A child-centric microbiology education framework

Microbes that eat rock

Uncle: why is the river Río Tinto in the south of Spain red and without fish?

Río Tinto

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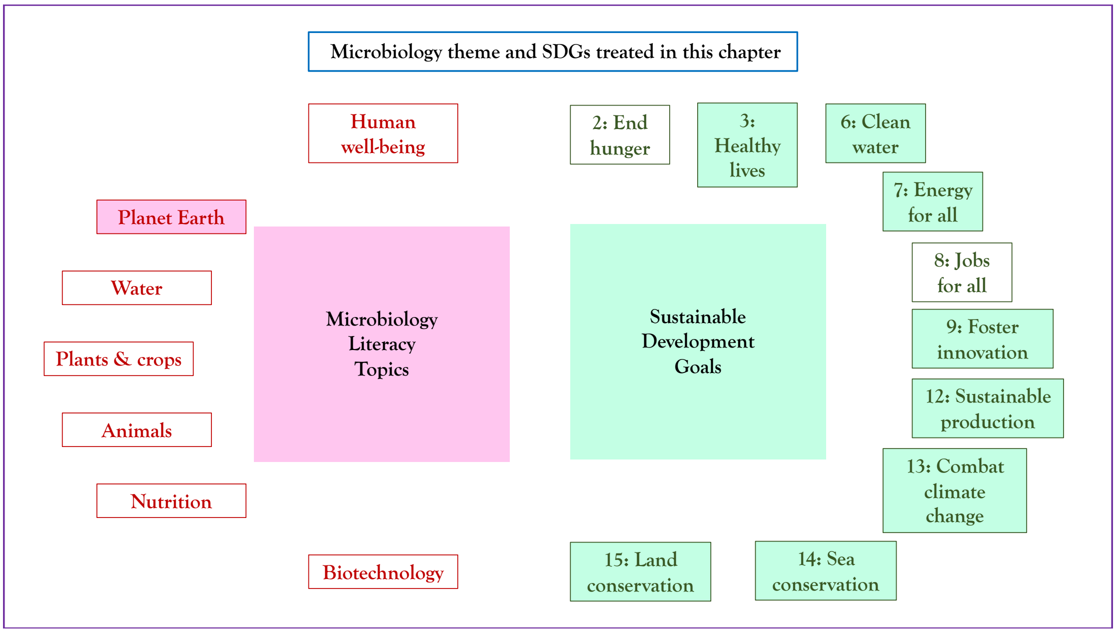
Storyline

The Río Tinto is a very special river that is as red as red wine and runs through a beautiful landscape (see recommended videos below) which is an exciting place to visit and take samples for microbiological experiments. The reason it is red is that it has a high concentration of oxidized iron in its waters (ferric iron), which is soluble due to the acidity resulting from the presence of sulfuric acid. Both the oxidized iron and the sulfuric acid are the metabolic products of microorganisms that are able to obtain energy from reduced energy-rich minerals like pyrite. The extreme conditions of acidity and high content of toxic heavy metals existing in the Río Tinto does not allow higher forms of life like fish to live in its waters. For many years we thought that the extreme conditions existing in the Río Tinto were the product of environmental contamination – Acid Mine Drainage (AMD) – created by the mining activities carried out in the area over the last 5000 years. Today we know that even though mining is a contaminating activity, Río Tinto and other acidic waters are the product of microbial activities of special microorganisms which use reduced minerals instead of carbohydrates or light to obtain the energy required for living. *They are real rock-eaters.* This topic is not only of fundamental interest, but also has important applications such as: biomining, that is the use of microbes as “microminers” to obtain metals (copper, nickel, cobalt, even gold); bioremediation, that is the use of microorganisms to eliminate toxic metals and other compounds from the environment; or to help us in searching for life in other planets such as Mars, where similar microorganisms could survive in similar conditions like those existing in Río Tinto.

The Microbiology and Societal Context

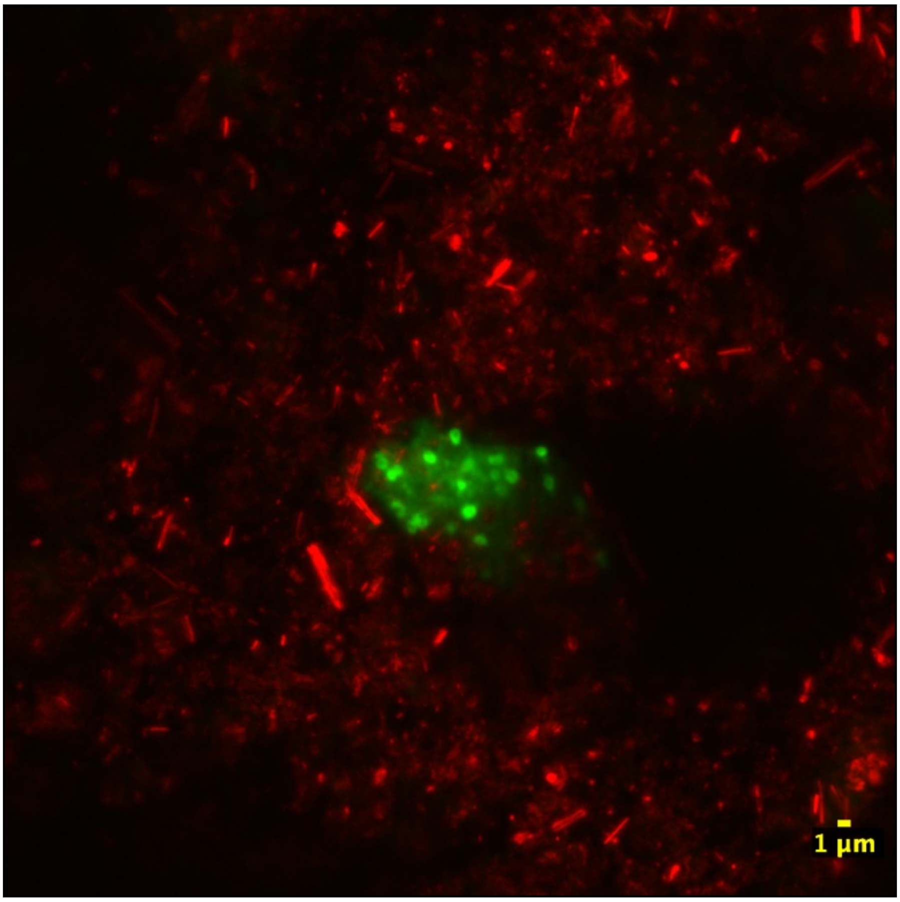


The microbiology: extreme habitats; extraction of energy from rocks; growth on CO_2; oxidation of ferrous iron to ferric iron and production of sulfuric acid; acid mine drainage; biomining; bioremediation; astrobiology. *Sustainability issues:* health; clean water; energy conservation; innovation; pollution and bioremediation; climate change; protection of land and aquatic systems from heavy metal pollution.

Microbes that eat rock: the Microbiology

1. ***Why some microorganisms obtain energy from reduced minerals instead of reduced carbohydrates like most living organisms.*** The answer requires to clarify how microorganisms generate useful cellular energy. Many microorganisms transform the chemical energy stored in reduced substrates like carbohydrates into electric power, using cellular metabolism, channeling the current to an electron sink (e.g. O_2) and storing it as an electric gradient (proton gradient) similar to that of an electric battery. With this energy, several cellular machines and metabolic processes are powered that require energy to mediate key cellular functions, such as uptake of nutrients, cellular movement to search for better environments, synthesis of ATP (the intracellular energy currency) to produce proteins, DNA and other components of the cell, and even the storage of energy to be used in times of scarcity.

In the case of rock-eating microorganisms, known as chemolithotrophs, their source of chemical energy is reduced (electron-rich/energy-rich) minerals, like pyrite, and the best characterized electron sink is O_2 (it is the transfer of electrons from an electron-rich 'donor' to an electron-poor 'acceptor', with the corresponding loss of energy, that liberates the energy stored in electron donors for use by the cell).



Iron oxidizing microorganisms in the deep subsurface of the Iberian Pyrite Belt identified using fluorescence in situ hybridization (FISH). The green dots are positive hybridization signals indicating the presence of a well characterized rock-eating bacterium: *Aciditihiobacillus ferrooxidans*. Sample from a depth of 420 meters below surface.

Some rock-eating microorganisms can use carbon dioxide – CO_2 – as a carbon source (chemolithoautotrophs) and fix nitrogen (diazotrophs). This means that these amazing microorganisms are nutritionally very undemanding, they do not require much to grow: minerals from the Earth and CO_2 and N_2 from the atmosphere.

2. ***Going back to the question posed to our uncle: why Río Tinto is red?*** The Río Tinto has its characteristic red color and acidity due to the activity of chemolithoautotrophic microorganisms growing on the pyrite (iron (II)-ferrous disulfide – FeS_2 – also known as fool's gold because of its brass yellow metallic appearance) of the Iberian Pyrite Belt and producing ferric iron (iron III, which absorbs light in the red part of the spectrum) and sulfuric acid.

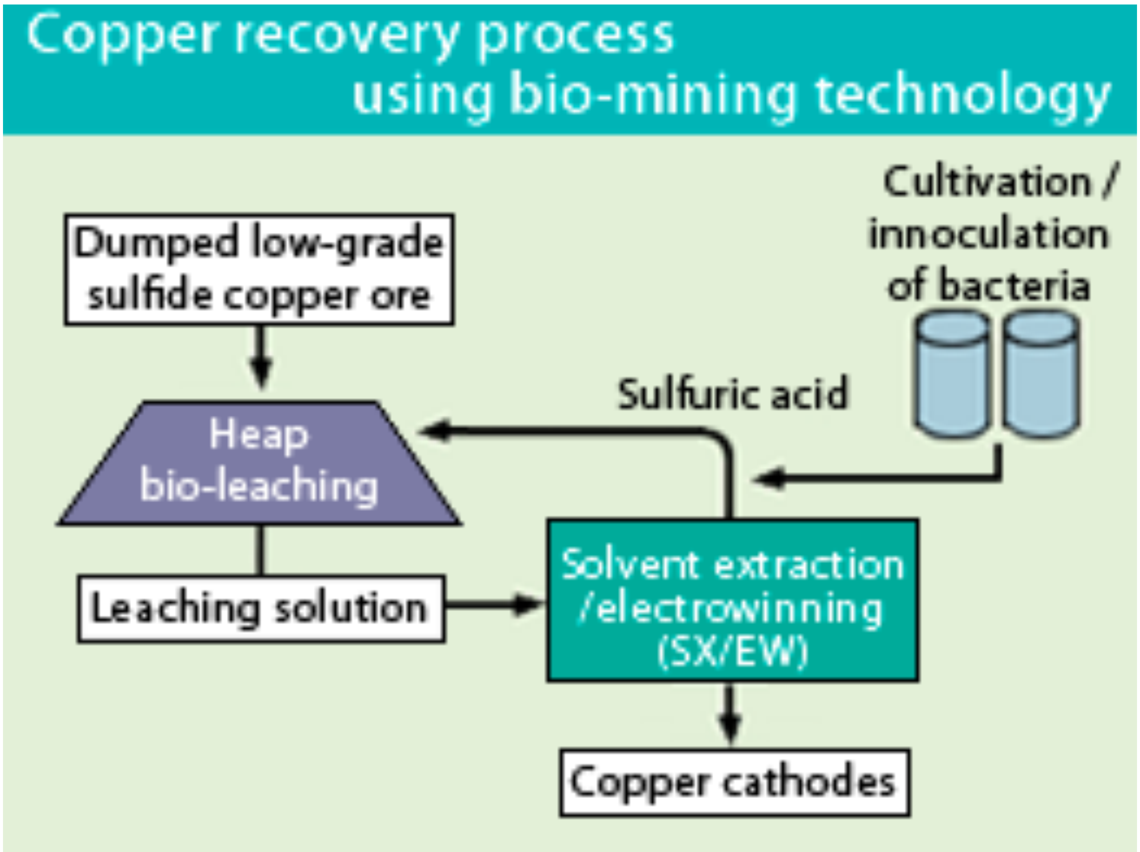
For many years, microbiologists have searched for microorganisms able to oxidize directly pyrite and other metal sulfides. But after many years of negative results, we understood recently why. There are no microorganisms that can directly obtain energy oxidizing pyrite. What they do is to oxidize ferrous iron Fe (II) to ferric iron Fe (III). The ferric iron, which has a strong oxidizing capacity, in turn chemically oxidizes pyrite (and other metal sulfides; this is the process underlying bioleaching, the microbial liberation of metals from minerals). The chemical oxidation of pyrite by ferric iron generates sulfuric acid and reduced iron (ferrous iron), which can be oxidized again by the iron oxidizing microorganisms, increasing the concentration of ferric iron in the system. Most chemolithotrophic microorganisms are also autotrophic, in other words they can use CO_2 as a source of carbon, and so are independent of reduced organic matter for its growth.

3. ***Chemolithoautotrophic microorganisms can generate a serious environmental problem.*** The metabolic activity of iron oxidizing microorganisms not only produces sulfuric acid and reduced iron from pyrite but also soluble metals from other metal sulfides, generating acidic waters with high content of toxic heavy metals that can contaminate aquifers. This is a serious environmental problem associated with conventional metal mining activities, which create large amounts of low-grade ores that, due to their low metal content and economical value, are discarded in waste piles on site. These microorganisms only require water to start generating ferric iron and sulfuric acid from pyrite in the waste piles which, in turn, starts the process of bioleaching of toxic heavy metals which accumulate in the acidic waters and drain into the environment, the problem of Acid Mine Drainage.

4. ***But bioleaching is also the foundation for the development of an environmentally-friendly biotechnology for obtaining needed metals: biomining - the mining of the future.*** Today a substantial amount of copper (one third of the world production) and other metals of economic and strategic value (nickel, cobalt, gold) are obtained by biomining. The methodology is rather simple, only requires the construction of appropriate mineral heaps and their irrigation with sulfuric acid to generate the optimal conditions for the microbial oxidation of ferrous iron to ferric iron needed for the solubilization of metal sulfides containing metals of interest.

The percolated solutions are collected and recycled until the concentration of the valuable metal is high enough for its recovery from the solution. The irrigation with sulfuric acid is only needed to kick-start the process; later on the biologically-produced acidity is sufficient to maintain the system.

An adequate construction of the heaps (dimensions, mineral size, irrigation, temperature, provision of required elements: O_2, and CO_2) is critical for an efficient operation. If the commercial value of the bioleached metal is high enough, heaps can be substituted by reactors in which is much easier to control the bioleaching conditions. Due to the complex nature of the mineral substrate and the characteristics of chemolithotrophic microorganisms, the efficiency of the bioleaching processes is dependent on a complex microbial ecology that still is not well understood. The introduction of molecular ecology methodologies to the study of these processes is helping to clarify the situation.



Scheme of a biomining process

5. ***Chemolithotrophic microorganisms can be used for bioremediation of waters contaminated with toxic metals.*** One important property that the microorganisms thriving in acidic waters require, in addition to being adapted to acidity (high concentration of protons, H^+), is their resistance to the high concentrations of the toxic heavy metals that their activity generates.

There are different strategies of adaptation to the presence of heavy metals: precipitation of the metal, thereby removing it from the system because it is no longer ‘bioavailable’; absorption to the cell wall, thereby sequestering it where it does no harm; transport and intracellular accumulation in a non-available form.

Based on these processes, different methodologies have been developed for the treatment of industrial heavy metal-contaminated waters. Although today most of the existing methodologies are directed at eliminating heavy metals from contaminated waters, the most promising for the future are those that allow specific recovery of precious or rare metals needed to maintain our quality of life, thus reducing the need for mining. Given that toxic metals cannot be destroyed like contaminant carbon pollutants, we need to learn how to extract them in a environmentally-friendly way and to recycle them, to reduce our dependency on mining and the environmental problems this creates.

6. ***From an evolutionary perspective, how old is chemolithoautotrophy?*** Considering that this type of metabolism does not have many requirements: the source of energy, reduced minerals, already existing since the formation of the planet; the source of carbon, CO_2, and nitrogen, N_2, both atmospheric components already existing in the Archaean period (from 4 to 2.5 billion ago), chemolithoautotrophy can be placed as one of the first metabolic systems on Earth.

Only one component was missing, O_2, the best-known electron acceptor (electron sink). But we know now that reduced iron and other metals can be oxidized in the absence of oxygen through the so-called anaerobic respiration, using other electron sinks like nitrate (NO_3^-). Furthermore, until recently we required the presence of photosynthetic cyanobacteria generating O_2 to explain the origin of the massive precipitation of oxidized iron (known as Banded Iron Formations) at the end of the Archaean (2.5 billion years ago). Today we know that this oxidation might have occurred under the then existing anaerobic conditions in the planet.

7. ***After the accumulation of O_2 in the atmosphere, is the aerobic oxidation of iron the only microbial activity to be considered for the solubilization of pyrite and other metal sulfides in our planet?*** The demonstration that iron and metal sulfides can be oxidized anaerobically in the absence of O_2 allows exploration of the possibility that these reactions are operating in the deep subsurface, an environment in which O_2 is absent due to its consumption by aerobic respiring microorganisms. These activities have been detected in the deep subsurface of the Iberian Pyrite Belt, which explains the origin of the Río Tinto, which has been dated much older (millions of years) than the oldest known mining activity in the area (5000 years).

As in the heaps, the microbial ecology associated with these activities is very complex and we are only now starting to learn how important this type of life in our planet is. Some authors claim that 80% of the prokaryotic biomass is under our feet, in the subsurface. This type of life corresponds to the so call "Dark Biosphere" and was predicted by Darwin. The dark biosphere is the new frontier of knowledge in microbiology, opening very interesting questions, such as: how important has it been in the origin and evolution of life in our planet? or in the geological transformation of the planet?

8. ***Astrobiological interest of rock-eaters.*** The existence of chemolithoautotrophic microorganisms able to thrive in the deep subsurface of our planet has important implications in the search for life in the universe. As an example, the main conclusion from the Viking mission was that life on the surface of Mars was unlikely due to the lack of water, low temperatures, lack of protection to sterilizing radiation and the extremely oxidizing conditions detected there. At that time, life in the deep subsurface of planet Earth was still unknown. Today we know that the dark biosphere in our planet is plentiful and diverse, so the probability that Mars and other planets and moons of the solar system and other planetary systems might hold living systems is increasing.

But the scientific method requires demonstration, so right now the probability exists but future space expeditions should demonstrate its past or present existence. The deep subsurface rock-eaters are shaping a new concept of habitability which cannot be centered on the existence of liquid water on the surface of a planet but should consider the existence of habitable zones in the subsurface of the planets, protecting life from the sterilizing conditions existing in the surface.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Healthy lives.** Heavy metal pollution from mining activities impacts drinking water supplies and hence human health. Microbial processes to remove and immobilize heavy metals at source, e.g. mining activities, can contribute to protection of humans and other organisms from heavy metal toxicity.
- **Goal 6. Clean water.** Heavy metal pollution from mining activities impacts drinking water supplies. Microbial processes to remove and immobilize heavy metals at source, e.g. mining activities, can contribute to efforts to provide clean drinking water.

- **Goal 7. Sustainable energy.** Traditional mining, involving ore smelting at high temperature, is energy intensive and polluting. Biomining, on the other hand, is energy-friendly, so contributes to sustainable energy use.
- **Goal 9. Innovation.** Biomining is, on one hand, technologically rather simple, but on the other, is complex because of its dependency on ecophysiological parameters. But the underlying biology is frontier land and exciting progress is regularly made that will enable considerable innovation.
- **Goal 12. Sustainable production.** Traditional mining is highly polluting; biomining is much less so. Better knowledge of how rock-eating microorganisms operate should improve the efficiency of biomining, ensuring the benefit of metals required for our quality of life using an environmentally-friendly biotechnology. Biomining is respectful of the environment and avoids the extremely contaminating methodologies used in conventional metallurgy. Society should regulate the use of methodologies to obtain metals favoring the use of those which are environmentally friendly and avoiding the extremely contaminating ones.
- **Goal 13. Climate change.** Traditional mining is extremely energy intensive, which means that it has a substantial carbon footprint. Biomining is energy-friendly, so contributes much less to global warming.
- **Goal 14. Conserve water.** Traditional mining is highly polluting of water bodies. Biomining is much less so, thereby contributing to the protection of our waters.
- **Goal 15. Biodiversity loss.** Environmental pollution with heavy metals impacts biodiversity at all levels of the food web, so environmentally-friendly biomining and heavy metal bioremediation processes will reduce biodiversity loss.

In addition to the SDGs, there are other important broad issues related to rock-eating microbes:

1. **Understanding the interaction mineral-microorganism.** Mineralogists believe that two thirds of the known minerals on Earth are biominerals, products of microbiological activity. Hence advances in our understanding of the role of microorganisms in the use of minerals as substrates, and the generation of secondary minerals as metabolic products, both of which have deeply transformed the geology of the planet, will advance the science of Geomicrobiology – microbiology underpinning geological changes on Earth.

2. **Astrobiology and astromining.** Better knowledge of terrestrial geomicrobiology should be beneficial for space exploration seeking signs of life elsewhere in the universe. Moreover, in the specific case of rock-eating microorganisms, understanding their metabolic activities and abilities may enable the development of new methodologies for metal extraction operations on other planets (space biomining), either to reduce environmental problems of mining on Earth and/or to obtain resources locally on planets to be colonized.

Potential Implications for Decisions.

1. *Individual*

a. Citizens should be informed about the importance of rock-eating microorganisms so they can evaluate and support the use of biotechnologies based on their properties

2. *Community policies*

a. Local environmental consequences of the use of contaminating metal extraction methods.
b. Health problems associated with bad management of toxic metals
c. Knowledge on the existence of alternative methodologies to obtain metals needed to maintain the quality of life.

3. ***National policies***
   a. Environmental metal pollution
   b. Ensuring safe irrigation and drinking water
   c. Investment in basic research on rock-eating microbiology to ensure the improvement and development of new methodologies
   d. Development of legislation favoring friendly environmental methodologies

Pupil participation.

1. ***Class discussion of the issues associated with rock-eating microorganisms***

2. ***Pupil stakeholder awareness***
   a. Rock-eaters have positive and negative consequences for the SDGs. Which of these are most important to you personally/as a class?
   b. Can you think of anything that might be done to reduce the negative consequences of heavy metal contamination, especially in the food supply chain and drinking water?
   c. Can you think of anything you might personally do to reduce the environmental footprint of the use of metals?

3. ***Exercise***
   a. Do you think that rock-eating microorganisms could live in the subsurface of Mars? Do they have all they need for growth? Follow the daily activities of the Perseverance and discuss them in class, trying to associate the discoveries with the potential habitat for rock-eating microorganisms**.**

The evidence base, further readings and teaching aids.

https://www.youtube.com/watch?v=VtNlQLYKHRQ
https://www.youtube.com/watch?v=aD4kFmHcEOM
https://www.brut.media/es/entertainment/why-the-red-river-in-spain-fascinates-scientists-1c58cbb4-7265-4400-8d40-c4af092058bb
https://www.youtube.com/watch?v=gZQGESnmaZc
https://www.youtube.com/watch?v=8obopaD8bXA
https://www.bbc.com/reel/video/p085k026/spain-s-otherworldly-red-river
https://www.youtube.com/watch?v=-vHyrwQrzZE
https://mars.nasa.gov/mars2020/
https://es.euronews.com/embed/433554 (interview on the astrobiological interest of Río Tinto)
https://www.rtve.es/alacarta/videos/el-cazador-de-cerebros/cazador-cerebros-viaje-marte/4233895/ (special program on the astrobiological interest of Río Tinto from RTVE, in Spanish)

and a collection of papers for teachers to get deeper inside on the subject:

- Darwin, C. Voyages of the Adventure and Beagle, volume III- Journal and remarks, 1832-1836. Henry Colburn, London (1839).
- Amaral-Zettler, L., Gómez, F., Zettler, E., Keenan, B.G., Amils, R., Sogin, M.L. (2002) Eukaryotic diversity in Spain`s "River of Fire". Nature, 137 doi:10.1038/417137a.
- Amils, R. (2016) Lessons learned from thirty years of geomicrobiological studies of Río Tinto. Res Microbiol, 167(7): 539-545, doi: 10.1016/j.resmic.2016.06.001.
- Escudero, C., Oggerin, M., Amils, R.(2018) The deep continental subsurface: the dark biosphere. International Microbiology, 21: 3-14. doi.org/10.1007/s10123-018-0009-y.
- Amils, R., Fernández-Remolar, D. (2020) Río Tinto: an extreme acidic environmental model of astrobiological interest. In "Extremophiles as Astrobiological models", Seckbach, J. and Stan-Lotter, H. (eds.), Wiley and Scrivener (Beverley, MA, USA), pp 21-44.

Glossary

Acidophiles, microorganisms thriving in acidic environments below pH 3
Astrobiology, an interdisciplinary science devoted to answer the fundamental question if we are alone in the universe.
Acid Mine Drainage (AMD), acidic waters generated by the biological oxidation of metal sulfides in mining operations
ATP, adenosine triphosphate: a molecule used as energy currency inside the cell
Aerobic respiration, production of cellular energy through metabolic oxidation reactions using O_2 as an electron acceptor (sink)
Anaerobic respiration, production of cellular energy through metabolic oxidation reactions in the absence of O_2 using different oxidized electron acceptors (nitrate, sulfate, CO_2)
Biomineral, mineral produced as a metabolic product of an organism
Biomining, biotechnology based on the use of microorganisms to extract metals from minerals
Bioleaching, chemical oxidation promoted by a metabolic product (e.g. ferric iron)
Biomass, total quantity of organisms in a given area
Cellular movement, movement of microorganisms searching for better environmental conditions.
Chemolithotrophy, metabolism generating cellular useful energy using reduced mineral substrates
Chemolithoautotrophy, metabolism of chemolithotropic microorganisms using CO_2 as a carbon source
Carbohydrates, reduced organic molecules that can be used as a source of energy or as structural cellular component (e.g. glucose)
CO_2, atmospheric gas component that can be used as a source of carbon by autotrophic organisms
Dark biosphere, the subsurface biosphere which is not dependent on the sun radiation
Energy, capacity of performing work
Electron, a negative charged component of any atom
Ferric iron, oxidized iron (Fe^{3+})
Ferrous iron, reduced iron (Fe^{2+})
Fluorescence in situ hybridization, methodology used to identify and quantify microorganisms using fluorescence probes designed to recognize nucleic acids sequences
Heavy metal, element of the periodic table exhibiting metallic properties
Habitability, currently conditions that allow the presence of liquid water on the surface of a planet
Heap bioleaching, bioleaching using a pile of minerals irrigated with sulfuric acid (biomining)

Iberian Pyrite Belt, a geological unit in the Southwest of Spain holding the highest concentration of metal sulfides existing on Earth.
Iron, an element of the periodic table (Fe) which is very important for living systems
Mars, a planet of the Solar System
Metal sulfide, most important group of ore minerals produced by the reaction of metals with hydrogen sulfide (H_2S)
Microbial ecology, an area of microbiology interested in understanding the composition and relations between the components of a microbial community
Molecular ecology, molecular biology methodologies used to study the ecology of microbial communities
N_2, atmospheric gas component used as a source of cellular compounds with nitrogen (e.g. amino acids, nucleic acids)
Nucleic acid, biopolymer storing genetic information (e.g. DNA, RNA)
Oxygen, atmospheric gas component used as an electron acceptor in aerobic respiration
Origin of life, hypothesis explaining the successful appearance of life on Earth
Pyrite, ferrous sulfide (FeS_2)
Proton gradient, a useful way used by many microorganisms to store cellular energy consisting in the generation of a different concentration of protons (H^+) at both sites of the cellular membrane. Is similar to an electric battery.
Protein, polymer made of amino acids
Substrate, compound used in a metabolic reaction
Sterile, absence of microorganisms after the application of conditions that eliminate their viability
Transport, movement of substrates through a semipermeable membrane
Viking mission, the space mission developed in the 70´s designed to detect signs of life on Mars. It is considered the first astrobiological space mission.