Physical and chemical conditions for colonization by *Euglena mutabilis*: case studies in two acid mine drainage sites

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Abstract

Acid mine drainage promotes high metals concentrations, low pH and high acidity and sulfate. In this way these waters are characterized by extreme environments with low biodiversity. *Euglena mutabilis* is unicellular alga specie that colonizes AMD sites. In order to understand the abiotic factors, necessary for colonization, two old sulphide mining sites were studied. Valdarcas and São Domingos represent different chemical and physical environmental conditions. Thus, it was concluded that lentic and low depth environments, situated between pH 2.8 and 3.0, and a maximum metals concentration of 20 mg/L, are favorable conditions to the abundant presence of *Euglena mutabilis*.

Keywords: Extreme Environments, Acidophilic Algae, Abiotic factors, *Euglena mutabilis*

Introduction

Sulphides are reactive minerals, which oxidation promotes acid mine drainage (AMD). This process, with strong biogeochemical component is focus of major concern in mining sites (Valente et al. 2012). Typical contamination is characterized by metal mobilization, and acidification of water and soil. The streams are commonly highly stressed ecosystems being described by low biodiversity, promoting in this way, degradation of freshwaters (Hogsden and Harding 2012). Although the stressful circumstances, it is possible to observe colonization by extremophilic organisms that have mechanisms to resist to such a contamination (Valente et al. 2016). In the recent decades, ecology of this type of extreme environments has been studied by several authors (e.g. Gross 2000; Nixdorf et al. 2001; Lottermoser 2003; Valente and Gomes 2007). According to Novis and Harding (2007), acidophilic algae can survive in these waters by developing a diversity of mechanisms to prevent metals entry into cells, namely forming metal complexes. So, these organisms interfere with the mobility of chemical species, both dissolved and particulate, playing an important role within the system (Valente and Gomes, 2007). In literature, Euglena genus has been consistently reported to occur in AMD sites (e.g. Freitas et al. 2011). In general, these studies cover identification and quantification of algae populations. In addition, it is necessary to provide a holistic perspective to understanding, not only the AMD effects, but also other abiotic factors, potentially critical for colonization and global health of the ecosystems. Therefore, the present work aims to assess the specific characteristics that may determine the occurrence and abundance of *Euglena mutabilis*, including ore paragenesis of the exploited mine, which is the first control factor of the system evolution. Then, effect of chemistry, hydrology, climate and physical properties should be analyzed to understand algae distribution, thereby promoting more comprehensive understand of ecology and help environmental monitoring. To evaluate the influence of above factors, the present study was carried out in two former sulphides mines: Valdarcas and São Domingos (NW and SE of Iberian Peninsula), representing different geological and bioclimatic contexts.

Materials and Methods

Studied sites

The study was carried out in two abandoned mining sites, with sulphide-rich ore deposits exploited for W (NW Portugal) and Cu, pyrite

(SE Portugal): Valdarcas and São Domingos, respectively (Figure 1). They are situated in two different metallogenetic provinces. The waste dumps have in common the presence of sulphides, but they have mineralogical differences, due to genetic type of deposits. Valdarcas, in the Northwest Portugal, is a skarn deposit with massive sulphides, mainly phyrrhotite and pyrite. Skarn contributes with neutralizing minerals, like calcite and Ca-silicates. It has a maritime temperate climate with July and August as the hottest (average temperature of 20.5°C) and January the coldest month (9.5°C). The annual precipitation is 1470 mm (Gomes et al. 2014), representing one of the rainiest region of Portugal.

São Domingos was one of the biggest mining complexes in Portugal that is located in the Iberian Pyrite Belt, one of the largest metallogenic provinces in the world, with massive sulphide deposits (pyrite, chalcopyrite, sphalerite; arsenopyrite and galena) with a diverse lithology of host rocks. Here the precipitation occurs mostly in the autumn and winter months, producing drought situations in summer and part of the spring. This is one of the driest regions in southwestern Europe, with typical characteristics of Mediterranean semi-arid climate (Gomes et al. 2018). The precipitation distribution is highly variable, with an average annual value of 595 mm.

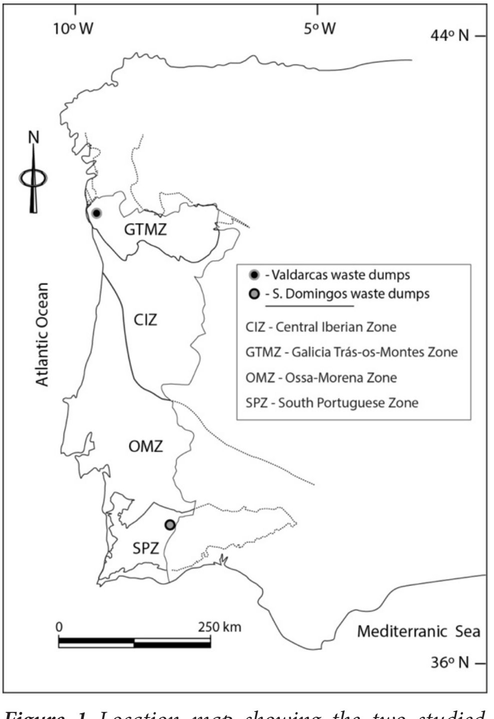

Figure 1 Location map showing the two studied mines, Valdarcas and São Domingos, in different geological contexts.

Both mines were closed without environmental rehabilitation and show manifestations of AMD (Valente et al. 2007; Gomes et al. 2017). As referred paragenetic and climate as well as physical/hydrological contexts of the receiving water system are different.

Water sampling and analysis

In both sites, there was a monthly sampling during an entire hydrological year. At Valdarcas, water was collected in a small mountain creek comprising 5 sites over a distance of about 900 m. At São Domingos there were 12 sampling sites. These locations are represented in Valente et al. (2007) and Gomes et al. (2017), respectively for Valdarcas and São Domingos. 1 L of surface water was collected in a sterilized polyethylene container at each point, to be used for the laboratory analyses. Other 50 mL was filtered with 0.45 μm membranes and acidified with acid nitric to obtain pH<2, in order to prevent the metals precipitation and bacterial growth. With a portable meter, Thermo Scientific Orion, temperature (°C), pH, electrical conductivity (EC μS/cm, at 25 °C), potential redox (Eh) and total dissolved solids (TDS) were recorded in situ.

The turbidimetric method (Standard Methods 4500 E) was used to analyze sulfate. Total acidity and alkalinity were immediately measured by volumetric determination (AWWA 1992). Fe, As, Cu, Zn, Mn, Cd, Pb, Co, and Ni were obtained by inductively coupled plasma-mass spectrometry (ICP/MS). All the reagents were of analytical grade or *Suprapur* quality (Merck, Darmstadt, Germany). The standard solutions were Merck AA Certificate. Milli-Q water was used in all the experiments. Metals and arsenic analyses were performed at Activation Laboratory, Lda (Actlabs, Canada), including analysis of duplicate samples and blanks to check precision, whereas accuracy was obtained by using certified standards.

Algae sampling and identification

Algae sampling was coincident with the water sampling points, in order to contemplate the observations in the field. They were obtained by scraping the substrates and by dredging the water surface through a polyethylene container. The taxonomic identification was achieved by optical microscopy (Leica Microscope CTR5000 with autofluorescence).

All samples were accommodated in a portable refrigerator at 4°C to avoid degradation. Some algae samples were identified immediately when arrived to the laboratory. In other cases preservation with Kew solution was carried out.

Results and discussion

Hydrochemical characterization and Euglena distribution

Table 1 presents annual average values of water parameters. At Valdarcas pH vary between 2.9 and 3.1. On the other hand, São Domingos shows a wide range, from extreme acid to circumneutral. The same different behavior occurs with other parameters as variation is considerably higher at São Domingos.

According to the Ficklin diagram (Figure 2), most samples from São Domingos are classified as High-acid, High-metal, whereas all the samples from Valdarcas are in the range High-acid, Low-metal and Acid, Low-metal.

At São Domingos *Euglena mutabilis* (Figure 3) was only observed in two sampling sites with similar hydrochemistry (PAT3 and PAT10 in table 1). Both sampling sites exhibited identical hydrology, with low water flow conditions, forming shallow lagoons (≈10 cm) with slight current (like puddles). At Valdarcas Euglena was found widely spread throughout the system. The only sampling site without observations of this unicellular alga presents high flow water regime and shadow conditions, thus with lower light incidence, and higher depth comparing to the others points. These observations are in accordance with the work of Smucker and Vis (2011), which refers that differences in water flow regime are decisive in the implementation and stabilization of primary organisms. Other abiotic factors, such as photic zone, photo-period, and sun exposure may have high importance. Also, Sabater et al. (2003), refers that Euglena genus attaches to substrate, and thus vigorous currents can pose difficulties to fixation. In this way, the lentic features,

Table 1 *Average parameter values from the two systems. Σ metals (Fe, As, Cu, Zn, Mn). Location/distribution of sampling points can be found in Valente et al. (2007) and Gomes et al. (2017)*

Mining Sites	Samples	pH	EC (µS/cm)	T (°C)	Eh (mV)	Acidity (mg/L $CaCO_3$)	SO_4 (mg/L)	Σ metals (mg/L)
Valdarcas	V3	3.0	1919	15.4	427	850	1089	448
	V4	3.1	1681	16.1	432	745	974	267
	V5	2.9	1710	16.6	464	765	1012	250
	V6	3.1	2289	16.1	404	1337	1763	710
	V9	2.9	1585	15.3	457	653	862	214
São Domingos	PAT 2	2.6	7297	21.9	552	3708	5873	1077
	PAT 3	2.7	2877	23.3	517	1274	1608	98.7
	PAT 4	2.7	2655	23.3	555	1228	1728	184
	PAT 5	2.5	4035	23.2	538	2841	3155	365
	PAT 6	3.0	1735	23.2	480	811	1002	106
	PAT 7	1.7	15133	24.4	517	169610	144352	50177
	PAT 8	2.8	3262	22.2	501	2036	2365	251
	PAT 9	2.9	2221	22.2	494	810	1285	87.3
	PAT 10	3.0	2601	20.2	486	774	1564	90.2
	PAT 11	4.4	654	20.9	345	103	255	22.9
	PAT 12	6.4	247	21.2	300	-	33.8	13.0

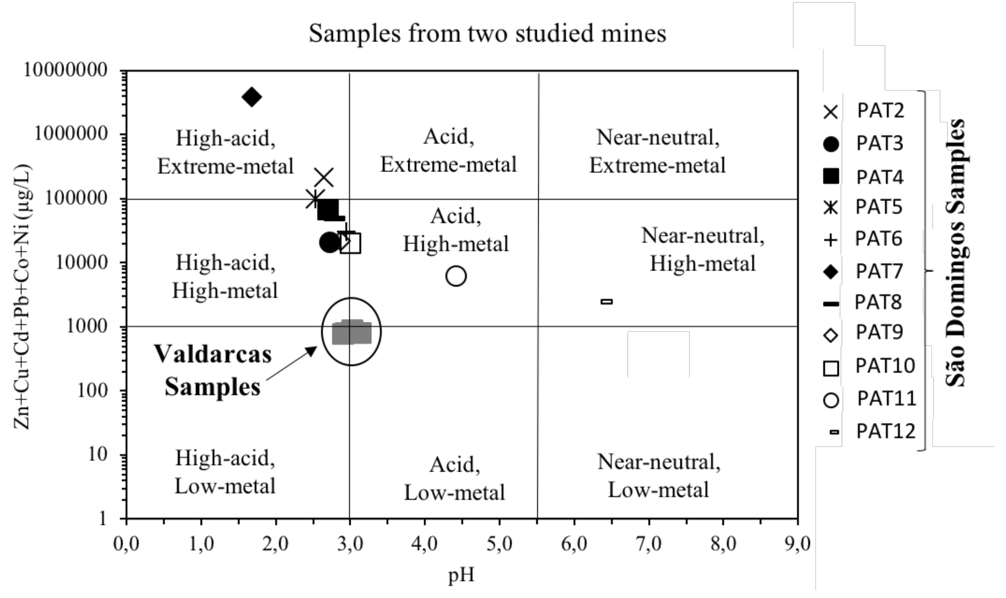

Figure 2 *Ficklin diagram revealing different classification for Valdarcas and São Domingos water samples.*

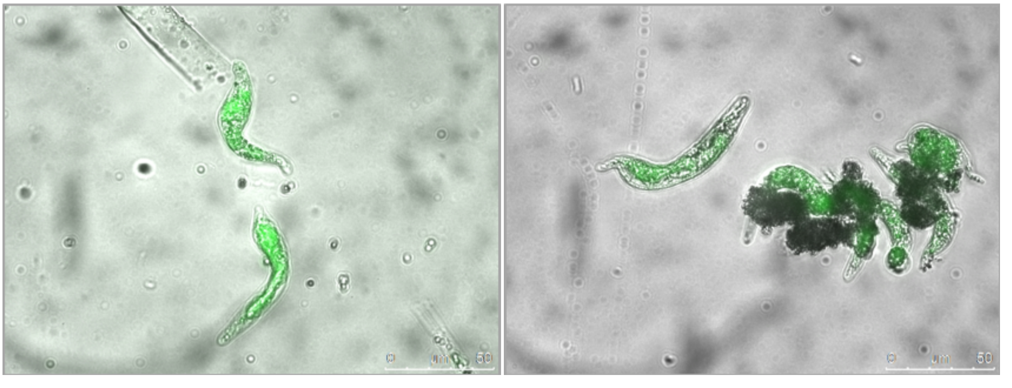

Figure 3 *Microscope Image of typical elongated Euglena mutabilis clear cells.*

with low depth (which allows light entry for photosynthesis) and low current speed in the lagoons of São Domingos and the calmer sections of Valdarcas stream appear to be more propitious to fixation of Euglena.

Contrarily to observations made by Wolowski et al. (2008), *Euglena mutabilis* was not found as an abundant alga in the São Domingos mine drainage. The presence of this specie only in PAT3 and PAT10 (Table 1) seems indicative of its survival range, namely regarding pH as stated by Verb and Vis (2000).

In terms of water properties the obtained results points to a minimum pH of 2.7 and metals concentrations until around 20 mg/L as most appropriate conditions for Euglena establishment. Several authors (e.g. Hogsden and Harding 2012) confirm that biota respond differently to the individual stressors. Niyogi et al. (1999) had already reported that AMD hydrochemistry has direct effects on the aquatic ecology. In the same way, Hogsden and Harding (2012) refer that low diversity is related to physiological sensitivity to extreme AMD conditions (high acid and/or high metals). On the other hand, the biomass production may reflect the tolerance of acidophilic algae to different levels of chemical contamination. For example, large biomass production, with blooms of Euglena and filamentous Chlorophyta (e.g.

Klebsormidium sp.) were reported in extreme AMD conditions (Valente and Leal Gomes 2007).

Climate may also act as limiting factor for Euglena survival. Since these algae are attached to humid substrates, at São Domingos they are subjected to additional stress, due to the dryness of the region, with frequent drought episodes (Gomes et al. 2018).

Conclusion

The abundance and distribution of *Euglena mutabilis* were analyzed in relation to abiotic chemical and physical factors in two different mining sites. The obtained results emphasize the influence of pH, metal concentrations, hydrology, depth of water column and solar incidence. High solar incidence, lentic, and low depth conditions seem to facilitate the establishment of Euglena communities. Moreover, pH in the small range of 2.8 and 3.0, and a metallic concentration (Σ (Fe, As, Cu, Zn, Mn)) around 20 mg/L were found as favorable environments for *Euglena mutabilis*.

Considering the factors analyzed in the two mining sites, the differences in water properties (conditioned by paragenesis and climate), and the hydrological conditions suggest that Valdarcas is more propitious for *Euglena mutabilis* survival and maintenance. At São Domingos this alga has a restrict distribution to some particular lentic environments.

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