Exploration of hidden Pb-Zn deposit through geomicrobiological studies at Irankuh area, Iran

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Abstract: Different methods have been developed for mineral exploration so far, amongst which biological-based methods known as geomicrobiological studies are of the most recent ones. Geomicrobiology as an interdisciplinary approach has achieved great progresses during the past two decades and involves the study of microbes in a number of fundamental geological processes, both in the past and present. The primary purpose of this study was to survey the possible relationship between soil bacterial populations with mineral deposit occurrence underground in order to find biogeochemical anomalies in mineral exploration. Irankuh Pb-Zn mining area located in central Iran was selected as the sampling site in order to evaluate the efficiency of geomicrobiology for exploration of blind deposits. Totally 32 soil samples were collected from 10-30 cm depth and then they are characterized by enumeration of total bacteria and microbial respiration, pH and electrical conductivity (EC) measurements. Furthermore, the isolated bacteria were identified using morphological characteristic tests and 16S rRNA technique. The results showed that while there was no significant relationship between most of soil characteristics (e.g. total bacterial count, pH, EC) and the hidden Pb-Zn mineralization, a class of bacterial colonies (identified as *Arthrobacter agilis*) was corresponded to the position of mineralization in the depth. Therefore, an effective biogeochemical exploration technique may be developed through targeting the concentration of *Arthrobacter agilis* counts in exploration of Pb-Zn mineralization for further detailed exploration activities.

Keywords: Blind mineralization; Geomicrobiology; *Arthrobacter agilis*; Irankuh.

1- Introduction

Mineral exploration is increasingly extending in areas covered by in-situ or transported overburden. However, due to the suppression by regolith materials, exploration in such areas is extremely difficult (Hallberg and Thompson, 1985; Smith, 1996; Mokhtari *et al*., 2009; Cohen *et al*., 2010). Two problems for geochemical exploration arise in areas of transported regolith. Transported regolith may create "false positive anomaly" due to concentration of elements not related to underling mineralization or may lead to "false negative anomaly" due to its prevention from migration of elements upward. Further complexities can be added to the situation where different transportation regimes are introduced into the area (Mokhtari, 2007).

Different geochemical techniques have been tested over known mineralized zones in order to evaluate different sampling media and analytical techniques in exploration of buried deposit. Revised geochemical methods span techniques from the collection of ions by induced electrical force, direct soil analysis (Anand *et al*., 2005), soil gas geochemistry (Highsmith, 2004; Sutherland, 2010), biogeochemistry (Hill and

Hill, 2003; Colin, 2007; Reid and Hill, 2010, 2013), hydrogeochemistry and application of partial and selective geochemical extraction techniques on regolith samples (Talapatra *et al*., 1986; Smith *et al*., 1993; Alekseev *et al*., 1996; Simonetti *et al*., 1996; Gray *et al*., 1999, Cohen *et al*., 2010).

Amongst developed methods in exploration of blind mineralization is microbial signature detection of such mineralization. Microbial methods of prospecting have a number of possibilities and advantages that can complement standard geochemical methods by relying on detecting the presence of indicator biological components. The idea of using microbes to explore natural resources dates back to the early studies focused on developing methods for oil and gas exploration (Sokolov, 1933; Mogilevsky, 1940). Following a significant development of petroleum exploration by geomicrobiological techniques, several isolated attempts were carried out to investigate indigenous microbial population associated with sulfide ore deposits (Brodskii, 1964; Salvanina, 1957), molybdenum (Kramarenko and Prizrenova 1961) and antimony (Lyalikova, 1974). Previous studies have shown that some microorganisms may also indicate the presence of buried deposits (e.g., Parduhn *et al*., 1985; Parduhn, 1991), because the composition of the microflora in natural and polluted environments is strongly influenced by the concentration of heavy metals and their bioavailability (Hassen *et al*., 1998). The use of the soil bacterium *Bacillus cereus* as an indicator organism for Au and other metals has been explored in studies at different terrains in Belgium, China, Argentina and Mexico (Parduhn., 1991; Neybergh *et al*., 1991; Melchior *et al*., 1994, 1996; Wang *et al*., 1999). Reith *et al* (2005) were used *Bacillus cereus* as an indicator organism for Au in Tomakin Park Gold Mine in southeastern New South Wales, Australia.

For soil microorganisms to be useful in prospecting for mineral deposits, two conditions must be met: (1) soil conditions in the vicinity of a mineral deposit must be chemically or physically different from adjacent soils, and (2) the make-up of the soil microorganisms must reflect these differences in a way that can be tested.

Amongst the techniques employed for detection of blind mineralization, geomicrobiological studies are younger and less practiced. In addition, as Iranian geological landscape is tectonically young and large areas display exposed rocks, most of the exploration activities are frequently directed towards the areas with exposed rock outcrops over these regions. However, there are considerable areas covered by in-situ or transported regolith over which no attention has been paid, especially with considering the recent advances in exploration of concealed mineralization.

Therefore, the aim of this research is to evaluate the efficiency of microbiological studies in exploration of blind Pb-Zn deposit covered by thick dolomite which is overlain by colluvium/alluvium cover. It is also intended to test geomicrobiology technique in a semi-arid environment in Iran where it more likely will be evolved for exploration of hidden mineralized zones.

2- Materials and methods

In order to test the hypothesis of the research, Pitchi (named after small scale pit in Persian language) Pb-Zn deposit is selected for sampling and analysis. The deposit is situated in Irankuh Mountain in Isfahan province, central Iran. The history of exploration in this region dates back to nearly 70 years ago. Lead and zinc in this area has been mineralized mainly inside dolomite, Mississippi Valley Type (MVT) (Ghazban *et al*., 1994; Ghaed Rahmati and Fathianpour, 2008), and have exposed in some part of the region but a significant portion is

covered by dolomite unit or colluvium / alluvium.

2.1- Description of the study area

The study area is located 25 km southwest of Isfahan city, central Iran, on the northern flank of the Irankuh Mountain and adjacent to flood plain (Fig. 1). The area is part of the Sanandaj–Sirjan tectonic zone which runs parallel to the Zagros main fault (Rastad, 1981; Teimoryacl *et al*., 2010).

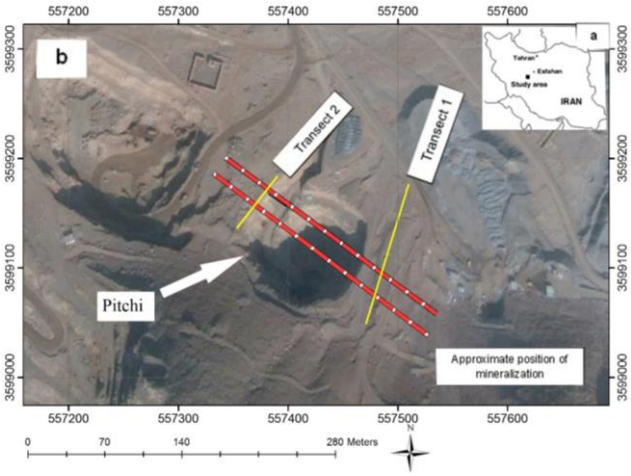

Figure 1a) Position of the study area in Iran and, b) position of Pitchi mine, sampling traverses and boundary of mineralization (Google Earth image).

The Jurassic shales (Shemshak formation) form the lowest stratigraphic unit and are overlain by a cretaceous sequence of dolostone and fossiliferous calcareous rocks (Ghazban *et al*., 1994; Ghaed Rahmati and Fathianpour, 2008). The dolostones host Mississippi Valley Type (MVT) Pb-Zn mineralization (sulfides and carbonates). A number of Pb and Zn deposits occur along ridges in the Irankuh Mountain with a strong spatial association between the degree of structural deformation, the extent of dolomitization and base metal mineralisation. Open cut mining of the Zn and Pb ores at different parts such as Gooshfil, Tapeh Sorkh and Kolahdarvazeh has occurred over the last 50 years. Extensive mine plant, tailings and waste rock areas are located on the southern flank of the range and are immediately adjacent to agricultural areas.

The region is arid, with 130 mm average annual rainfall. The soils are generally calcic, weakly alkaline (pH~7.8), of low electrical conductivity and low organic content (Solhi *et al*., 2005). Solhi *et al*. (2005) have described the soil as fine, loamy, mixed, typic, Torrifluent.

2.2- Sampling procedures

Chemical analyses on core and powder drillings samples were used in order to delineate the boundary and depth of Pitchi mineralization. Coring was done by BAMA company.The area was recently excavated for open cut mining and recently an access tunnel was dug to develop mining underground. For this research, two transects were selected after site investigation where it was assumed mining or contamination had no effect on results. The position of transects and mineralization boundaries are depicted in Fig. 1. The first and second transect were elongated north-easterly with 125 m and 60 m long, respectively, both perpendicular to the mineralization trend. On the base of drilling data mineralization is occurred at depth about 80 m under Transect 1 (T1) and 30 m below the surface under Transect 2 (T2). Cross sections corresponding to T1 and 2 are displayed in Fig. 2.

Sampling was carried out by collecting approximately 500 g of soil at 10-30 cm depth from regolith along each transect in every sampling site. Sampling distance was almost every 5 m over T1 and every 10 m over T2, resulting 25 and 7 samples from T1 and T2, respectively. Sampling positions are marked by measuring tape; however, where there was a possibility for contamination, they were repositioned accordingly. All samples were sieved to <2 mm size fraction for microbiological analysis at the sampling sites, using flame sterilized sieves (Reith *et al*., 2005). Samples were stored in sterile plastic bags, and were transferred to the laboratory and stored at 4 ^{o}C in refrigerator.

2.3- Chemical and biological analyses of soil samples

Soil pH and EC were measured in 1:2.5 water: soil ratio with a glass electrode and conduct meter device respectively (Thomas, 1996). Soil respiration as an index of soil microbial activity was measured without adding substrates in order to show a measurement of active populations in their entirety (Bloem *et al*, 2005). For this purpose, a plastic pots each one containing 20 g dried soil sample was put into the incubation vessel containing 20 mL NaOH 0.025 M. The vessel was equipped with a special lead to avoid CO_2 adsorption from ambient air flowing into the flask. The same conditions were considered for the blanks which had only solutions (without soil samples). After a week, the vessels were removed and the sodium hydroxide solution was titrated immediately. Before that, 1 mL of $BaCl_2$ 0.5 M was added to each sample for precipitation of CO_3^{-2}. Then 4 drops of phenolphthalein solution, as an indicator, were added and finally the solution was titrated with HCl 0.025 M until decoloration of solution. Consequently, according to the consumed volume of HCl, the amount of CO_2 released due to microbial activity was calculated.

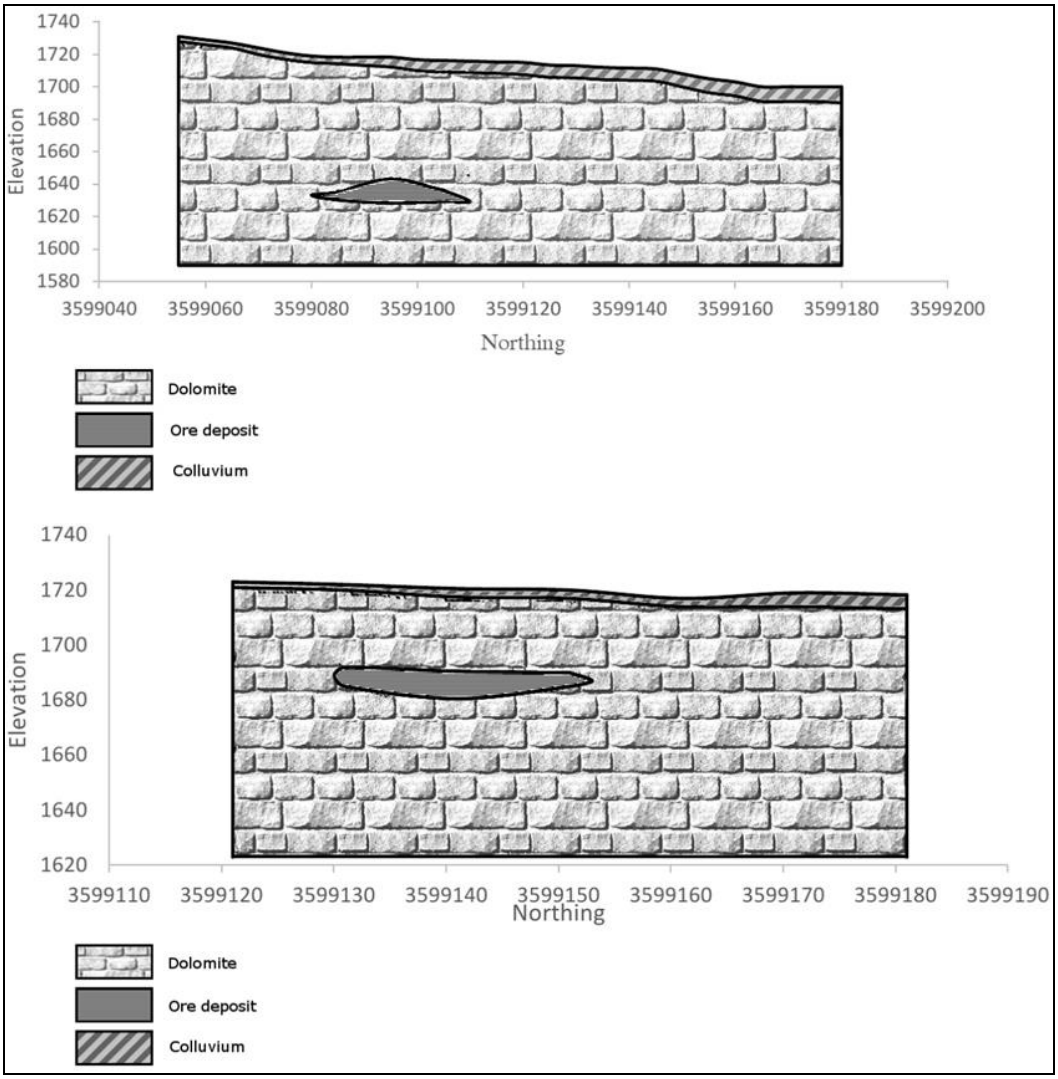

Figure 2) Cross sections of mineralization at positions Transect 1 and 2.

In addition, the number of soil bacterial population was counted using plate count technique (Bloem *et al*, 2005). Briefly, 1 g of each soil sample was diluted in 9 mL sterilized water and diluted 6 folds (serial dilution method). Subsequently, a 100 µL aliquot of

each dilution was spread on nutrient agar plates. Then the samples were incubated for 24-48 h at 30 ºC. Finally, the total bacterial count was reported as colony-forming units (CFUs).

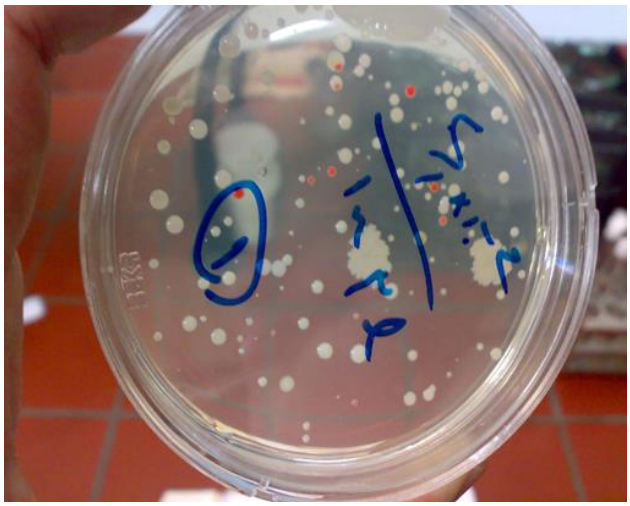

Figure 3) Cultured bacteria on nutrient agar. The red color colony grown amongst other colonies is highlighted by its distinct color.

All analyses were done in 3 replicates considering that the relative deviation was <10% (Ellis *et al*. 2003). It should be mentioned that after culturing colonies, a group of colonies were appeared in red color which was clearly notable (Fig. 3). So, it was decided to count CFU of this type of bacteria as well.

2.4- Characterization and identification of bacteria

In order to determine the bacterium type of red color colony that was displaying correlation with Pb-Zn mineralization, it was purified and isolated on nutrient agar. A sample was sent to Persian Type Culture Collection (PTCC). Analyses included macroscopic and microscopic features identification completed in Persian Type Culture Collection (PTCC) and DNA extraction carried out in Qiagen Inc. Physiological and biochemical characteristics of isolated bacterium are presented in Table 1.

To determine 16S rRNA gene sequence, the genomic DNA were extracted from bacterial single colonies using DNeasy Blood & Tissue Kit. Polymerase chain reaction (PCR) amplification was performed using 2 primers including 27f: 5'-GAGTTTGATCCTGGCTCAG -3' and 1541r: 5'- AAGGAGGTGATCCAGCCGCA -3'. The PCR program was run in 35 cycles with the following conditions: initial denaturation step at 96 ºC for 3 min, denaturation step at 93 ºC for 45 min, annealing step at 58 ºC for 60 min, and extension step at 72 ºC for 90 min.

Table 1) Physiological and biochemical characteristics of isolated bacterium having red color colony.

	Test /Characteristic	Results		Test /Characteristic	Results
1	Oxidase	-	11	Growth at 37˚C	-
2	Catalase	+	12	Growth at 7.5% NaCl	-
3	OF	Oxidative	13	Growth on Simmons citrate	-
4	Nitrate reduction to nitrite	-	14	Motility	-
5	Aerobic acid from Glucose	-	15	Lipase (Tween 80)	-
6	Esculin Hydrolysis	-	16	Gelatin hydrolysis	-
7	Arginine dihydrolase	+	17	Voges-Proskauer (Acetoin)	-
8	Urease	-	18	Methyl Red	-
9	Indole	-	18	Beta- Galactosidase (ONPG)	+
10	Growth at 20˚C	+	19	Hemolysis	-

DNA concentration in this step was 77.5 ng µL-1 (λ=260 nm).The PCR products were purified using Qiaquick PCR Purification Kit. Subsequently the electrophorese was conducted

which confirmed the 1500bp sequences (Band 12671) (Fig. 4). Purified products of PCR was sequenced in both side (ddNTP fluorescence method) using ABI 3730XL DNA Analyze device considering the following primers:

27f : 5'- GAGTTTGATCCTGGCTCAG -3'

16r339: 5'-

CTGCTGCCTCCCGTAGGAG -3'

16f358: 5'-

TCCTACGGGAGGCAGCAG-3'

704f: 5'- GTAGCGGTGAAATGCGTAGA-3'

The 16S rRNA gene sequence was compared with the information of GenBank, RDP and Eztaxon. Furthermore, the physiological and biochemical characteristics of isolated bacteria including oxidase, catalase, oxidation-fermentation (OF), urease, lipase, beta-Galactosidase, indole, motility, color, and growth at 20 ^{o}C, 37 ^{o}C and 7.5% NaCl were measured.

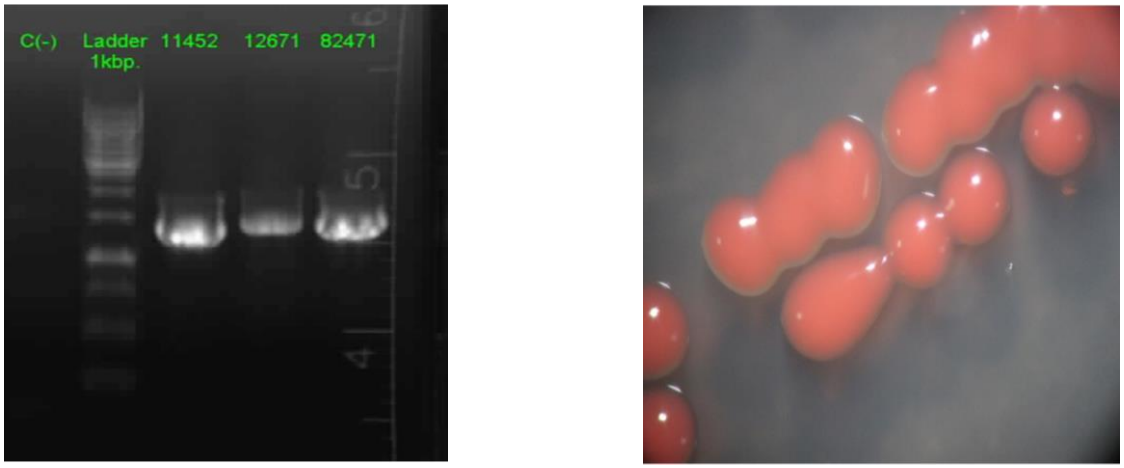

Figure 4) 16S rRNA-PCR amplification of isolated bacterium (left) of purified red colony (right).

3. Results and discussion

3.1- Soil characteristics

The pH values demonstrate alkaline condition ranging from 7.9 to 8.8 and EC varying from 146 to 171 µS cm^{-1}. In order to investigate their lateral variations and whether presence of mineralization has led to noticeable changes in these two variables, pH and EC profiles over both transects are shown in Fig. 5. As it can be seen no specific variations can be related to the position of mineralization underneath.

3.2- Microbiological characteristics

The determination of respiration curves provides information on the microbial biomass in soil and its activity. Basal respiration gives information on the actual state of microbial activity in the soil. The results of the respiration tests did not show significant variations in both transects (Fig. 5). Since the respiration rate has direct relationship with the total activity of bacteria in soil samples, so using this parameter as an indicator to identify areas susceptible to mineral deposit is inefficient (Özkanca and Flint 1997; Popelářová *et al*., 2008).

According to the results obtained from enumeration of bacterial population, anomalies area could not be determined at first and second transects with the total number of bacteria (Fig. 5) which could be due to the influence of various soil characteristics. As it was stated above, plate count technique revealed various colonies, which one of them had a red color. In order to evaluate the variations of this type of bacterium, CFU at every site was counted for it and its fluctuations were mapped (Fig. 5). As it can be seen, populations of red colony significantly corresponds to the position of mineralization under the colluvium and dolomite cover. The comparison of 16S rRNA

gene sequence of selected bacterial colonies to the sequence of GenBank, RDP and Extazon demonstrated that the sequence have 99.61% similarity to *Arthrobacter agilis* (T); DSM 20550 as accessions X80748 in NCBI. It seems that *Arthrobacter agilis* has a high potential to be used as an indicator of hidden Pb-Zn mineralization; however, more researches are needed to prove this potential, especially in various field conditions. There is not any Microbial study at this area.

The correlation coefficient between the number of Red colonies, PH and EC are shown in tables 2 and 3.

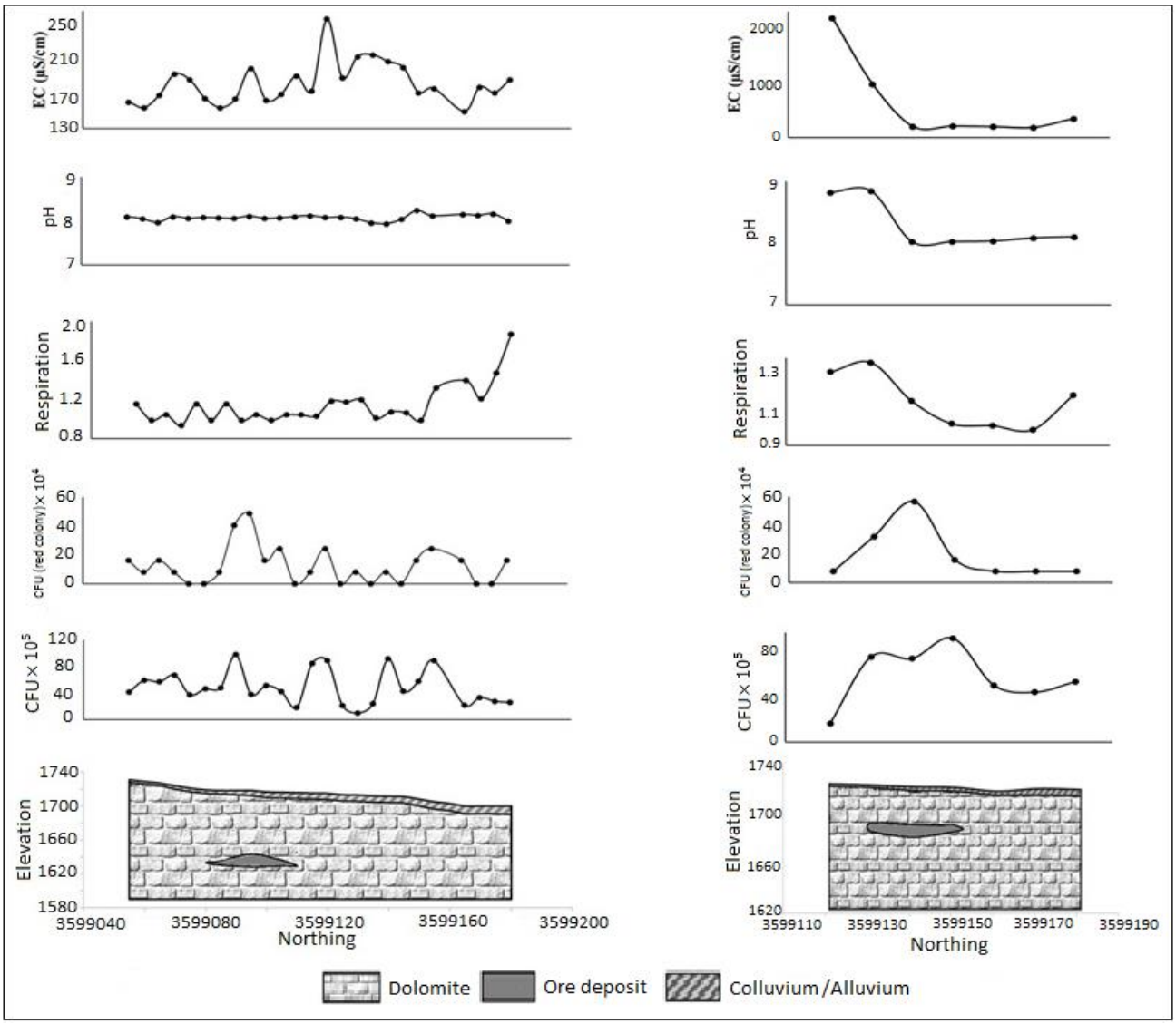

Figure 5) pH, EC, respiration and number of total and red colony variations over T1 (left) and T2 (right) transacts, Pitchi mineralization. Schematic cross sections of mineralization at T1 and T2 are shown in the bottom.

Table 2) Correlation between parameters in transect 1

Correlation coefficient (Transect 1)			
	Red colony	**PH**	**EC (µs)**
Red colony	1	0.09	-0.20
PH	0.09	1	-0.57
EC (µs)	-0.20	-0.57	1

Table 3) Correlation between parameters in transect 2

Correlation coefficient (Transect 2)			
	Red colony	**PH**	**EC (µs)**
Red colony	1	-0.35	-0.62
PH	-0.35	1	0.88
EC (µs)	-0.62	0.88	1

4. Conclusion

Since there was a considerable relationship between the presence of Arthrobacter agilis and buried Pb-Zn mineralization, this bacterial strain may be considered as a potential indicator of Pb and Zn deposits. Therefore, an effective biogeochemical exploration technique may be developed, where Arthrobacter agilis counts are measured in the field and used as a pre-screening method to target areas useful for further sampling and complete geochemical analyses.

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