bacteria.

Fig. 3: Black Colonies of *Desulfovibrio* growing on API medium

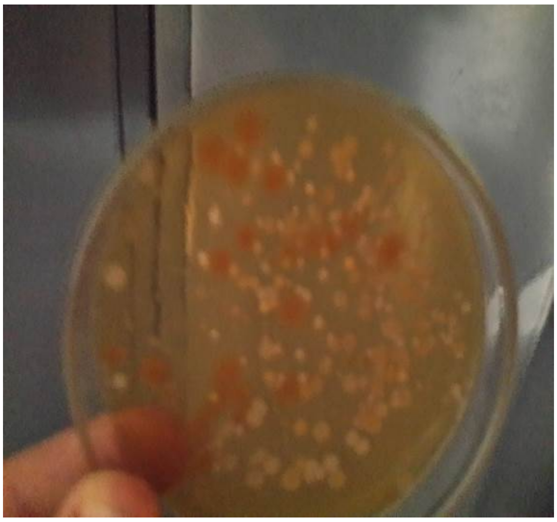

Fig. 4: Brown Colonies of *Desulfovibrio* growing on TSA medium

Biochemical tests were done to identify SRB. Three species of *Desulfovibrio* were isolated, they are *D. desulforicans*, *D. vulgaris*, *D. piger* Table (1). The percentages of these three species were (15%, 7% and 8%) respectively. As it is shown in Table (2), *D. desulforicans* was isolated from all locations except hot water spring and sediment of Tigris river. This may due to this bacteria is anaerobic while these locations are aerobic.

Table 1: Biochemical tests of SRB isolated from water and sediment samples

Test	*D. desulforicans*	*D. vulgaris*	*D. piger*
Catalase	-	-	-
Indol	+	+	-
Urea	+	-	-
Motility	+	+	+
Nitrate	+	-	-
SIM	+	+	+

Table 2: Types of *Desulfovibrio* spp. isolated from four locations

Sampling		*D. desulforicans*	*D. vulgaris*	*D. piger*
Water Samples	Hot water spring	-	-	+
	Cold water spring	+	-	+
	Tributary water	+	+	-
	Tigris river	+	+	+
Sediment Samples	Hot water spring	+	-	+
	Cold water spring	+	+	+
	Tributary water	+	+	+
	Tigris river	-	+	-
Winogradsky (1)		+	+	-
Winogradsky (2)		+	+	+

Fig. 5: Winogradsky column after incubating for two weeks

The enrichment of *Desulfovibrio* species is easy in magnesium and ammonium sulfate medium containing ferrous iron Fe^{2+} (Madigan *et al*., 2012). A reducing agent, such as thioglycolate or ascorbate, is required to achieve reduction potential in the medium. When sulfate reducing bacteria grow, the H_2S formed from $SO4^{2-}$ reduction combines with the ferrous iron to form black, insoluble ferrous sulfide. This blackening not only indicates sulfate reduction, but the iron also binds and detoxifies the H_2S, making possible growth to higher cell densities (Loubinoux *et al*., 2003).

Over 40 genera of these organisms, collectively called the dissimilative sulfate-reducing bacteria and sulfur-reducing bacteria, are known. The word dissimilative refers to the use of SO^{2-} or S^0 as electron acceptors in energy generation instead of their assimilation as biosynthetic sources of sulfur (Shihab and Hashim, 2006). Sulfate ($SO4^{2-}$) and sulfur (S^0) are electron acceptors for a large group of anaerobic bacteria that utilize organic compounds or H_2 as electron donors in anaerobic respirations. Hydrogen sulfide (H_2S) is the product of both SO_4^{2-} and S^0 reduction. The range of electron donors used by sulfate-reducing bacteria is fairly broad. Hydrogen (H_2), lactate, and pyruvate are almost universally used and many species also oxidize certain alcohols (for example, ethanol, propanol, and butanol) as electron donors. Some strains of Desulfotomaculum utilize glucose, but this is rare among sulfate reducers. Desulfovibrio species typically oxidize lactate, pyruvate, or ethanol to acetate and then excrete this fatty acid as an end product (Al-Naqib and Al-Dabbagh, 1992).

The organisms grow very slowly, taking 3 to 5 days to produce tiny transparent, non hemolytic colonies on anaerobic blood agar plates and therefore are easily missed or overgrown in mixed cultures (Odom and Singelton, 1993).

Desulfovibrio organisms belong to a heterogeneous group of sulfate-reducing, motile, anaerobic bacteria with more than 30 proposed species, some of which infrequently cause a variety of human infections (*D. desulfuricans*, and others).The bacteria may be carried asymptomatically in the human gastrointestinal tract, or they may act as opportunistic pathogens associated with primary bacteremia and abdominal infections. Fig. (6) shows the variety in colors of the sulfur springs in Hammam Al-Alel region.

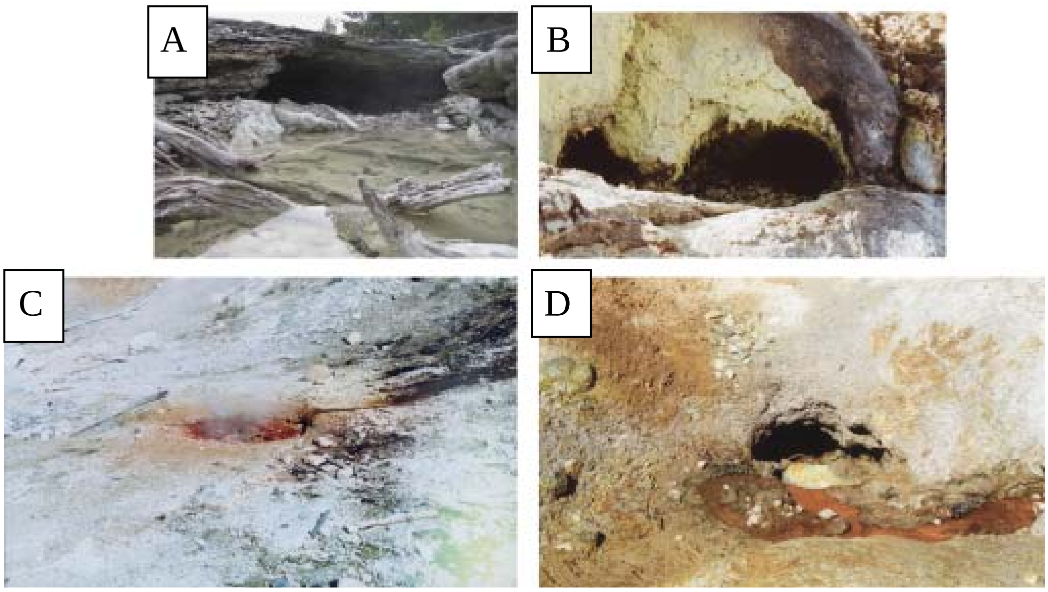

Fig. 6: Variation of colors of the sulfur spring area

Bacterial numbers represented the total plate count (TPC) variation along the tributary and its junction with Tigris river in each location, Table (3) shows TPC; coliform & feacl coliform for four locations of Hammam Al-Alel region. In any raw water certain types of microorganism are blooms which will play vital role in biochemical reaction. In seeping and tributary waters small number of bacteria (sulfur bacteria) are occurred where the pollutants can reach there, in addition to the effects of biological activity of sulfur compound cycle (Warren *et al*., 2005). Fig. (6) shows the activity of microorganism and the changing of rocks and sediment color.

Table 3: TPC, Coliform and Fecal coliform count in Hammam Al-Alel region

Parameter (CFU/ml)	Location (1)	Location (2)	Location (3)	Location (4)
TPC CFU/ 1ml	20×10^3	8×10^3	14×10^3	40×10^3
Coliform Bacteria	Non	Non	+	+++
Fecal Coliform	Non	Non	+	+++

Location (1): Hot water seepage springs Location (2): Cold water seepage springs
Location (3): Tributary of cold spring Location (4): Tigris River water.
+ :weak growth +++ : heavy growth

An understanding of the survival of fecal indicator organisms and the enteric pathogens in water is basic to the meaningful interpretation of sanitary water quality data. This is so because the isolation of fecal coliform bacteria (Odem and Singelton, 1993) is commonly used to signify the potential presence of intestinal pathogens. Although detection of indicator bacteria suggests occurrence of pathogenic organisms in water, the potential health hazard independent on retention of critical density levels and associated virulence for the pathogens in a given time frame during transmission via the water route. Furthermore, once these bacteria are deposited into the water they are in an environment that is not favorable to the maintenance of viability of most bacteria

Sulfur bacteria were detected at the surface, while at the tributary bed desulphurization phenomena occurred by anaerobic bacteria which gave the mud near the springs black color.

The following biochemical reactions are occurred in the presence of sulfate-reduction bacteria.

$$\mathbf{CaSO_4 + CH_4 \xrightarrow[\textit{desulfuricans}]{\textit{Desulforibrio}} CaCO_3\downarrow + H_2S\uparrow + H_2O}$$

$$\mathbf{SO_4^{-2} + CH_4 + 2H^+ \xrightarrow{\text{bacteria}} H_2S\uparrow + CO_2 + 2H_2O + 22.8kCal}$$

(Madigan *et al*., 2012)

(Mcfeters *et al*., 1974) mentioned that survival of 51 cultures of indicator bacteria and an *Aeromonas* species was studied in the un chlorinated well water. A total of 29 coliform cultures, both fecal and non fecal, isolated from water and from fecal samples of man and animals were used. Persistence of the various coliform types that were examined varied by the amounts .There was a lack of close agreement in survival pattern among coliform bacteria that were grouped together according to species, type, source, or characterization as fecal or non fecal.

The results of the experiments describing the survival of indicator bacteria in the spring water demonstrated that the fecal coliform bacteria remained viable to a similar extent under these conditions, There was also no important difference in the persistence of fecal as compared with non fecal coliform cultures. On the other hand, (Geldreich, 1970) reported that a fecal coliform culture had a more rapid die-off than a non fecal variety at 20 ^oC. However, this lack of close agreement in survival potential should not be surprising among bacteria that are as diverse as the coliforms (James and Evison, 1979).

Physical properties

Generally, temperature of hot springs was ranging from 40 - 50 ^oC with an average of 43^oC. This may due to the dissolution of sulfur in deep layers, with high concentration of sulfur and the high rate of exothermal metabolic activity (Al-Sawaf, 1997). While in the cold springs the temperature decreased to 18 ^oC as an average, (Table 4). Temperature variation is limited compared with surface of water. Consequently, these could be related to the shallowness of the tributary despite the oxidation reduction reaction. These results were agreed with Zanquba location east of Tigris river which is enrichment with sulfur springs (Al-Tayyar *et al*., 1992).

Significant drop in pH values (6.7- 6.38) as an average took place at two spring locations. Such drop could happen as far as consuming acid materials are available. This occurred mainly in the presence of limestone, dolostone and calcareous claystone. Also H_2S in water formed H_2SO_3 acid which is weaker than H_2CO_3 at pH below 8, as explained in the equation (Al-Tayyar, 2007).

$$\mathbf{H_2S + 1.5O_2 \rightarrow H_2SO_3 + energy}$$

pH and E.C. values were agreed with the results of (Kanna, 2001), who found that many wells and springs in Hammam Al-Alel, Al-Meshraq and the surrounding areas were weakly acid.

The seeping water has highly electrical conductivity (EC) gained from the dissolved inorganic matter in the rocks as gypsum, limestone and gypsi ferous marl. Its values were ranged from (2680 to 2300) Mhos/cm for the sulfur springs water (Table 4). On the other hand the tributary water has noticeable fluctuation in conductivity which is gradually decreased towards the main junction with Tigris river, due to the dilution caused by increase discharge of Tigris river, also as (Mahmood, 1994) mentioned that the high precipitation of dissolved salts within tributary during summer season and biological exhausting of theses salts. Large amounts of elemental sulfur are deposit, considered to be an area where oxygenated water mixed with hydrogen sulfide gas, consisting yellow sediment of sulfure, Fig. (7). Next equation explain that:

$$\mathbf{H_2S + 0.5O_2 \rightarrow S + H_2O + energy}$$

The colored sediment was also shown in Zanquba sulfur springs by (Al-Tayyar *et al*., 1992).

Table 4: Average and the range values of the physical water quality parameters of Hammam Al-Alel region locations

Parameter		Location (1)	Location (2)	Location (3)	Location (4)
Temp. ^oC	Average value	43	18.0	18.0	18
	Range	50-40	8.6-25	9.1- 25	10-28
pH Unit less	Average value	6.7	6.38	6.51	7.6
	Range	6.1-7.2	5.8-7.0	5.8- 7.2	7.0-8.1
EC μhos/cm	Average value	2680	2300	2210	460
	Range	2000- 3100	1900- 2520	1800-2480	380-510
Turb. Ntu	Average value	25	35	41	28
	Range	16.7- 38.5	15.8- 45.0	28.0- 55.g2	23.2- 55.8
D. O. mg/L	Average value	0.8	1.1	1.8	6.6
	Range	0.0-1.2	0.2-1.8	0.6- 2.5	5.6-8.1

* Location (1); Hot water seepage springs
* Location (2); Cold water seepage springs
* Location (3); Tributary of cold spring
* Location (4); Tigris River water.

Water in the studied area was turbid, the turbidity is mainly due to suspended matter which is freshly precipitated CaSO4, this is shown noticeably in the tributary samples in location (3).

Materials causing turbidity range from pure inorganic substances to largely organic in nature (Al-Obaidy and Al-Nima, 2013).

Dissolved oxygen (D.O.) in water is another pollution indicator. Ground water have low concentration of oxygen, especially with high concentration of minerals. Hot springs almost without oxygen, with a concentration not exceed 1.2 mg/l. The average value was 0.8 mg/l, while in the cold springs the average value of (D.O.) increased to 1.1 mg/l. The results agree with (kanna, 2001) who confirmed the low concentrations of oxygen, especially in sulfur wells which reached anaerobic condition.

Chemical properties

Geologically, most of the rocks in Mosul district regions are enriched with sulfur compounds having the ability to dissolved in water (Al-Naqib and Al-Dabbagh, 1992). As sulfur has many valiances though reducing compounds are expected from ground water. These have toxic effect like H_2S, which is formed in the absence of oxygen. Under anaerobic condition the sulphate ion is reduced to sulfide and vice versa. It is responsible for temporary hardness in natural water and mainly occurs as $CaSO_4$ and $MgSO_4$ which are considered as vital factors in water quality evaluation (Al-Tayyar *et al*., 1992). Table (5) explain the chemical properties of the studied locations.

At the first and second locations the sulphate concentrations were (215 and 185mg/l) respectively. These springs are feeding from Tigris river which is diluted. In the third location (tributary), the shallow thickness of gypsum layers attributed to high concentration of sulphate which exceed (255mg/l). While in the fourth location the dilution of Tigris river to the springs water was noticeable (65 mg/l).

Springs water have high hardness due to dissolution of rock minerals and salts. In the first and second locations were (1580,1120 mg/l) respectively comparing with third location which was dropped to (1080mg/l). The declination of hardness concentration along the tributaries are primarily due to super saturation and low oxidation of different sulfur compounds to sulphate form and precipitated as gypsum by chemical and biochemical reaction at the terminal end of the streams. The low temperature of water springs precipitate the dissolved minerals. Therefore, the total hardness in the cold springs reached (1210 mg/l).In the hot water springs the higher recorded value was (1650 mg/l), Table (5).

The main source of alkalinity is Na^{+1} and K^{+1} ions. These ions are leached by acid water of springs. As completely dissolved ion, with fixed valiances, no change in their concentration occurred, unless dilution takes place. It is useful to mention that, in many parts of Iraq Na^{+1} ion is greater than K^{+1} ion (Al-Rawi *et al*., 1990). In these springs Na^{+1} concentration is greater than K^{+1} with at least ten times (540-640 mg/l) for Na^{+1} and (40-48 mg/l) for K^{+1}. These increments lead to increase alkalinity values in the springs (140-148 mg/l) comparing with Tigris water (110 mg/l).

Chemical oxygen demand (COD) as a pollution indicator was also increased in springs water (55-61mg/l) comparing with Tigris river water (18mg/l) due to the high mineral concentrations, with their ability to oxidize. As the high concentration of sulfur is toxic to microorganism, seeding were not available to support biochemical oxygen demand (BOD5). Therefore, these values were decreased to (14-22mg/l) in the springs water comparing with the tributary (24 mg/l). This agree with (Al-Tayyar,2007), who found that the difference between COD and BOD5 values were more than 200% in sulfur springs water, comparing with the differences in Tigris river water which not exceed 100%.

Table 5: Average and the range of the chemical studied water quality parameters of Hammam Al-Alel region locations

Parameter		Loca. (1)	Loca. (2)	Loca. (3)	Loca. (4)
$SO4^{-2}$ mg/L	Average value	215	185	255.0	65
	Range	170-295	140-205	180- 320	44-75
S mg/L	Average value	175	210	200	10
	Range	150-210	180-255	175- 250	0-25
Total hardness as $CaCO_3$ mg/L	Average value	1580	1120	1080	220
	Range	1410-1650	1000-1210	980-1200	180-245
Ca hardness as $CaCO_3$ mg/L	Average value	940	720	695	110
	Range	810-1100	670-830	640- 800	85-125
Ca^{+2} mg/L	Average value	376	288.5	268.4	60
	Range	310-390	260-295	240- 300	48-69
Mg^{+2} mg/L	Average value	143.4	89.6	86.5	17
	Range	120-156	78-105	70- 99	12-21
Na^{+} mg/L	Average value	540	640	650	20
	Range	410-730	430-810	400- 800	18-32
K^{+} mg/L	Average value	40	48	41.0	5.8
	Range	28-58	36-64	34- 68	4.1-8.2
Total alkalinity as$CaCO_3$ mg/L	Average value	140	148	150	110
	Range	128-146	108-195	110- 210	95-125
COD mg/L	Average value	55	61	60	18
	Range	41-68	52-71	50- 74	8-22
BOD_5 mg/L	Average value	14.0	22.0	24.0	5.8
	Range	8-24	18- 32	12-36	4.2-6.8

* Location (1); Hot water seepage springs
* Location (2); Cold water seepage springs
* Location (3); Tributary of cold spring
* Location (4); Tigris River water

REFERENCES

Ahmed, M.R. (2009). Ground water quality of selected wells in Zaweta District, Northern Iraq. *J. Tik. Eng. Sci.*, **17**(1), 28-35.

Al-Naqib, S.K.; Al-Dabbagh, T.H. (1992). Some physical and geotechnical properties of the new rock type Ninivite, 26th Annual conference of the engineering Group. Sept. 9th-13th 1990, edsi J. C. Gripps and C. F. Moon.

Al-Obaidy, M.; Al-Nima, B. (2013). Turbidity and removal efficiency in the main water purification plants of Neneveh province. *J. Raf. Sci.*, **24**(3), 39-53.

Al-Rawi, S.M.; Al-Azzo, S.I.; Abbawi, S.A. (1990). "Hydro Geochemical Evaluation of Ground Water in some Parts of Mosul City Suitability For Irrigation". 2nd scientific conference of Saddam Dam Research Center. 224-239.

Al-Sawaf, F.D. (1997). Sulphate reduction and sulphur deposition in the lower Fars formation, Northern Iraq. *J. Eco. Geo.*, **72**(4), pp. 608-618.

Al-Tayyar, T.A. (2007). "Water Quality of Natural Sulfur Springs in Selected Sites in Mosul". 1st Sci. Conf. of EPCRC, Mosul Univ., pp. 35-43.

Al-Tayyar, T.A.; Mustafa, M.A.; Al-Naqib, S.K. (1992). Investigation of Tigris river pollution. 18th WEDC conference Nepal, water, environment, and management. pp. 51-54

APHA; ASTM. (2007). Standard method for the examination of water and wastewater.

Benson, H.J. (2002). "Microbiological Applications, Laboratory Manual in General Microbiology". 8th ed. The McGraw-Hill companies, Inc. pp. 323-445.

Fadhel, M. (2013). The impact of water oscillation on hydrophytes and macro-algae growth in Tigris river within Mosul area. *J. Raf. Sci.*, **24**(1), 17-30.

Geldreich, E.E. (1970). Applying bacteriological parameters to recreational water quality. *J. Amer. WaterWorks Ass.* 62,113-120.

James, A.; Evison, L. (1979). "Biological Indicators of Water Quality". John Wiley and Sons, Great Britain. Chapter 5.

Kanna, A.M. (2001). The study of the quality of the sulfur ground waters in Ninevah goverenment. M.Sc. Thesis, College of Science, Mosul University.

Lodowska, J.; Wolny, D.; Jaworska-Kik, M.; Kurkiewicz, S.; Dzierzewicz, D.; Weglarz, L. (2012). The Chemical composition of endotoxin isolated from intestinal strain of *Desulfovibrio desulfuricans*. *J. Sci. Wor.*, Article ID 647352, 1-10.

Loubinoux, J.; Jaulhac, B.; Piemont, Y.; Monteil, H.; Le Faou, A.E. (2003). Isolation of sulfate-reducing bacteria from human thoracoabdominal pus. *J. Clin. Microbiol.*, **41**(2), 1304–1306.

Madigan, M.T.; Martinko, J.M.; Stahl, D.A.; Clark, D.P. (2012). "Brock Biology of Microorganisms". 11th ed. Prentice Hall. Upper Saddle River NJ. San Fransisco pp. 510-512.

Mahmood, F.Y. (1994). Physical-chemical evaluation of ground water in some wells in Ninevah district used for drinking and domestic purposes. *J. Raf. Eng. Sci.*, **2**(2), 35-44.

Mcfeters, G.A.; Bissonntte, G.K.; Jezeki, J.J.; Thomson, C.A.; Stuart, D.G. (1974). Comparative survival of indicator bacteria and enteric pathogens in well water. *J. App. Microbiol.*, **27**(5), pp. 823-829.

Odom, J.M.; Singelton, R. (1993). "The Sulfate-Reducing Bacteria: Contemporary Perspectives". Springer-Verlag, New York, N.Y. 289 p.

Sacks, L.A. (2002). Report of water resources of Florida, approach: U.S. Geological Survey WRIR 02-4192. www.florida-water.com.

Shihab, A.S.; Hisham, A. (2006). Cluster analysis classification of ground-water quality in wells within and around Mosul city/Iraq. *J. Environ. Hydrol.*, **4**(1), 1-11.

Vester, F.; Ingvorsen, K. (2014). Improved most probable number to detect sulfate reducing bacteria with natural media and aradiotracer. *J. Appli. Environ. Microbiol*. **64**(5), 1700-1707.

Warren, Y.A.; Citron, D.M.; Merriam, C.V. (2005). Biochemical differentiation and comparison of *Desulfovibrio* species and other phenotypically similar genera. *J. Clin. Microbiol* ., **43**(8), 4041-4045.