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The role of two acidophilic algae as ecological indicators of acid mine drainage sites

El papel de dos algas acidófilas como indicadores ecológicos de zonas de drenajes ácidos de minas

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Abstract

Acidic effluents, emerging from four abandoned mines (Valdarcas, Carris, Adoria and Cerquido) in Northern Portugal were analyzed regarding physical-chemical, mineralogical and ecological characteristics. Such effluents present distinct properties since they were mobilized from wastes of different primary ores and mining procedures. Results show that acidophilic algal colonization is dominated by *Euglena mutabilis* and *Klebsormidium* sp.. Abundance and distribution of both algae are related to different intensity of acid mine drainage (AMD) at each mining site. Mineral-alga interactions influence metal deposition at Valdarcas and probably contribute to iron mineral precipitation. *Euglena mutabilis* displayed a preference for schwertmannite-rich AMD precipitates, which may suggest a mineralogical control on algal colonization. Spatial distribution of *E. mutabilis* can be used to qualitatively assess water quality improvements along the effluent channel.

Keywords: acid mine drainage; acidophilic algae; *Euglena mutabilis*; *Klebsormidium*; ecological indicator; mineral-alga interaction

Resumen

Las aguas residuales ácidas que emergen de cuatro minas abandonadas (Valdarcas, Carris, Adoria y Cerquido) en el norte de Portugal fueron analizadas según sus características físico-químicas, mineralógicas y ecológicas. Estas aguas residuales presentan distintas características ya que se desplazaron desde escombreras con estériles de diferentes tipos genéticos de depósitos minerales y procedimientos de minería. Los resultados muestran que la colonización de algas acidófilas está dominada por *Euglena mutabilis* y *Klebsormidium* sp.. La abundancia y la distribución de ambas algas está relacionada con la diversa intensidad de los drenajes ácidos de mina (AMD) en cada sitio de explotación minera. Las interacciones de tipo mineral-alga influyen en la deposición metálica en Valdarcas y probablemente contribuyen para la precipitación de minerales del hierro. *Euglena mutabilis* muestra una preferencia por los precipitados ricos en schwertmannite, que puede sugerir un control mineralógico en la colonización de algas. La distribución espacial de *E. mutabilis* puede servir para evaluar cualitativamente las mejoras en la calidad del agua a lo largo del canal receptor de AMD.

Palabras clave: Drenajes ácidos de mina; algas acidófilas; *Euglena mutabilis*; *Klebsormidium*; indicador ecológico; interacción mineral-agua

1. Introduction

From an ecological point of view, the acid mine drainage (AMD) sites are extreme environments because impose stress on the majority of organisms. Very low pH values (pH<3), high metal solubility, presence of iron colloids provoking water turbidity and deficiency in inorganic carbon and phosphorus are general features of AMD that contribute to stress conditions.

The nature of stress on aquatic systems, particularly on those affected by AMD, as well as the special strategies regarding survival in these media have been studied over the recent decades (Whitton, 1984; Gross, 2000; Fyson, 2000; Fogg, 2001; Gaur and Rai, 2001; Gimmler, 2001; Nixdorf *et al*., 2001). The influence of acidity in decreasing the biological diversity is broadly documented, although an increased response in biomass is occasionally registered (Niyogi *et al*., 2002). Therefore, AMD is structurally a simple ecosystem, dominated by acidophilic and acid-tolerant organisms. Functionally, the relations between autotrophs, and between them and the environment, control such ecosystems. These organisms, either prokaryotes or eukaryotes, play important roles, ensuring primary production and interfering with the mobility of chemical species dissolved in the aquatic medium. There is extensive bibliography concerning prokaryotes in mining environments, such as the chemoautotrophic *Acidothiobacillus ferrooxidans* and other acidophilic Bacteria and Archeaon microorganisms (Evangelou and Zhang, 1995; Ehrlich, 1996; Banfield *et al*., 2000; Benner *et al*., 2000; Bond and Greg, 2000; Robbins, 2000; Johnson *et al*. 2002). The general subjects covered by these studies include the identification and quantification of microbial populations, their participation in the sulphide oxidation processes and in the generation and treatment of AMD.

Among eukaryotes, photosynthetic acidophilic algae have also been deserving of careful attention (Lessmann *et al*., 1999; Gross, 2000; Brake *et al*., 2001a; Verb and Vis, 2001; Valente, 2002; Sabater *et al*., 2003). From a monitoring perspective, some algae, mainly the macroalgal communities, have a great deal of importance. They have optimal growth in acidic conditions, most are also mesophilic, and especially they are easy to recognize macroscopically. Therefore, although there are inherent problems with spatial and temporal heterogeneity when compared with microaalgal community, they can be used as expeditious indicators in analysing systems impacted by AMD. This is the case of *Klebsormidium* and *Euglena* genus (Hargreaves *et al*., 1975; Whitton, 1984).

Filamentous algae of *Klebsormidium* genus are well known as metal resistant and have been related with metal-rich polluted waters. Particularly *K. subtile*, *K. rivulare* and *K. flaccidium* are often referred species in AMD (Nixdorf *et al*., 2001; Verb and Vis, 2001; Sabater *et al*., 2003).

Euglena genus has been consistently reported in literature to occur in natural or anthropogenic acidic and metal-rich waters. The competitive advantage of *Euglena mutabilis* in AMD, in comparison with other *Euglena* species, is well documented (Olaveson and Nalewajko, 2000).

Uptake of metals by algal communities and mineral-algae interactions, in general, induce more or less discrete modifications in aquatic environments. These interactions may yield to biomineralization processes. Although biomineralization is broadly documented, references to such a process involving acidophilic algae in AMD are much rarer (Mann *et al*., 1987; Brake *et al*. 2001b, 2002). These articles report the contribution of Euglenophyta to the formation of iron-rich minerals. Besides their role promoting mineral precipitation, algae can interfere with geochemical cycles in several ways. Extra-cellular metal adsorption is known as a common strategy to limit access of toxic elements to the interior of the cells and, in such a way, to survive in stressful conditions (Gaur and Rai, 2001).

As a result, algae may control acidity and metals in solution leading to natural attenuation of the AMD environmental impact (Lawrence *et al*., 1998; Elbaz-Poulichet *et al*., 2000; Valente, 2002; Casiot *et al*., 2004).

The roles of acidophilic algae in acid mine drainage are pointed out in this paper through the characterization of acidic effluents from four abandoned mines in Northern Portugal: Valdarcas, Adoria, Carris and Cerquido (Fig. 1).

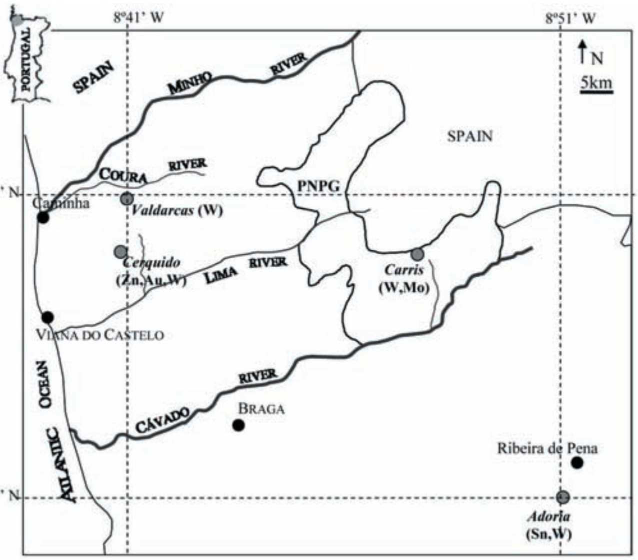


Fig. 1.- Location of the abandoned mines under consideration in Northern Portugal; Information about respective mineralization is indicated. PNPG, Gerês National Park.

Fig. 1.- Localización de las minas abandonadas objeto de estudio en el norte de Portugal; incluye información sobre la respectiva mineralización. PNPG, Parque Nacional da Peneda- Gerês.

Mine	Ore deposit	Properties of the mine wastes
Valdarcas	Skarn with sulphides (tungsten mineralization)	Fine tails from milling and hydrogravitic separation; abundant sulphides (pyrrhotite, pyrite, arsenopyrite), carbonates (calcite and siderite), phosphates (apatite) and calcium silicate minerals
Carris	Quartz veins in granite (tungsten and molybdenum mineralization)	Fine sulphide-rich residues from ore beneficiation; pyrite, arsenopyrite and molybdenite are the most abundant sulphides
Adoria	Quartz veins in granite and metassedimentary rocks (tin and tungsten mineralization)	Coarse grain size residues; there is a dominance of sulphide assemblages with arsenopyrite, pyrite and galena
Cerquido	Quartz veins in shear zone (tungsten, zinc and gold mineralization).	Rough fragments resulted from crushing; the mineralogical assemblages are rich in quartz and sulphides (arsenopyrite, pyrite, chalcopyrite, sphalerite and galena)

Table 1. Properties of sulphide-rich wastes in relation with the genetic type of ore deposit.
Tabla 1. Propiedades de los estériles ricos en sulfuros relacionados con los distintos tipos genéticos de depósitos minerales.

Combination of ecological, geochemical and mineralogical data from those effluents provides evidence of the importance of acidophilic algal colonization for environmental monitoring and impact qualification procedures in AMD sites.

2. Methods

2.1. Selection of mining sites

The studied mining sites were selected because they represent different genetic types of ore deposits. All of them possess abandoned waste-dumps with sulphides exposed to weathering processes generating AMD. The paragenesis of mining wastes is the main distinguishing factor among the sites, namely sulphide species and respective mineral proportions are different (Table 1). Besides mineralogy, inherited from primary ore, grain size resulting from ore processing are important distinguishing features.

2.2. Water sampling

The sampling scheme used for effluent monitoring was optimised for each mining site, taking into consideration the main source of acid mine drainage, the heterogeneity of the effluents and the hydrologic regime. Valdarcas is the only site with permanent drainage, which allowed a more frequent sampling (monthly between 2000 and 2002). At Carris, Adoria and Cerquido superficial drainage has an ephemeral nature and therefore three sampling campaigns were performed (in the autumn of 1999, 2001 and 2002). Figure 2 presents the location of sampling stations at Valdarcas, Carris and Adoria. At Cerquido, the absence of drainage did not allow the establishment of a fixed scheme. Therefore sampling during rainy periods took place in the main gallery entrance and in the waste-dump surrounding runoff. Samples of unpolluted superficial regional waters were seasonality collected in order to describe background characteristics.

2.3. Algae sampling and identification

The sampling schedule for algae was coincident with the one described for water sampling. Therefore, at Valdarcas samples were collected monthly. At the other sites they were collected only during autumn and winter, although field trips were performed during the four seasons of the year in order to register the presence or absence of algal mats. Samples for algal study were collected at the stations used for water sampling (Fig. 2) always at the same time of the day (early in the morning) and observed within 24h.

Where benthic algae were macroscopically visible, biological material and the sediment on which they grow were collected. If algae were not visible, than effluent filtrates were qualitatively obtained and examined for the presence of suspended cells.

Taxonomic identification was achieved by optical microscopy, based on morphological features and simple coloration tests (amide presence) (Round, 1975).

2.4. Water chemical analyses

pH, electric conductivity (EC), redox potential (Eh), dissolved oxygen (O_2) and temperature of the water were measured in the field with a multi-parameter meter (Orion, model 1230). The following Orion probes were used: combined pH/ATC electrode *Triode* ref. 91-07W, conductivity cell *DuraProbe* ref. 0133030, redox combination electrode ORP ref. 96.78 and a galvanic oxygen immersion probe, ref. MSR 083010.

Laboratory analyses were performed for fluoride and chloride by ion chromatography (IC) with suppressed conductivity detection (761 Compact IC Metrohm). Sulphate was measured by turbidimetry and total acidity by volumetric determination (Standard methods for water analysis reference 4500E, 2310B, respectively). Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was used for metals. IC and ICP–AES analyses were preceded by sample filtration through 0.2 µm pore-diameter cellulose ester membrane filters. For metal analysis, filtration was followed by acidification with HNO_3 65% *suprapur* Merck.

2.5. Mineralogical analysis

Mineralogical composition of AMD precipitates, used by algae as attachment surfaces, was analysed by x-ray powder diffraction (XRD) with a Philips X'pert Pro-MPD difractometer, using Cu-K_α radiation. Sample preparation procedures and the appropriated XRD conditions for these kinds of samples, particular leading with low crystallinity and mineral mixtures, are described in Valente (2004). Scanning electron microscopy (on carbon or gold coated samples), with a LEICA S360 microscope, combined with an energy dispersive system (SEM-EDS), allowed the observation of morphological and compositional aspects of mineral-alga interactions.

3. Results and discussion

3.1. Physical and chemical characterization

At Valdarcas, Carris and Adoria, AMD emerges at the base of the enriched-sulphide waste-dumps. Acidic seepages and surface runoff are naturally drained into small permanent (Valdarcas) or ephemeral streams, which represent the main effluent channels (Fig. 2). At Cerquido, there is no evidence of persistent acidic effluents arising from the waste-dumps. Signs of AMD can be detected in the surface runoff and in the water flowing from a mine gallery, but only during the most intensive and lasting rainy periods.

Table 2 lists the main features of these effluents concerning the field parameters and specific chemical composition. A comparison of the effluents is provided through a radial representation of some common physical-chemical indicators (Fig. 3). This representation uses mean values and regards the effluent collected at and near the waste-dumps up to a distance of approximately 500m.

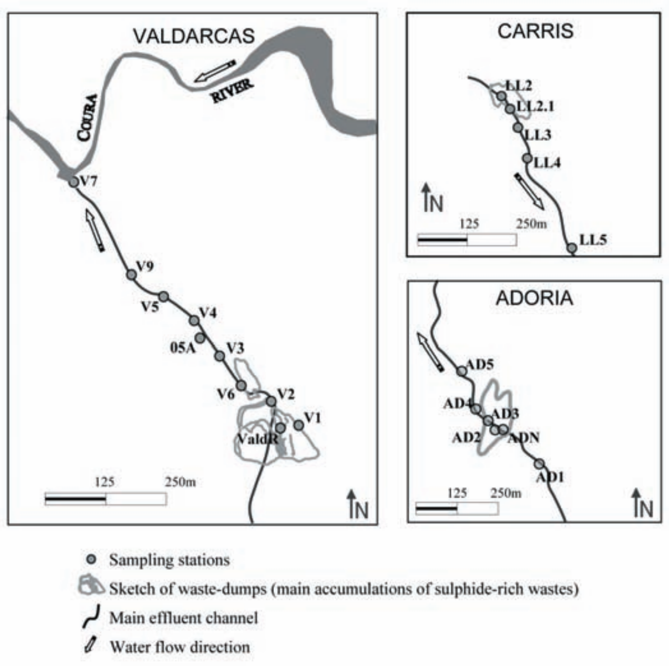


Fig. 2.- Location of water sampling stations.
Fig. 2.- Localización de los puntos de muestreo de agua.

At Cerquido, the local hydrology, which determines the absence of water during most of the year, and the coarse grain size of residues limit the interaction between water and enriched-sulphide rough residues. As a result, physical and chemical indicators reveal the less contaminated effluent.

The most acid and generally degraded effluent is observed at Valdarcas. Contamination extends downstream, affecting the Coura River, where effluent discharges (Fig. 2). An important attribute of this effluent is its strong chemical heterogeneity. Highest pollutants concentrations, corresponding to maximum values presented in Table 2, are generally obtained in seepages at the base of the waste-dumps during the first autumn rains. In contrast, lowest concentrations are measured after long raining periods, during winter, which allow dilution to occur, at the more distant sampling station (V7, Fig. 2). The fine grain size of residues and the abundance of very reactive sulphides (pyrite and pyrrhotite) are the main promoters of such chemical characteristics. Carris and Adoria are the intermediate situations: either because of coarser grain size or the greater stability of the major sulphides, in contrast to the strong reactivity of pyrrhotite and pyrite.

Physical-chemical indicator		Valdarcas n=175	Carris n=15	Adoria n=18	Cerquido n=12
pH	Mean	3.00	4.13	4.67	4.69
	Min	2.07	3.49	4.18	4.12
	Max	3.79	4.96	5.85	5.20
EC(μS/cm)	Mean	1760	156	71.7	31.4
	Min	196	50.0	29.0	22.0
	Max	11870	253	202	37.2
Eh (mV)	Mean	470	nd	382	373
	Min	133		304	304
	Max	627		440	429
O_2 (mg/L)	Mean	6.50	nd	6.34	nd
	Min	1.80		5.35	
	Max	17.0		7.05	
SO_4^{2-} (mg/L)	Mean	1412	40.5	30.5	4.10
	Min	25.0	21.3	6.49	3.40
	Max	21630	54.2	113	4.81
F^- (mg/L)	Mean	23.7		0.51	
	Min	0.23		0.15	
	Max	835	<0.01	0.64	<0.01
Acidity (mg/L $CaCO_3$)	Mean	885	-	-	-
	Min	28.0	-	-	-
	Max	9017	-	-	-
Fe (mg/L)	Mean	370	2.57	0.16	0.029
	Min	0.90	0.08	0.09	0.027
	Max	15000	6.90	0.21	0.032
Cu (mg/L)	Mean	1.89	0.66	0.17	0.012
	Min	0.02	0.23	0.03	0.010
	Max	65.0	0.94	0.30	0.013
Zn (mg/L)	Mean	1.07	0.29	0.92	
	Min	0.02	0.18	0.21	
	Max	45.0	0.42	1.20	<0.063
As (mg/L)	Mean	0.92	0.0097		
	Min	<0.0063	<0.0063		
	Max	57.0	0.014	<0.0063	<0.0063
Al (mg/L)	Mean	312	1.88	1.25	0.25
	Min	1.00	1.08	1.00	0.21
	Max	42000	2.71	1.50	0.30

nd – not determined; - absence; n – number of samples.

Table 2.- Summary of the effluent chemistry regarding some selected indicators.
Tabla 2.- Resumen de la composición química de las aguas residuales según algunos indicadores seleccionados.

3.2. Acidophilic algal colonization

A comparison of algal colonization is given in Table 3. None of the algae was detected in unpolluted regional waters, and most of them have been recognized as acidophilic, or at least, acid-tolerant (Gimmler, 2001). Qualitative information about abundance near waste-dumps (up to a distance of 100m) is provided. Three classes of dispersion were distinguished, considering the distance that algae are capable to extend colonization.

Acidophilic colonization is more important at Valdarcas, where a maximum of six genera were identified. Especially well-succeeded colonizers belong to Euglenophyta and Chlorophyta (*Euglena mutabilis* and *Kleb-*

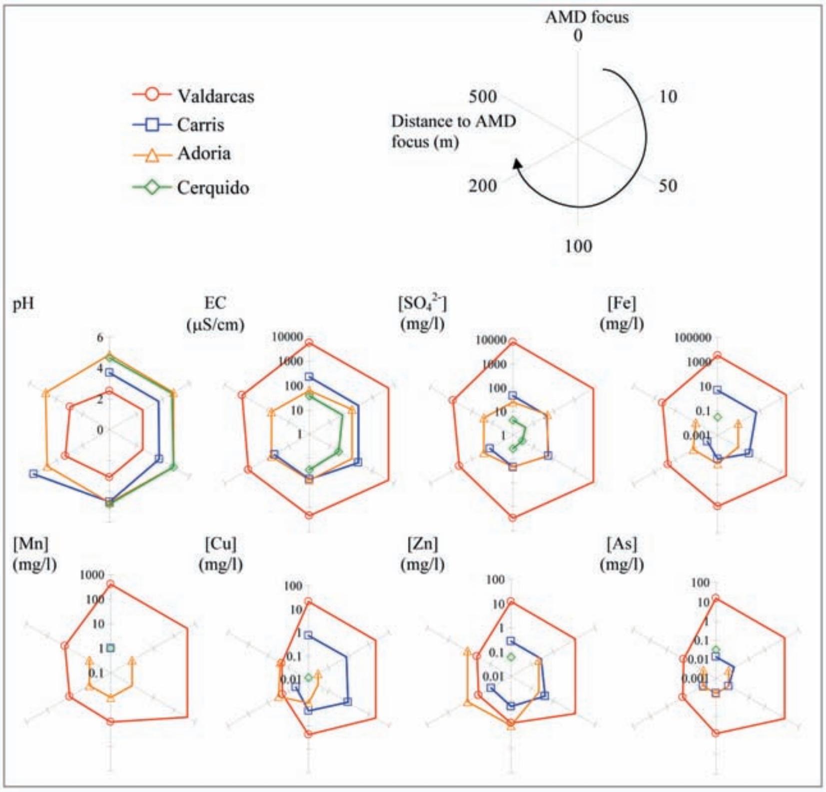


Fig. 3.- Radial representation of physical-chemical parameters reflecting the effluent quality near the waste-dumps (up to a distance of 500m) (average concentrations).

Fig. 3.- Representación radial de los parámetros físico-químicos que reflejan la calidad de las aguas residuales cerca de las escombreras (hasta una distancia de 500m), (concentraciones medias).

sormidium sp., respectively) are the most abundant and persistent ones.

3.3. Occurrence and distribution of Euglena mutabilis *and* Klebsormidium *sp.*

The abundance and distribution of both taxa suggest a differentiation among the effluents. *Klebsormidium* sp. is present in all them, indicating a wide tolerance to different levels of chemical contamination. It forms green benthic communities in flowing shallow water (Fig. 4a). At Valdarcas, it is highly abundant and widespread along the entire extension of the effluent channel, until discharging point in the Coura River (V7, Fig. 2). These algal mats, primarily composed by *Klebsormidium* sp., also have *Euglena mutabilis* cells and minor filamentous *Mougeotia* sp., presenting a structure similar to the one reported by Stevens *et al.* (2001) from AMD waters in Ohio.

Because they are densely populated and act as barriers to the flux of iron oxyhydroxide particles, these green mats appear, sometimes, with an ochre coloration (Fig. 4a). It can also be observed on the waste-dump surface, binding mineral particles (Fig. 4b). Long interwoven filaments form labyrinth algal mats, which precede more developed biological crusts, like the ones described by Lukesová (2001).

At Carris, communities are sparse and restricted to the area near waste-dumps. They are even more restricted in Cerquido, being detected only during winter in a mine gallery entrance.

A rather different situation is accounted for *Euglena mutabilis*. This species is a particular attribute of Valdarcas, since it was not detected in the other effluents. It appears mainly as submerged benthic green mats covering the effluent channel. It also forms exposed communities in acidic seepages. Brake *et al*. (2001b) describe similar occurrences at the abandoned Green Valley coal mine site in western Indiana, USA. These authors use the thickest of biofilms and the level of channel coverage to assess relative population density. At Valdarcas, these parameters visually indicate a decrease in the effluent colonization with distance relative to the waste-dump. In fact, very densely populated communities are found in the seepages at the base of the waste-dump and in the upper section of the effluent channel (from V6 to V4, Fig. 2). Here, several millimetres-thick biofilms, covering the entire channel width, can be observed during the period of the most intensive productivity (between spring and summer) (Fig. 5). However, for higher distances, communities begin to be sparser, until complete absence at V7.

3.4. Factors controlling algae distribution

Field observations at Valdarcas about *E. mutabilis* distribution indicate a preference for growing on two types of geological material. One, a white colour, has a restric-

	VALDARCAS		CARRIS		ADORIA		CERQUIDO	
ALGA	Abund.	Disp.	Abund.	Disp.	Abund.	Disp.	Abund.	Disp.
Characium sp.	+++	CII	n.d.		n.d.		n.d.	
Mougeotia sp.	+++	CII	n.d.		n.d.		n.d.	
Klebsormidium sp.	++++	CIII	++	CI	+++	CI	+	CI
Characiopsis sp.	+++	CII	n.d.		n.d.		n.d.	
Euglena mutabilis	+++++	CII	n.d.		n.d.		n.d.	
Eunotia sp.	i.e.		n.d.		+	CI	+	CI

Qualitative scale of abundance is between rare (+) and very abundant (+++++). Classes of dispersion: CI – algal colonization is restricted to the area near waste-dumps (up to 100m); CII – algal colonization extends up to a distance of approximately 500m from the waste-dumps; CIII – algal colonization is widespread at full length on the effluent channel (approximately 800m); n.d. – not detected; i.e. – isolated specimen.

Table 3.- Acidophilic algae identified in the effluents.
Tabla 3.- Algas acidófilas identificadas en las aguas residuales.

ted occurrence in acidic seepages at the base of the waste-dump (V2). It is composed of amorphous iron phosphate and supports the larger observed specimens, living in air exposed communities (Fig. 5a). Other growing surfaces, with ochre colours, are iron-rich precipitates, hard coating the effluent channel. XRD analysis indicates that those precipitates are mixtures of variable proportions of goethite, schwertmannite and jarosite, which is in accordance with reported results from similar AMD environments (Bigham and Nordstrom, 2000; Murad and Rojík, 2003). Densely populated communities are more often associated with fresh precipitates that are low in crystallinity and composed of major schwertmannite and jarosite and minor goethite. Fig. 5b shows submersed communities, colonizing a yellow ochre mixture and avoiding the darkest one, which is goethite richer.

Table 4 summarizes the range of some physical and chemical parameters, derived from where *Euglena mutabilis* and *Klebsormidium* sp. form more densely populated communities. Fig. 6 represents the relationship between sulphate and pH for AMD at Valdarcas. Conditions that support both taxa are marked, discriminating two fields of algal preferences. *E. mutabilis* occurs at the highest sulphate levels and, in comparison with *Klebsormidium* sp.*,* seems to have preference for more highly contaminated conditions. This explains its abundance in seepages and at the upper section of the effluent channel. In contrast, the improvement in water quality along the channel, related with natural attenuation processes, like dilution, adsorption and precipitation, may be the explanation to its absence at the channel mouth (V7).

Acidophilic algal colonization was not detected in some located, very oxidizing and acidic seepages, where sulphate and iron are rather high (ValdR, Fig. 2). However, the concentration of these constituents is compatible with growth ranges reported in literature for *Euglena* and *Klebsormidium* species in AMD (Sabater *et al*., 2003). This absence could result from a toxic effect caused by other potentially toxic elements, like aluminium and fluoride, which present here the highest concentrations.

Another hypothesis is based on Brake *et al*. (2001b) statements regarding iron speciation. These authors suggested that *E. mutabilis* prefers environments where aqueous phases of iron (II) are in excess relative to iron (III), which is not the case of such seepages.

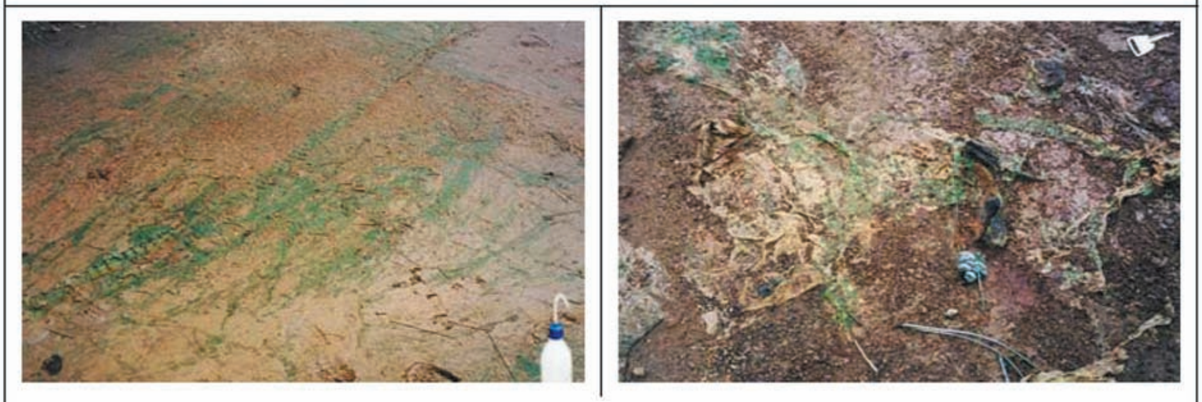
Other than chemical factors may constrain algal colonization at these seepages. For instance the absence of flowing shallow water probably limits *Klebsormidium* fixation. Another possibility concerns a mineralogical effect, once field observation suggests that *Euglena* prefers to grow over schwertmannite-rich precipitates. However schwertmannite is rather rare at these seepages, where jarosite is the major component of iron precipitates.

To understand geochemical and mineralogical control of algae distribution needs further investigation. Particularly, laboratory experiments using isolated organisms are necessary to identify and quantify chemical preferences. Growing experiments using synthetic AMD solutions and different mixtures of iron precipitates may be useful to confirm or evaluate the influence of schwertmannite on *Euglena mutabilis*.

4. Mineral-alga interactions

At Valdarcas no clear evidence of intracellular iron-rich precipitates was found. However it is common to observe

a) Densely populated communities in the effluent channel (Valdarcas).
These green algal mats act as barriers, retaining ochre particles.



b) Algal communities in the surface of the waste-dump, leading to the formation of algal crusts.

Fig. 4.- Colonization by Klebsormidium species.
Fig. 4.- Colonización de las especies Klebsormidium sp.

accumulations of iron precipitates outside the alga cells, while the interior stays clean and transparent. Deposits of ochre precipitates can be observed at *Klebsormidium* sp. cell walls and at the extra-cellular polymers segregated by *Euglena mutabilis* (Fig. 7). In that way these algae may have ability to modify the effluent chemistry, particularly concerning iron and related elements.

E. mutabilis and schwertmannite form a typical assemblage at the Valdarcas effluent. It is common to observe *Euglena* cells and sometimes their paramylon grains (which are the typical food reserve polymer of Euglenophyta) surrounded by schwertmannite spheres (Fig. 8). This kind of relationship suggests that schwertmannite may to precipitate from a biological nucleus, related to *Euglena* presence.

Oxygenation through photosynthetic activity by algae may also interfere with iron speciation at Valdarcas. Distribution of dissolved oxygen is related to the cycles of biomass productivity (Fig. 9). Elevated levels of dissolved oxygen between spring and summer create geochemical microenvironments that may be favourable to iron

Parameter	*Euglena mutabilis*	***Klebsormidium* sp.**
pH	2.5 – 3.4	3.0 – 3.5
Temp. (ºC)	13.0 – 18.0	11.5 – 22.0
EC (µS/cm)	1700 – 7000	500 – 2000
Eh (mV)	275 – 475	200 – 500
SO_4^{2-} (mg/L)	1000 - 5200	180 – 1200
máx (∑Cu, Zn, As) (mg/L)	4.0	4.0

Table 4.- Range of selected physical-chemical effluent parameters, measured where algal colonization is better succeeded. Data are from Valdarcas mine.

Tabla 4.- Gama de parámetros fisico-químicos de aguas residuales medida donde la colonización de algas ha proliferado más. Los datos proceden de la mina de Valdarcas.

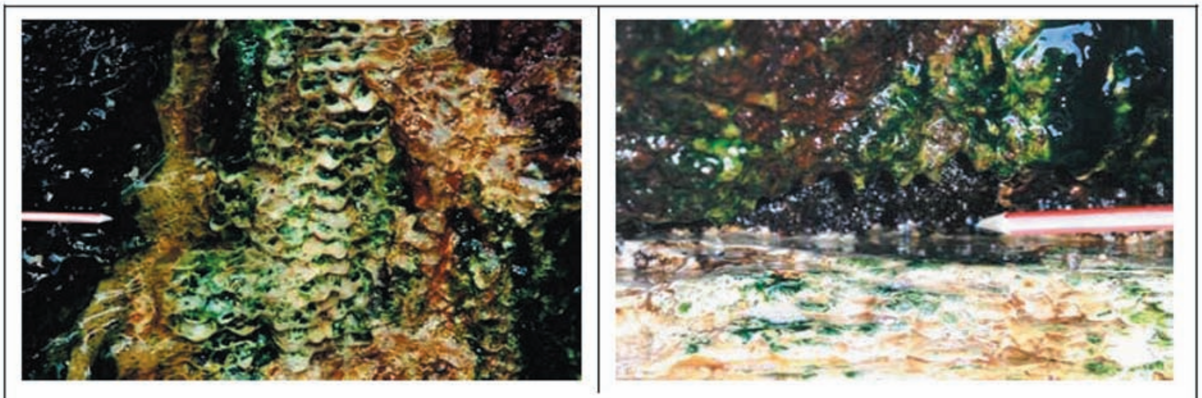
precipitation. Where *E. mutabilis* forms densely communities it is possible to observe laminated deposits with honey-comb like texture, just like the ones described by Brake *et al*. (2002) (Fig. 5b), referring to the contribution of this alga to the formation of iron-rich stromatolites in acid mine drainage systems.

5. Conclusion

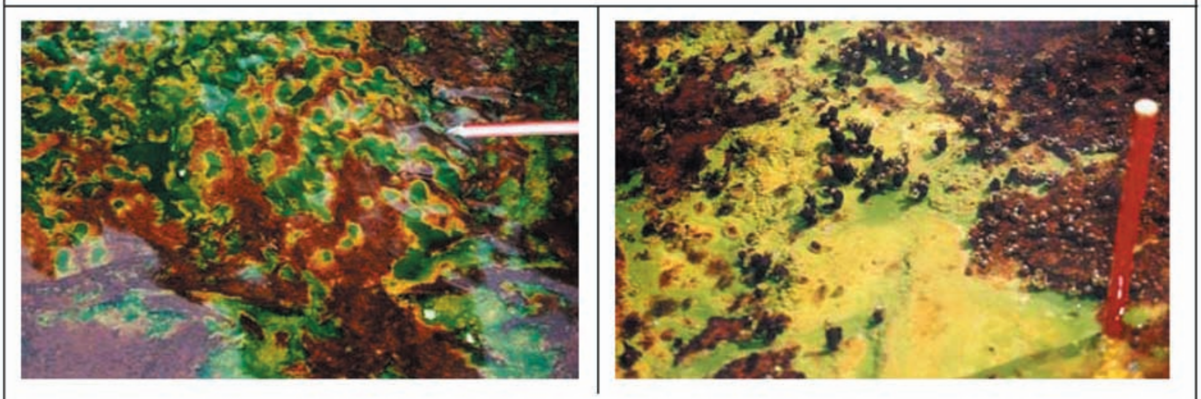
The four AMD sites studied present different degrees of contamination. This difference is expressed by physical and chemical parameters. For instance, the mean pH value varies between 3.0 (at Valdarcas) and 4.7 (at Cerquido). The pH and the chemical composition generally reflect a decrease in acid contamination from upstream to downstream due to natural attenuation. Such pattern is also expressed by acidophilic algal colonization. At Valdarcas, the abundance and strong reactivity of sulphide wastes induce the most acidic and metal-rich environment. Indeed, this is the only effluent with *E. mutabilis* colonization. The spatial distribution of *E. mutabilis* can be used to qualitatively assess water quality improvements along the effluent channel. Densely populated communities are established where effluent is more acid and metal-rich polluted.

Klebsormidium sp. is established in all the effluents. However, its abundance and dispersion can also be allied with effluent chemistry. Valdarcas presents widespread and densely populated communities while they are sparse and restricted to the upper section of the effluent channels at Adoria and Carris. *Klebsormidium* sp. is scarcely detectable at Cerquido.

In relation to the factors that control algae distribution, results indicated that *E. mutabilis* prefers to grow up on schwertmannite-rich precipitates. This statement allied to field observations about the existence of oxygenated microenvironments created by algal activity suggest that algae influence iron minerals precipitation, especially schwertmannite.



a) *E. mutabilis* in the seepages at the base of the waste-dumps, growing over a white colour precipitate of amorphous iron phosphate.



b) Submerged benthic green mats in the effluent channel, , growing over ochre colour iron-rich precipitates.

Fig. 5.- Colonization by *Euglena mutabilis*.
Fig. 5.- Colonización de la especie *Euglena mutabilis*.

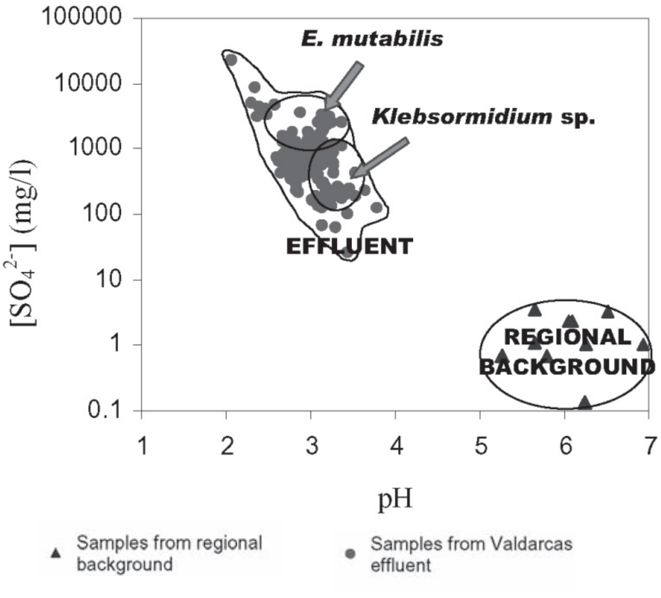
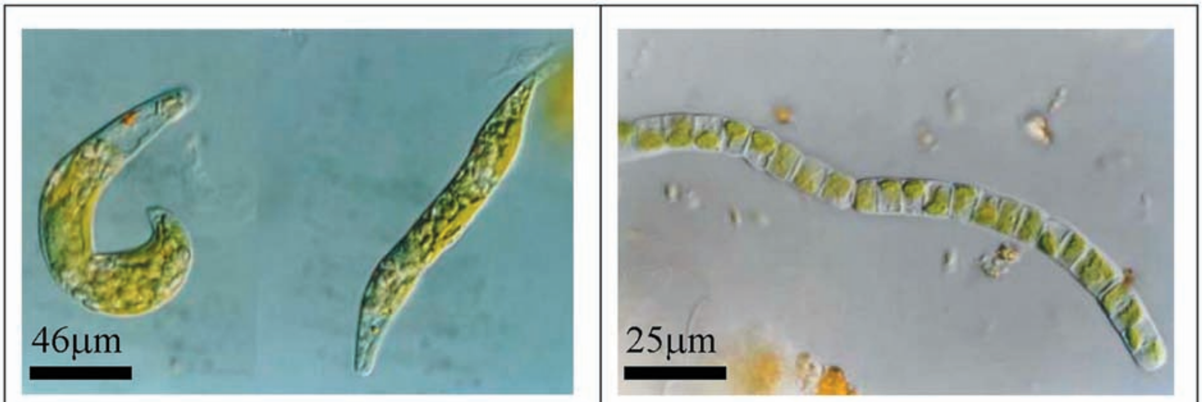


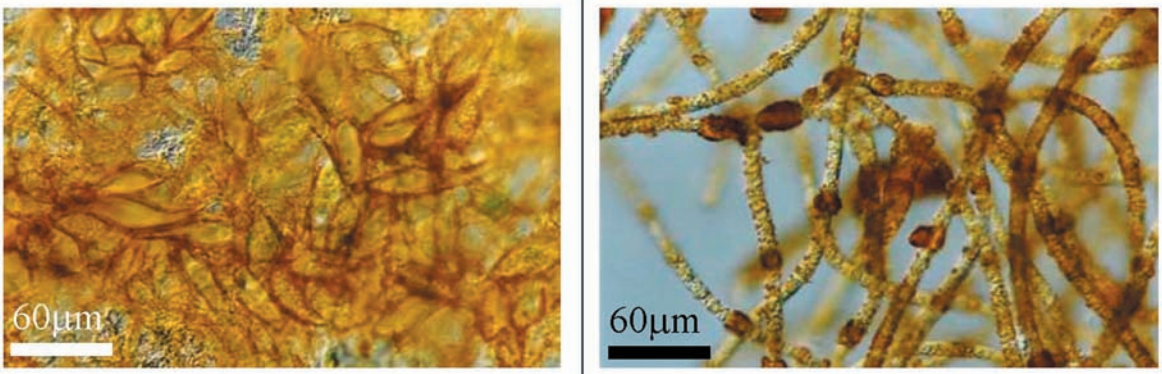
Fig. 6.- Relation between sulphate and pH at Valdarcas with projection of the conditions that support *Euglena mutabilis* and *Klebsormidium* sp.

Fig. 6.- Relación entre las cifras de sulfato y el pH en Valdarcas, con la proyección de las condiciones que favorecen las especies *Euglena mutabilis* y *Klebsormidium*



a) View of typical elongated *Euglena mutabilis* clear cells.

b) View of a typical *Klebsormidium* kinked filament.



c) Deposits of ochre precipitates at the extra-cellular polymers segregated by *Euglena mutabilis*.

d) Deposits of ochre precipitates at *Klebsormidium* cell walls.

Fig. 7.- Photomicrograph showing algal morphology and deposition of ochre iron-rich precipitates.

Fig. 7.- Fotografía con microscopio óptico de la morfología de las algas y de la deposición de precipitados ocres ricos en hierro.

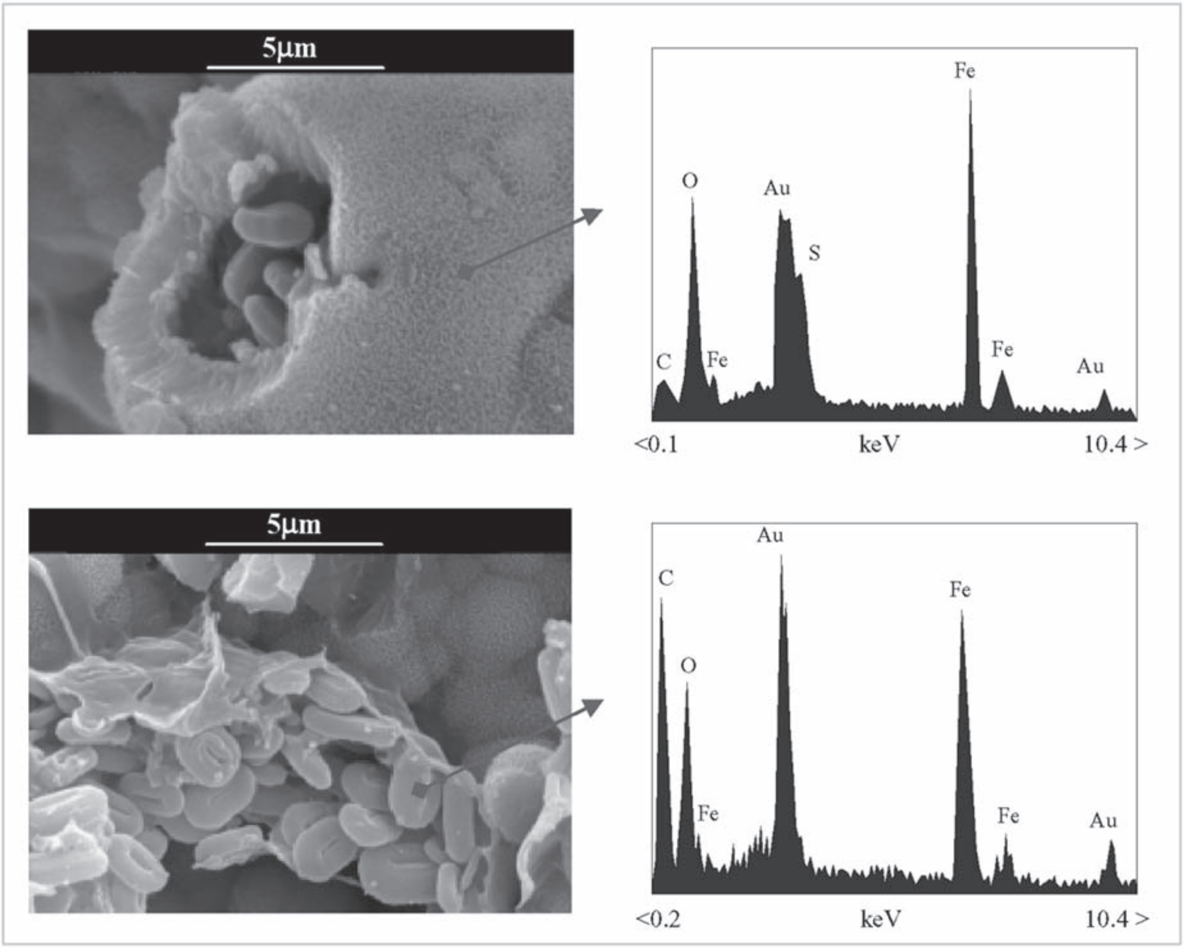


Fig. 8.- SEM (ES) micrographs and EDS analysis showing the relation between Euglenophyta and schwertmannite. The schwertmannite spheres occur with its typical "pin-cushion" morphology; inside the mineral spheres there are abundant paramylon grains.

Fig. 8.- Fotografías con microscopio electrónico de barrido (SEM) y análisis dispersivo (EDS) mostrando la relación entre las Euglenophyta y schwertmannite. Las esferas de schwertmannite aparecen con su típica morfología de "pin cushion"; en el interior de las esferas del mineral aparecen abundantes granos de paramiylon.

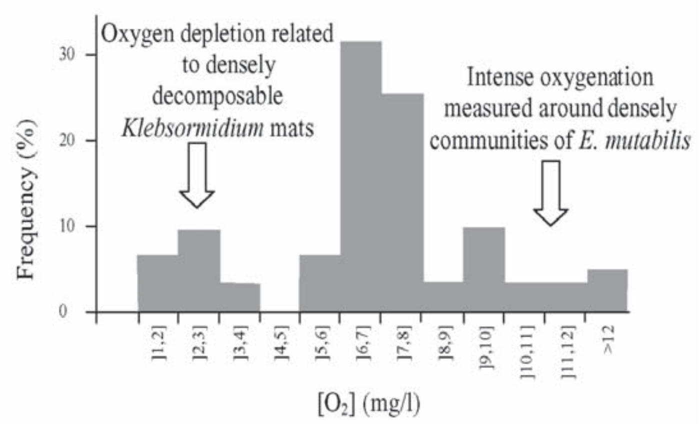


Fig. 9.- Dissolved oxygen distribution of samples from Valdarcas effluent.

Fig. 9.- Distribución del oxígeno disuelto en las muestras de aguas residuales de Valdarcas.

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