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Microbio-ecology and hydro-geochemistry of saline sulfur springs of Ghale-Madreseh, Khuzestan, Iran

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ABSTRACT: Ghale-madreseh is the first point that the saline and sulfurous streams flow into Tembi River, one of the well-known saline rivers in Khuzestan province, Iran. This river is one of the main sources of increasing Karun River's salinity, which is the largest river in Iran in terms of discharge. There are three saline and sulfurous springs (Shour-1, Shour-2m and Namak Springs) as well as a drinkable one (Shireen spring) in Ghale-madreseh region. Normally, most probable number counting of sulfate reducing, sulfur oxidizing and nitrate reducing bacteria showed that there are different patterns of microbial populations in the springs of Ghale-madreseh region. The observed differences are highly attributed to the hydro-geochemical properties of the springs. It is assumed that the groundwater which streams in the Gachsaran formation receives considerable amounts of SO_4^{2-} (0.09-0.1 M), Na^+ (1.654-3.604 M), and Cl^- (1-548-3.775 M) by halite and gypsum dissolution, resulting in the saline and sulfurous springs on the Gachsaran formation. Also, due to the low depth of local oil reservoirs, activity of sulfate reducing bacteria in the close vicinity of oil reservoirs and groundwater streams is highly probable. Hence, the microbial sulfate reduction may be responsible for the production of H_2S, probably playing a role in the souring of local oil reservoirs. Besides, the groundwater that reaches the Bakhtiary formation shows different characteristics as detected in Shireen spring.

Keywords: Gachsaran formation, Khuzestan, Sulfate reducing bacteria, Tembi River, Thrust-fault.

INTRODUCTION

The Tembi River, one of the most saline rivers in Khuzestan province, Iran, (EC= 8513 ms/cm) is in the class C_4-S_4. Thus, it is known as an unusable and unusual water stream. The Tembi flows into the Karun, the most effluent and the only navigable river of Iran. So, one of the significant causes of the increasing salinity of Karun is Tembi, the salinity of which is mainly controlled by groundwater streams of Gachsaran formation origin as well as the

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gypsum and evaporite based cap rock of Asmari oil and gas reservoir (Bavarsad et al., 2010; Chitsazan et al., 2012; Papizadeh et al., 2013).

Ghale-madreseh (Qaleh-madreseh), an abandoned rural region between Mordeh-fel and Asmari Mountains, is the point at which the saline sulfurous springs flow into the Ghale-madreseh rivulet, one of the main rivulets of Tembi. Ghale-madreseh is situated in the simple-folded zone of Zagros Mountains. The region has sediments with the most important hydrodynamic characteristics. The major geological units outcropping in the study area are Pabdeh-Gurpi, Asmari, Gachsaran, Mishan, Aghajari, Lahbari, and Bakhtiary formations, not to mention alluvial deposits. From an environmental point of view, the most important formation in the study area is the Gachsaran formation with some plastic characteristics. Calcium sulfate minerals such as gypsum and anhydrite are the main constituents of this formation. Dissolution of the evaporites, mainly through joints and fractures increases the total amount of solids, dissolved in local groundwater, resulting in the significant salinity of the water. Gachsaran formation is also well-known as the cap rock of the Asmari reservoir with Pabdedeh formation acting as the bed rock (Papizadeh et al., 2012; Torabi-Kaveh & Miri, 2012; Alizadeh et al., 2007).

Because of the tectonic stresses, the Asmari limestone formation is intensely fractured and hydrocarbons and oil residues can be seen in the fractures, caves, and drilling samples. Gachsaran formation is not very consistent, which causes a significant weathering and dissolution. Considering the basins water quality in Zagros, Khuzestan, Gachsaran formation causes various undesirable hydro-geochemical phenomena like H_2S, natural gas, and oil seepages as well as saline, saline sulfide, or saline sulfur springs that can be seen in the area. These phenomena affect Tembi River, effectively. Gachsaran formation is separated from the Bakhtiari formation by a thrust; the morde-fel thrust fault. The Bakhtiari formation composes the gravel calcareous envelopes, more or less, in a clayey matrix. Also, the quality of the water streams with the Bakhtiari formation origin is suitable for Agriculture and other uses (Papizadeh et al., 2012; Torabi-Kaveh & Miri, 2012; Alizadeh et al., 2007; Aghdam et al., 2012).

The Asmari reservoir is a shallow hydrocarbon body, served by the highly plastic Gachsaran formation as its cap rock. Furthermore, H_2S, natural gas, and oil seepages, along with sulfur springs can be detected in the Ghale-madreseh, where Gachsaran formation can be observed on the surface as gypsum clays (Alizadeh et al., 2007). Thus, Tembi River shows various kinds of pollution, mainly related to the Gachsaran formation origin of the ground waters that stream into this river. However, the amount of H_2S seems alarming in this area, with the probable cause of this increasing rate of H_2S production in low-depth Asmari reservoir, not elucidated exactly. The thermogenic and/or biological H_2S production can be assumed as the possible causes of the Asmari reservoir souring, at least in MIS oilfield, though the biological souring has not been studied in Iran (Papizadeh, 2012; Papizadeh et al., 2013).

The hydro-geochemical and biological parameters are in a close association and any changes in these; e.g. likely fluctuations of ground water hydrochemistry or its penetration in to the Asmari reservoir, can encourage the SRB to grow. Thus, SRB proliferation may lead to the production of huge amounts of H_2S in the reservoir that can result in reservoir souring. That is why many groups of microorganisms such as bacteria, diatoms, and microalgae can be used as environmental bio-indicators (Olliver & Magot, 2005; Ansari, 2013; Saba et al., 2016a, 2016b).

Regarding the recent alarming increases of not only salinity and draught, but also air and water pollution in Khuzestan province, Iran (Papizadeh et al., 2010; Papizadeh & Roayaei Ardakani, 2010; Papizadeh et al., 2011; Papziadeh et al., 2017), local authorities are trying to elucidate the causes and processes of such pollutions. This study first shows that there is a close relation between the concentration of sulfate-NaCl and the geological origin of the studied ground water. Further, it studies the probable correlation between the hydro-geological properties of the springs (in Ghale-madreseh region) and the abundance of SRB, SOB, and NRBs. Also, it is shown that SRB can play a significant role in the production of H_2S in the reservoir or at least in its vicinity and the hydro-geochemical parameters may be related to the microbioecological changes, detected in the springs.

MATERIALS AND METHODS

Ghale-madreseh is located between Mordeh-fel and Asmari Mountains on Gachsaran formation (Fig. 1). In Masjede-Soleiman great oilfield, Gachsaran formation is covered by Mishan formation at the same slope with the former covering the Asmari limestone oil reservoir, stretching from North West to South East. Also, a complex branch of Bakhtiari-Lahbari/Mishan formations has emerged in the location between Mordeh-fel and Asmari Mountains. A saline sulfur spring (Shour-1), two saline sulfide springs (Shour-2 and Namak), and a drinkable spring (Shireen) are detected in this region (Fig. 1) (Papizadeh, 2012; Papizadeh et al., 2013).

The sampling points were selected by studying the geographical data, kindly offered by Khuzestan Water and Power Authority (KWPA). Sampling took place in 2011 and 2012 by documenting the GPS, EC, Eh, and pH. Water and the sediment samples were analyzed (Table 1). Kept in an ice box, all samples were transferred to the laboratory in 6 hours (triplicate samples of a sampling point for each analysis). After nearly 24 hours they were analyzed by Zagros-Abshenas Hydrochemistry Corporation (Papizadeh, 2012; Papizadeh et al., 2013).

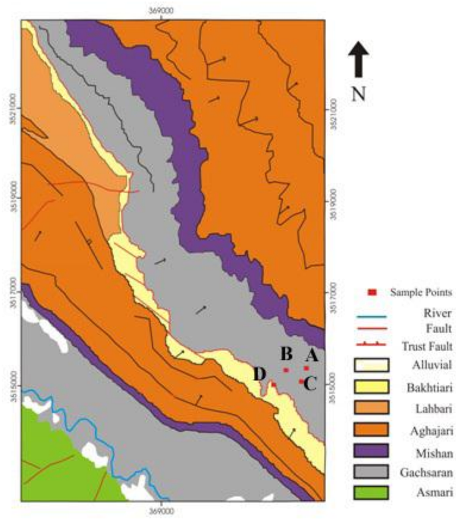

Fig. 1. (Left) Sampling points. A, Shour-1. B, Shour-2. C, Namak and D, Shireen Springs in Ghaleh-madreseh region; (Right) High salt depositions around Namak spring

Table 1. A brief survey of hydro chemical properties of water samples of the springs A: Shour-1, B: Shour-2, C: Namak, and D: Shireen Spring.

Springs	EC (ms/cm)	Na^+ (M)	Cl^- (M)	SO_4^{-2} (mM)	NO_3^- (ppm)	TDS (ppm)	pH	Eh (mv)	TOC (mg/L)	Mg^{2+} (ppm)	Ca^{2+} (ppm)	HCo_3^- (ppm)
A	118540	1.508	1.65	90	2.64	100314	7.22	-17.5	85.6	0.037	0.1	0.0028
B	339830	3.604	3.775	100	1.73	224508	7.10	-33.7	97	0.018	0.107	0.0044
C	462420	3.99	4.45	90	2.35	256828	6.55	-49.3	78.4	0.013	0.112	0.003
D	912	0.0051	0.0043	1.5	17.38	682	7.95	1.2	2.1	0.0007	0.004	0.0041

Anion and cation concentrations of water samples, such as Na^+, K^+, Ca^{+2}, Mg^{+2}, HCO_3^-, Cl^-, NO_3^-, and SO_4^{-2} were analyzed by a Metrohm Ion Chromatography apparatus model Compact IC 761 for anion and cation analysis. Anion and cation columns were: Hamilton prp-x100 (code 6-1005-000) and Metrosep cation 1-2 (code: 6-1010-000), respectively. Standard ion solutions were injected into the apparatus to gain standard curves and the samples were analyzed, based on the standards. Also the water samples were analyzed for anion and cation moiety by flame photometry (Table 1; Ansari, 2013; Ghadimi et al., 2013; Negarestani et al., 2014; Mirhosseini et al., 2015).

To investigate the possible causes behind the differences in elemental composition of the studied springs' water samples, the sediment samples were analyzed too (samples S1-S4, respectively). Binalood- Kansaran Company has analyzed the samples for major and trace elements with an X-Ray Fluorescent (XRF) instrument (Philips-X Unique) (Table 2; Sola et al., 2013; Przylibski & Gorecka, 2014).

Table 2. XRF analysis and mineral composition of the sediment samples of the springs A: Shour-1, B: Shour-2, C: Namak, and D: Shireen

Elements (%)	A	B	C	D
Si	31.03	14.77	28.86	33.64
Al	7.13	2.46	5.06	8.48
Fe	4.91	6.11	5.08	3.66
Ca	16.87	22.31	31.85	29.52
Na	4.55	4.85	5.2	0.13
K	1.92	0.87	1.75	2.31
Mg	2.38	4.81	5.31	1.39
S	0.365	14.25	1.068	0.017
L.O.I	25.93	18.71	22.81	21.23
Cl	1.48	1.89	2.12	0.09
Ti	0.478	0.262	0.5	0.108
Mn	0.055	0.014	0.051	0.016
P	0.191	0.096	0.153	0.256
Elements (ppm)				
As	0.984	3.108	2.316	0
Cu	9	31	26	0
Zn	30	67	60	0
Pb	18	31	26	0
Ni	70	133	122	0
Cr	22	105	84	0
V	37	75	69	0
Zr	96	113	119	0
Rb	35	52	58	0
Sr	459	685	787	43

Hydro-geochemistry information was analyzed and the abundance of SRB, SOB, and NRB in both the water and sediment samples measured, accordingly. N-MPN counting of the aforementioned metabolic groups of prokaryotes helped us explain the probable effects of Gachsaran formation on the abundance of various metabolic groups in the studied springs.

SRB was enumerated via the Most Probable Number (MPN) technique at three different NaCl concentrations (1, 2 and 4 M of) for each sample. This technique used is a conventional MPN rate (normal MPN or N-MPN), in which the presence of sulfate reducing bacteria in MPN tubes is detected by blackening the medium and precipitation of black Iron sulfide. N-MPN enumerations were done in iron-rich synthetic minimal media, supplemented with organic electron donors: i.e. Acetate and Lactate, which are known as the main electron donors for SRB in sediments. The media consisted of (per liter): 3 g $MgSO_4$, 0.4 g $MgCl_2.7H2O$, 0.5 g KCl, 0.7 g NH_4Cl, 0.7 g KH_2PO_4, 0.4 g $CaSO_4$, 0.1 g $MnCl_2.2H_2O$, and 0.3 g yeast extract as well as trace element solution SL-10, itself made of 1.5 g $FeCl_2{\cdot}4H_2O$, 0.2 g $CoCl_2{\cdot}6H_2O$, 0.1 g $MnCl_2{\cdot}4H_2O$, 0.07 g $ZnCl_2$, 0.04 g $Na_2MoO_4{\cdot}2H_2O$, 0.02 g $NiCl_2{\cdot}6H_2O$, 6 mg H_3BO_3, 2 mg $CuCl_2{\cdot}2H_2O$, and 2 ml HCL (25% V/V) 10 ml. The medium was prepared in three different NaCl concentrations, namely 1M, 2M, and 4M, and the media were made anoxic, autoclaved, and dispensed into anoxic glass-made stopper tubes. The following medium constituents were subsequently added from sterile stock solutions to obtain the final enumeration media (final concentrations per liter): 0.5 g of $FeSO_4.7H_2O$ and 1.2 g of $NaHCO_3$. A mixture of sodium salts of acetate and lactate were added as electron donors (3 g/L each) separately. The pH of the media was finally adjusted to 7 by adding 1N NaOH (Papizadeh et al., 2012; Ollivier & Magot, 2005; Vester & Ingvorsen, 1998; Hollibaugh et al., 2006; Fukui et al., 1999; Brandt et al., 2001; Foti et al., 2007; Thamdrup et al., 2000; Petrie et al., 2003; Maturrano et al., 2006).

Moreover, a mineral salt N-MPN medium was prepared, using sampling point water in place of distilled water. Acetate or lactate, as electron donors, as well as sulfate or thiosulfate, as terminal electron acceptors, were added to the sterilized sampling point water and the same procedure as that of N-MPN dilution was carried out.

SOB was enumerated via MPN Technique at different medium salinities for each sample. This technique is an N-MPN procedure, in which the presence of SOB in MPN tubes is evaluated by the turbidity as well as the medium acidification in a selective medium for chemolithotrophic SOB. N-MPN enumerations were performed in synthetic minimal media, which consisted of (per liter): NaCl, 58 g (1M), 116 g (2M), or 232 g (4M), with 3 g of $Na_2S_2O_3$ as the main energy and electron source, 0.6 g of NH_4Cl as the nitrogen source, 0.5 g of KH_2PO_4 as the phosphate source, 0.8g $MgCl_2$, and 0.08 g $FeSO_4.7H_2O$. The media were made, autoclaved, and dispensed into glass-made stopper tubes. The following medium constituents were subsequently added from sterile stock solutions to obtain the final enumeration media (final concentrations per liter): 2.8 g of $NaHCO_3$, as the sole carbon source and alkaline buffer and 0.15 g of Na_2S as the alternative sulfur-based energy and electron source. The pH of the media was finally adjusted to 7 by adding 1N HCL (Sorokin et al.; 2001a; 2001b; 2003; 2006a; 2006b; 2008a; 2008b).

Moreover, a mineral salt N-MPN medium was prepared, using sampling point water instead of distilled water. Thiosulfate as electron donor and $NaHCO_3$ as the sole carbon source were added to the sterilized sampling point of the water samples and the same procedure as N-MPN dilution was performed.

NRB Enumerations were performed by MPN technique at different medium salinities for each sample. This technique was a normal MPN, in which the presence of NRB in MPN tubes was evaluated by NO_2^-, while gas production and NO_3^- disappearance happened in a selective medium for NRB. N-MPN were enumerated in synthetic minimal media, consisted of (per liter): 0.5 g, NaCl 1, 2, or 4M in separate flasks, 3 g KNO_3, 2 g glycerol, 2 g glucose, 1 g $(NH_4)_2SO_4$, 0.9 g $K_2HPO_4 \cdot 3H_2O$, and 0.54 g KH_2PO_4. The media were made anoxic, autoclaved, and dispensed into glass-made stopper tubes with the following medium constituents subsequently added from sterile stock solutions to obtain the final enumeration media: 10 ml of $MgSO_4 \cdot 7H_2O$ 2 g/L solution and 10 ml of trace salt solution, containing $CaCl_2 \cdot 2H_2O$, 1 g $FeSO_4 \cdot 7H_2O$, 0.5 g $MnSO_4 \cdot H_2O$, 0.1 g $CuSO_4 \cdot 5H_2O$, and 0.1 g $Na_2MoO_4 \cdot 2H_2O$ in 100 mL HCl (0.1N solution). Finally, denitrification was evaluated, using a denitrification detection kit (Sorokin et al., 2001a; 2001b;, 2003; Atlas, 2005).

All the N-MPN tubes were incubated for 45 days in triplicate series for each sample and each NaCl concentrations.

RESULTS AND DISCUSSION

To begin with, the hydrochemical analysis of water samples showed that the mineral composition of Shour-1, Shour-2, and Namak springs differ much from that of Shireen spring (Table 1). Also, the main anions, present in Shour-1, Shour-2, and Namak springs included Cl^- and SO_4^{2-}, whereas Cl^- and NO_3^- existed in Shireen spring.

These differences were also confirmed by EC, SAR, and TDS analyses. Considering Table 1, SO_4^{2-} water content of the the studied saline sulfurous springs tended to be sufficient (0.09 to 0.1 M) for microbial dissimilatory sulfate reduction. However, the water of Shireen spring was not rich in minerals with the only detected component in its water, capable of acting as a probable terminal electron acceptor being NO_3^-. Additionally, XRF analyses showed that the sediments of Shour-1, Shour-2, and Namak springs seemed to differ from thoe of Shireen spring (Table 2). Consequently, the differences may be highly associated with the geochemical properties of the formations, through which the ground water streamed (Table 2). Interestingly, it was shown that Shireen spring was situated just on the margin of Morde-fel thrust, separating Bakhtiari and Gachsaran formations.

SRB's most probable number counting showed that their abundance was highly affected by the concentration of sulfate and nitrate as well as the salinity itself (Fig. 2). Considering the results from hydrochemistry analyses, the highest sulfate concentration was detected in Shour-2 spring, which was followed by the highest SRB rates. MPN rates showed that not only was SRB abundance controlled by sulfate and nitrate concentrations, it was also controlled by Eh and salinity.

However, according to the analyses, the most critical factor belonged to sulfate concentration, (Table 1, Figs. 3, 4, and 5), with dissimilatory sulfate reduction by SRB being the major kind of metabolism in Shour-1, Shour-2, and Namak springs. This kind of metabolism, however, was a minor one, active in Shireen spring as MPN rates showed (Fig. 6).

According to our MPN rates, SRB, SOB, and NRB showed different reactions to higher and lower salinities than the detected springs' salinities (Fig. 6). Moreover, there was about 144 ppm of sulfate in the water of Shireen spring with a mean Eh of 1.2, though the mean nitrate concentration was also 17.38 ppm in this spring's water, nearly 10 times more than the highest nitrate concentration recorded in the water samples of the studied saline springs (Fig. 4).

The MPN rates showed that the sulfate reducers were barely active in the water of Shireen spring (Figs. 5 and 6). According to these results, there was a relation between hydrogeochemical properties of the above-mentioned springs and the abundance of SRB in their water. Also, MPN rates in various salinities showed that SRB had high potentials to resist a considerable range of salinities. So, their presence and activity can be predictable in the brines of local oil and gas reservoirs and these activities may play a role in reservoir souring.

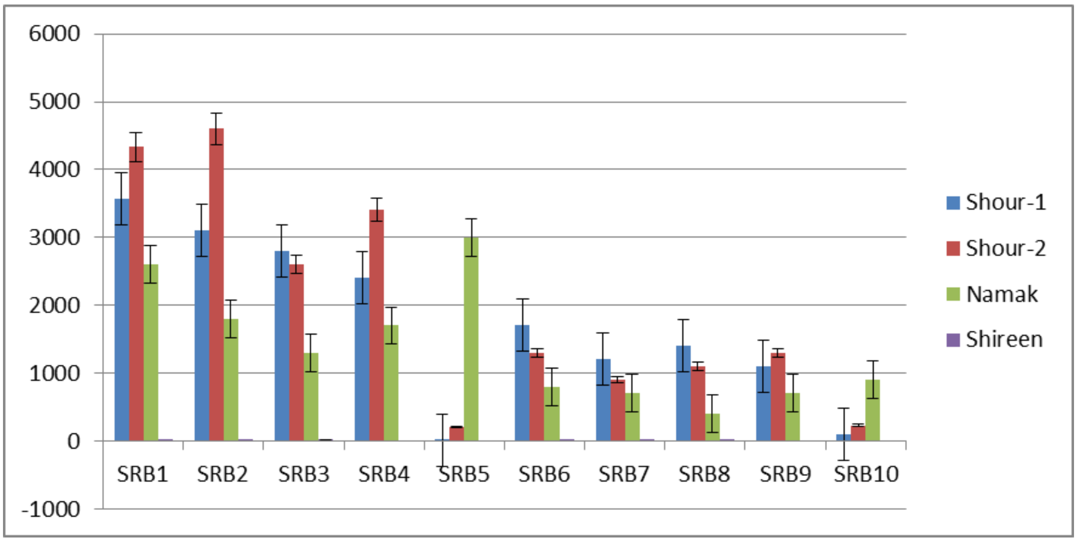

Fig. 2. MPN rates of acetate and lactate oxidizing SRB of water samples in different salinities of MPN test tubes. The media are shown as SRB1 (Acetate+sulfate), SRB2 (Acetate+thiosulfate), SRB3 (Acetate+sulfate+1M NaCl), SRB4 (Acetate+sulfate+ 2M NaCl), SRB5 (Acetate+sulfate+4M NaCl), SRB6 (Lactate+Sulfate), SRB7 (Lactate+thiosulfate), SRB8 (Lactate+sulfate+1M NaCl), SRB9 (Lactate+sulfate+2M NaCl), and SRB10 (Lactate+sulfate+4M NaCl). Error bars indicate 95% confidence limits for MPN rates.

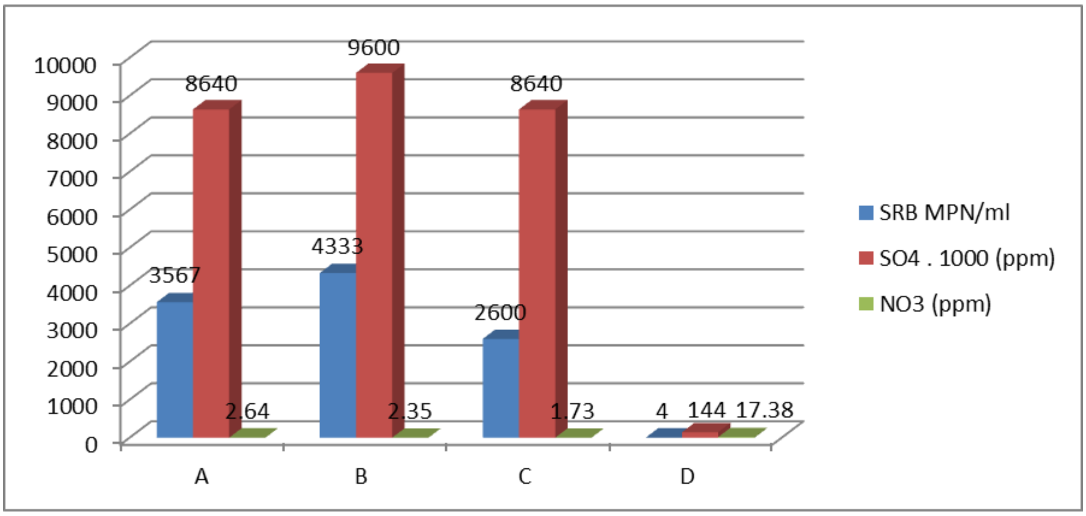

Fig. 3. MPN rates of sulfate reducing bacteria in water samples of the springs A: Shour-1, B: Shour-2, C: Namak, and D: Shireen and its relation to the soluble sulfate and nitrate concentrations

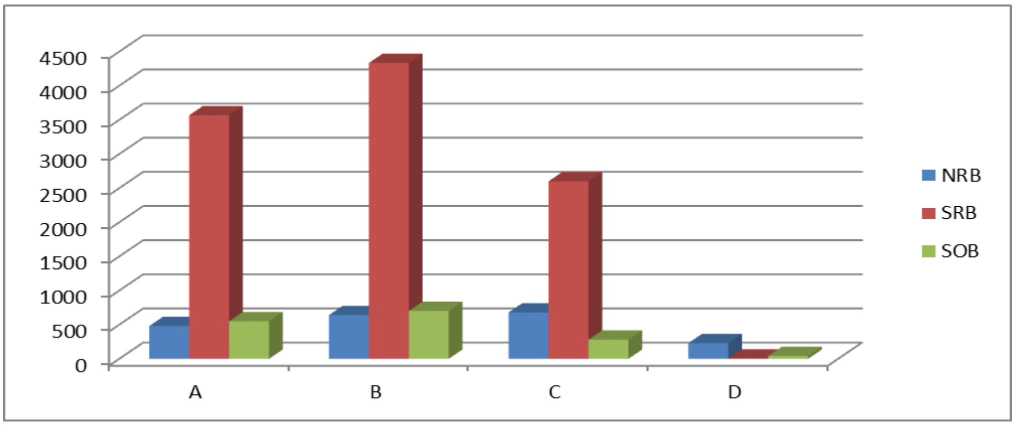

Fig. 4. SRB (Acetate oxidizing), SOB and NRB rates of water samples of the springs A: Shour-1, B: Shour-2, C: Namak, and D: Shireen

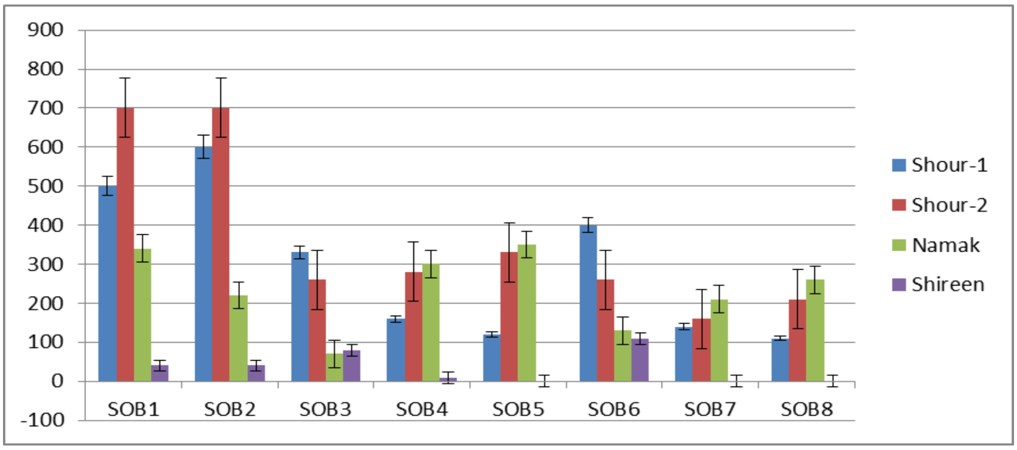

Fig. 5. MPN rates of SOB of water samples in different salinities of MPN test tubes in the presence of thiosulfate or sulfide as the sole electron and energy source. The media are shown as SOB1 (thiosulfate), SOB2 (sulfide), SOB3 (thiosulfate+1M NaCl), SOB4 (thiosulfate+2M NaCl), SOB5 (thiosulfate+4M NaCl), SOB6 (Sulfide+1M NaCl), SOB7 (Sulfide+2M NaCl), and SOB8 (Sulfide+4M NaCl). Error bars indicate 95% confidence limits for MPN rates

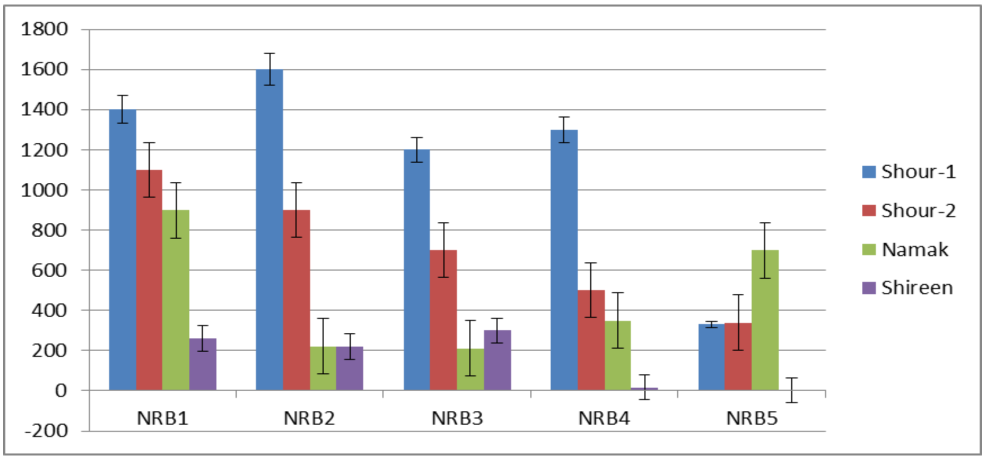

Fig. 6. Measurement of NRB of water samples in different salinities of MPN test tubes. The media are shown as NRB1 (NRB medium made of the given spring water), NRB2 (NRB+0.5M NaCl), NRB3 (NRB+1M NaCl), NRB4 (NRB+2M NaCl), and NRB5 (NRB+4M NaCl). Error bars indicate 95% confidence limits for MPN rates.

The abundance of sulfur oxidizing bacteria followed a pattern, observed in SRB, though the abundance of SOB was not affected by sulfate availability as much as SRB was (Table 1, Fig. 6). Furthermore, their abundance was not very sensitive to salinity as observed in SRB. The highest rates of SOB were recorded in the water samples of Shour-1 and Shour-2 springs.

The oxygen- and nitrate-based oxidative metabolisms may be the major metabolic groups in Shireen spring (Fig. 6). According to MPN rates, Sulfate reducers and sulfur oxidizers were very scarce in this spring. In comparison to the saline springs, nitrate reducers were more abundant in Shireen spring. It is interesting that nitrate reducers were also present in Shour-1, Shour-2, and Namak springs and even their abundance was more than the Shireen spring. Having studied the results of NRB MPN rates for Shour-1, Shour-2, Namak, and Shireen springs, we found out that the abundance of NRB was not highly affected by sulfate availability, though it was affected by the presence of nitrate and salinity (Table 1, Fig. 6). Dissimilatory nitrate reduction was shown as the dominant form of anaerobic respiration, occurring in Shireen spring. Also, our results showed that the nitrate reducers were the dominant group in the Shireen spring (of course, among the studied microbial groups) and could be assumed as the most tolerant population in case of exposure to higher salinity.

The high concentration of sulfide inside the waters of Shour-2 and Namak springs was a critical factor, controlling hydro-geochemistry of the springs. Because, together with high TDS, high sulfide concentration can reduce the oxygen solubility and Eh, likely to result in a microbioecological deviation in advantage of some metabolic groups, capable of anaerobic respiration and fermentation.

Additionally, sulfate and Iron were the most available electron acceptors in the water and sediment samples of the Shour-1, Shour-2 and Namak springs, which could support specific metabolic pathways, based on sulfate and Iron reduction. Further, considering the high sulfate concentration, the high level of H_2S gas, bobbled from Shour-2 and Namak springs, and the salinity of Shour-1, Shour-2, and Namak springs, it can be assumed that the sour gases from Asmari oil reservoirs spilled through the fractures of Gachsaran and Asmari formations. Such spills are the reason behind contamination of local groundwater streams with petro-based hydrocarbons. These phenomena are concomitant with a high sulfate availability and a low Eh, which can easily enrich sulfate reducing bacteria in the vicinity of the ground water. The above-mentioned phenomenon could contribute to specific microbioecological characteristics of Shour-1, Shour-2, and Namak springs. Such a characteristic was seen only in the springs Shour-1, Shour-2 and Namak in the Ghale-madreseh region, located on Gachsaran formation. The hydro geochemical difference of Shireen spring from that of Shour-1, Shour-2, and Namak springs was proven to cause microbioecological differences.

Also sediment geochemistry analysis showed that higher concentration of NaCl in the water increased the metal moiety of the sediments. Also it is very interesting that higher concentrations of specific heavy metals and elements like S, Fe, As, Ni, V, Pb, Zn, Cu, Fe, and Cr in the clays were recorded at the saline sulfide spring Shour-2, where that the highest rates of sulfate reducing and sulfur oxidizing bacteria were measured.

MPN rates showed that the acetate oxidizing sulfate reducers were dominant among lactate oxidizing sulfate reducers. According to Figure 1, the highest record of sulfate reducers can be seen in Shour-2 spring. The sulfate reducing population was the main metabolic group, active in the water of Shour-1, Shour-2, and Namak springs. Also the sulfur oxidizing population has a significant proportion in saline springs, which can be related to the close and complementary correlation of the metabolism of sulfate reducing and sulfur oxidizing populations. Although the sulfur cycling populations were the dominant active metabolic groups in the saline springs, the nitrate reducing bacteria were also active in these springs. There was no significant density difference of nitrate reducing bacteria in the water of the saline springs, though the mean density was higher than that of Shireen spring. The higher density of NRB in saline springs, in comparison to Shireen spring, can be related to the lower nutrient and organic carbon composition of the Shireen spring.

Considering the MPN rates of SRB, SOB, and NRB, it was indicated that the sulfate reducing population was highly affected by higher nitrate availability and lower sulfate concentration in the Shireen spring. These parameters were restricted by the rate of sulfate reducers (Ollivier & Magot, 2005; Grigoryan et al., 2008; Hubert et al., 2007; Greene et al., 2006; Kleikemper et al., 2002).

Also, it was clear that the enzymatic system for the dissimilatory sulfate reduction was very sensitive, even to smallest traces of nitrate. So, even very low concentrations of nitrate affect the rate of sulfate reducers (Pe´Rez-Jime & Kerkhof et al., 2005; Allen et al., 2007; Papizadeh et al., 2013). Furthermore, sulfate reducers were among strictly-anaerobic microorganisms, with their desired microenvironment being beneath the multi-layers of biofilms, developing on particles, clays, and rocks in the sediments and usually somewhere far from oxygen penetration. Thus, they were very sensitive to environmental changes, while being released into the water stream from the developing biofilms (Ollivier & Magot, 2005; Elshahed et al., 2003; Gieg et al., 2011).

Considering the metabolic and physiological demands of sulfate reducers, the best microenvironment for sulfate reducers can be found not in shallow sediments of the saline springs of Ghale-madreseh, but deeper in the clays, through which the ground water stream the gypsum and anhydrite rich clays of Gachsaran formation. Such a place may be found in close vicinity to Asmari gas and oil reservoir, capable of supplying the sulfate reducers with organic matter (Papizadeh et al., 2012; Ollivier & Magot, 2005; Kleikemper et al., 2002; Kniemeyer et al., 2003; Hulecki et al., 2009; Bødtker et al., 2008; Azadpour et al., 1996; Orphan et al., 2000).

Asmari reservoir and indeed great MIS oilfield is an old and- to some extent-abandoned shallow reservoir, whichh at the moment shows a high H_2S discharge and soured products (Alizadeh et al., 2007). The H_2S gas, sensed around Shour-2 and Namak springs in Ghale-madreseh region as well as Garo springs in Golgir region, may be caused by the souring, itself likely to be a result of the reservoir that the microbial sulfate reduction at this level. It is very probable that SRB activity by itself or together with thermal phenomena caused the souring of the reservoir. Although Alizadeh et al. (2007) declared that there was no sign of microbial souring due to the high temperature of Asmari and Bangestan reservoirs up to nearly 80 °C and their chromatography analysis of the oil samples showed no sign of the microbial souring, the present study showed a probable role of SRB for so doing.

Moreover, there are reports of sulfate reducers, capable of growth at such a high temperature (Ollivier & Magot, 2005; Azadpour et al., 1996; Orphan et al., 2000; Gittel et al., 2009; Struchtemeyer et al., 2011; Al-Raei et al., 2009; Korenblum et al., 2010). Also, this microbial phenomenon seems very probable due to expected low depth and low temperature of Asmari reservoir in the great MIS oilfield (Azadpour et al., 1996; Orphan et al., 2000;

Papizadeh, 2012). It was indicated that was a very diverse microbial community with highly flexible metabolic properties, living subsurface and affecting the oil reservoirs characteristics. Such a microbial life was analyzed mainly by studying the production water. These investigations were used to monitor the reservoir production and for MEOR in some oilfields all around the world and it could be tested on the great MIS oilfield (Ollivier & Magot, 2005; Kumaraswamy et al., 2011).

Regarding the results, gained from XRF analysis of sediments and hydrochemical examinations, there was a close relation between water salinity and sulfate concentration in the water along with the metal moiety of the sediments. Shour-2 spring in Ghale-madreseh was the point, at which such a close relation was observable between hydro-geochemical and Microbiological data. Although, we did not examine the Asmari gas and oil reservoir in great MIS oilfield for SRB presence and activity, our results further confirmed the theory of the presence of SRB activity subsurface in the vicinity of the Asmari reservoir in great MIS oilfield and ground water streams of ghale-madreseh region, likely to cause the local H_2S seepages that are very common in the great MIS oilfield. Such biogeochemical phenomena made people evacuate their houses (Alizadeh et al., 2007).

In place, sulfur oxidizing population was not affected by sulfate, nitrate, and NaCl concentrations as much as sulfate reducing population was. Indeed it can be related to nitrate dependence of some groups of sulfur oxidizing bacteria and the ability to change metabolic pathways. Although sulfate reducers are chemoorganotrophic and/or chemolithotrophic bacteria and their metabolism is not very confined, the metabolism of sulfur oxidizing bacteria can be assumed more flexible. Some sulfur oxidizers are facultative nitrate reducers and some of them can grow with oxidative chemoorganotrophic metabolism if chemolithoauto-trophic growth is not desired. Therefore, the growth of sulfur oxidizers was not influenced by the presence of nitrate.

Nitrate reducing population was not affected by sulfate, nitrate, and NaCl concentrations; however, the rate of nitrate reducers was decreased in Shireen spring, which can be related to its lower nutrient composition. Considering the fact that most nitrate reducers are chemoorganotrophic, their growth production was highly restricted with low nutrient and organic carbon composition of Shireen spring, originating from the sand-based Bakhtiary formation. According to MPN rates of sulfate reducing and nitrate reducing bacteria, nitrate reduction was not affected by higher concentrations of sulfate and lower concentrations of nitrate. This is due to the fact that most of the nitrate reducing bacteria are chemoorganotrophic facultative aerobic/fermentative bacteria whose ability to reduce nitrate is an alternative to metabolism and can be triggered when NRB experiences a very limited oxygen concentration at the presence of available nitrate. These facultative aerobic bacteria have a very flexible metabolic system that can flip to various metabolic pathways, depending on the microenvironment they live in. So, in the limiting presence of oxygen and nitrate they can grow through fermentation or alike and such metabolic flexibility is one of their advantages to grow in various hydro-geochemical conditions, being the reason behind the fact that the rate of NRB was not very sensitive to highly different hydro-chemical properties of Ghale-madreseh springs.

CONCLUSION

The results of the present study show that the studied metabolic groups of microorganisms have different reactions to environmental changes, caused by Morde-fel thrust-fault. It was indicated that the dominance of the studied metabolic groups in the spring's water was highly controlled by the concentration of anions that can be used as terminal electron acceptor; such a control was caused by geochemistry of the springs and the formations, through which the groundwater streams. Sulfate reducing group was the dominant group of the saline springs and these water streams are richer in minerals. MPN rates indicated that the SRB population highly depended on the sulfate moiety of the water; however, both nitrate and salinity had influenced the SRB population, as well. Sulfate reducing group was shown as the major microbial group of saline springs along with the minor microbial group of Shireen spring. What is more, Sulfate reducing group of all the saline springs resisted 4M NaCl. The abundance of sulfur oxidizing group followed the same pattern as sulfate reducing group, yet their abundance was not as sensitive as SRB. In comparison, it was shown that abundance of NRB was not highly affected by sulfate availability, but it was influenced by the presence of nitrate and salinity. Nitrate reduction was indicated as the dominant form of anaerobic respiration in Shireen spring.

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