

# Biogeochemical characterization of surface waters in the Aljustrel mining area (South Portugal)

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## Abstract

Aljustrel mining area (South Portugal) is a part of the Iberian Pyrite Belt and encloses six sulfide mineral masses. This mine is classified of high environmental risk due to the large tailings' volume and acid mine drainage (AMD)-affected waters generated by sulfides' oxidation. The use of biological indicators (e.g., diatoms) revealed to be an important tool to address the degree of AMD contamination in waters. Multivariate analysis has been used as a relevant approach for the characterization of AMD processes. Cluster analysis was used to integrate the significant amount and diversity of variables (physicochemical and biological), discriminating the different types of waters, characterized by the high complexity occurring in this region. The distinction of two main marked phenomena was achieved: (1) the circumneutral-Na-Cl water type (sites DA, PF, BX, BF, RO, CB), expressing the geological contributions of the Cenozoic sediments of Sado river basin, with high diatom diversity (predominating brackish diatoms as *Entomoneis alata*); and (2) the acid-metal-sulfated water type (sites BM, JU, RJ, AA, MR, BE, PC, AF), reflecting both the AMD contamination and the dissolution of minerals (e.g., silicates) from the hosting rocks, potentiated by the extremely low pH. This last group of sites showed lower diatom diversity but with typical diatoms from acid- and metal- contaminated waters (e.g., *Pinnularia aljustrellica*). In addition to these two water types, this hierarchical classification method also allowed to distinguish individual cases in subclusters, for example, treated dams (DC, DD), with alkaline substances (lime/limestone), that changed the physicochemical dynamics of the contaminated waters.

**Keywords:** Acid mine drainage, Heavy metals, Diatoms, Cluster analysis, Iberian Pyrite Belt, Aljustrel

## Introduction

The Iberian Pyrite Belt (IPB) is one of the greatest provinces hosting massive sulfide deposits which extend from Grândola (Portugal) to Seville (Spain). These massive sulfide ore bodies contain mainly pyrite, with minor phases as pyrrhotite ( $\text{Fe}_{1-x}\text{S}$ ), galena ( $\text{PbS}$ ), sphalerite ( $\text{ZnS}$ ) and chalcopyrite ( $\text{FeCuS}_2$ ) associated (Sa´ez et al. 1999). They have been exploited in the Iberian Peninsula for more than 2000 years (Mil-Homens et al. 2016). Nowadays, numerous traces of this activity remain, such as extensive mining works and millions of tons of former tailings and slags (Matos and Martins 2006).

Acid mine drainage (AMD) is the result of sulfides' oxidation, that occurs when these minerals are exposed to air and water at the surface, which is largely promoted by mining activities. During this process, sulfuric acid is generated accelerating mineral dissolution and the release of heavy metals such as Al, As, Cd, Cu, Fe, Pb and Zn in waters. Also, some bacteria (e.g., *Acidithiobacillus ferrooxidans*) play an important role in accelerating the rate of acid generation by catalyzing the oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  (Johnson and Hallberg 2003). Thus, the AMD process is widely described in the scientific literature because it is a global and huge problem associated with mining activities that affect very seriously a large volume of water resources all over the world (e.g., Letterman and Mitsch 1978; Gray 1997; Grande et al. 2010b, 2011, 2018; van der Ent and Edraki 2018).

The impact of the AMD contamination can be assessed through the monitoring of diatom communities (Bacillariophyceae), a successful microalgal group with more than 62,000 diatom genera, species and taxa at infraspecific ranks (Fourtanier and Kocielek 2011). The ubiquity, the specific ecology of diatom species and the sampling easiness allow diatoms to be used as environmental indicators of water quality (i.e., abiotic changes, including pH—Van Dam et al. 1994). Diatoms are already well described in several mining areas of IPB (Lu´ıs et al. 2009, 2011, 2012, 2016; Valente et al. 2016a, b). Since AMD-affected waters are physicochemically variable, it has implications in the type and number of diatoms they might have. The acidophilic diatom *Pinnularia aljustrellica* Lu´ıs, Almeida et Ector (Lu´ıs et al. 2012), is an example of a well-adapted species to AMD-affected media, presenting strategies to overcome the high  $\text{H}^+$  concentration that may lead to rapid acidification of the cytosol (Gross and Robbins 2000; unpublished results). In addition, diatoms that have been pre-exposed to low pH values seem to have a greater tolerance to metals and hence are more adapted to the prevailing extreme chemical environment (Lu´ıs et al. 2014). However, diatom species can be different and respond differently in other mining areas (e.g., metal mines without AMD generation), presenting, for example, deformed (teratological) valves due to metal stress, as for example *Fragilaria capucina* var. *rumpens* (Ferreira da Silva et al. 2009).

Multivariate statistics (e.g., cluster analysis) proved to be an important method for the environmental assessment of this type of impacted scenarios due to the high diversity of geochemical parameters involved (e.g., Grande et al. 2003, 2015). Given the complexity of the area (acidic-/metal-contaminated sites vs. chlorinated sites), and the high abundance and diversity of geochemical and biological indicators, the central objective of the present work was to apply cluster analysis to characterize the surface waters surrounding the Aljustrel mining area (IPB, South Portugal) and better discriminate cause–effect relationships between the dissolved pollutants and the abundance/diversity of diatoms.

## Study area

### Geological setting

Aljustrel is located in the Alentejo province, in the south of Portugal. This region is characterized by a Paleozoic basement from the South Portuguese Zone and by a more recent sedimentary cover from the Sado tertiary basin. The NE–SW Messejana fault defines the SE border of this basin, which is confined to the NW block of the fault. This fault shows an Iberian dimension and it was responsible for the formation of a graben by the abatement of the NW block where the sediments from Sado river were deposited. Along this major structure, a

dolerite intruded in Jurassic period (Schermerhorn et al. 1987) is also observed. The Aljustrel's Paleozoic stratigraphic sequence is formed (from the oldest to the most recent) by the following main units (Andrade and Schermerhorn 1971; Schermerhorn et al. 1987; Silva et al. 1990; Leitão 1998; Oliveira et al. 2006): The IPB lower unit, the sedimentary *Phyllite–Quartzite Group* (Frasnian– Upper Famennian), mainly composed of shales and less abundantly quartzites. The IPB superimposed unit, the *Volcano–Sedimentary Complex* (VSC) (Upper Famennian–Upper Viséan), which is represented by the following units: *Paraíso Fm.*—siliceous shales, phyllites, tuffites, purple shales, jaspers and cherts; *Aljustrel Volcanic Fm.*—divided in felsitic/mine metavolcanics and megacryst/green metavolcanic sequences and globally composed of sericitic felsic volcanics, felsites, felsophyres, volcanic breccias, massive sulfides, feldspar megacryst volcanics and lavas. The Baixo Alentejo Flysch Group (Upper Viséan), a monotonous turbidite sequence mainly composed of shales and graywackes.

Structurally, the VSC in Aljustrel is represented by an anticlinorium, defining a NW–SE lineament (4.5 km length and 1.5 km across). The Aljustrel thrust is one of the main structures and materializes the SW contact between the VSC and the Flysch group. Late variscan faults with NNE–SSW direction produced important dextral strike-slip movements and are probably conjugated faults of the Messejana fault.

### **The Aljustrel mine**

The Aljustrel ore is one of the greatest volcanogenic massive sulfide deposits of IPB and well described by many authors (Barriga et al. 1997; Carvalho et al. 1999; Matos and Martins 2006; Oliveira et al. 2006; Tornos 2006). This deposit encloses six mineral masses (S. João do Deserto, Moinho, Algares, Estação, Fetais and Gavião), disposed around the city, and defining three parallel alignments (Andrade and Schermerhorn 1971; Leitão 1998). The massive sulfides are predominantly composed of pyrite and associated sphalerite, chalcopyrite, galena, antimony, sulfosalts, tin minerals and arsenopyrite (Andrade and Schermerhorn 1971; Gaspar 1996). These mines went through several periods of exploitation, one of the most important during the second half of the twentieth century for the pyrite recovery to the acid sulfuric production, which caused significant environmental impacts due to the high amounts of tailings, vol. 5 Mt (Matos and Martins 2006) disposed on the surface and exposed to weathering conditions. This promoted the easy oxidation of sulfides and the metal (loid) mobilization to soils and waters, which was intensified when these mines were closed in 1993. A rehabilitation program has been conducted by EDM (a public capital company) since 2006 until nowadays, focused on the mining-affected areas prior to 1990 (Matos and Martins 2006). After more than one decade of inactivation, in 2008 the mine restarted its activity for Zn and Cu concentrates' exploitation. Nowadays, the modern mines operating in Portugal follow the environmental normatives imposed by the E.U (Directiva nº 2006/21/CE) and by the National Portuguese Legislation (Decreto-Lei nº 31/2013) that transposed the European legislation.

### **Climate, relief and drainage system**

The region of Aljustrel is characterized by semiarid climatic conditions, with mean temperatures of 17 °C, but which can reach 49 °C in summer. The annual mean precipitation is 450 mm and rarely surpassing 600 mm. Precipitation occurs mainly during winter and more than 75% between October and March (source: IPMA). The high temperatures during summer originate very high values of evapotranspiration, surpassing 700 mm, mainly during the period from July to September (Chambel and Almeida 1998).

Generically, the region is characterized by soft reliefs; however, in the Paleozoic basement, greater altimetric amplitudes are recognized and are conditioned by the fault systems that occur there, which is also responsible for the large density of water courses (Schermerhorn et al. 1987). Therefore, the drainage system of this region is well developed and hierarchized, formed by a set of low slope and shallow ephemeral streams, which dry during summer: Água Azeda and Barranco do Farrobo. Água Forte stream is larger and is the most affected by the mine (Fig. 1), converging to the main water course of this area, the Roxo stream, draining into the Sado river.

### **Methods**

Sampling, preservation and preparation of samples

Sampling of surface waters and diatom assemblages was carried out in 18 sites (Fig. 1) during spring, and after the rainy season. Both surface waters under the mine influence (with clear evidence of AMD contamination) and outside the mine influence were collected. The ephemerality of many of these streams does not allow a seasonal sampling per hydrological year in all of the sites, since this is a region subjected to a negative hydric balance, where some of the channels only carry water during the rainy season and, in case of streams coming from tailings, a few more weeks, in response to the discharge of accumulated water in pounds or from the underground mine. Also for diatoms, spring is the best season to collect, due to the best stability and diversity of the communities.

To determine physicochemical parameters of surface waters, a volume of 1 L was collected in acidrinsed polyethylene bottles, and sampling was made as close as possible to the central part of the stream. Temperature (T, °C), pH and electrical conductivity were recorded on site, using a multiprobe WTW® Multiline P4 SET. Water samples were then returned to the laboratory in a cool box. Immediately after samples' arrival to the laboratory, a volume of 250 mL was taken from each and filtered through 0.45  $\mu$ m Millipore membrane filters using an all-plastic pressurized filtration system. A subsample of these filtered waters, preserved with ultra-pure nitric acid (HNO<sub>3</sub>) to prevent metal precipitation and bacterial growth, was used for trace and major cation analysis, and another portion, non-acidified, for anion analysis. Samples were then stored at 4 °C until being analyzed.

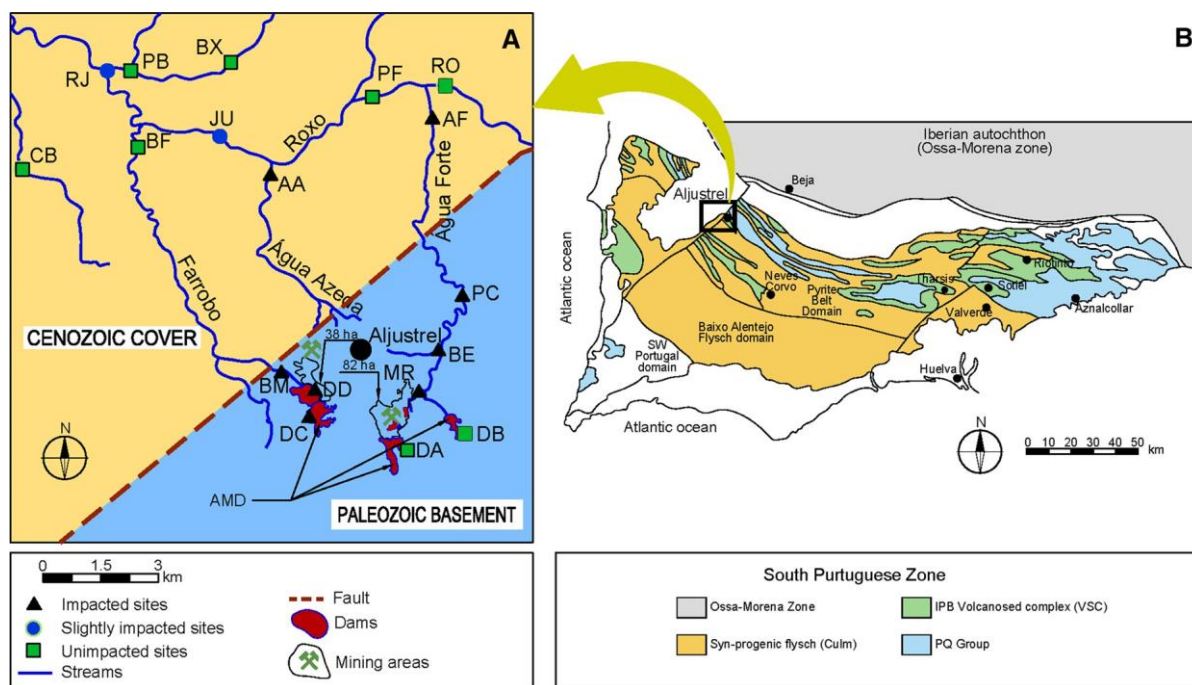


Fig. 1 a) Simplified geological map of the location of the sampling sites in the streams of Aljustrel mining area. b) Location of Aljustrel in the Iberian pyritic belt (IPB), showing IPB geology evidencing the three existent structures in Aljustrel: VSC, PQ and Culm

Epipsammic diatom samples were collected by removing the top layer of the sediment surface with a syringe. Following the sampling protocol (Prygiel and Coste 2000), pools of stagnant water and shaded sites were avoided. Two samples were taken, one kept alive and the other preserved with formalin solution (5% final concentration).

#### Analytical methods

The chemical analysis of water samples was carried out by inductively coupled plasma mass spectrometry (ICP-MS) for determination of 14 elements (Al, As, Ca, Cd, Cl, Cu, Fe, K, Mg, Mn, Na, Pb, Si and Zn) in ACME laboratories (ACME Anal. ISO 9002 Accredited Lab, Canada). Sulfate was determined by ion chromatography

and total N by spectrophotometry in the Geochemistry Laboratory of the Geosciences Department at the University of Aveiro. A rigorous quality control program was implemented during water chemical analysis, which included reagent blanks, duplicate samples and certified reference materials (STANDARD WASTWATERD5). The precision and bias error of the chemical analysis were less than 10%.

Live diatom samples were examined to exclude the possibility of the presence of dead diatoms to avoid abundance errors. From the other set of samples (preserved with formalin), an aliquot (after cleaning off formalin) was treated with HNO<sub>3</sub> (65%) and potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) at room temperature for 24 h, followed by three centrifugations (1500 rpm) to wash off the excess of acid. Then, permanent slides were prepared using Naphrax<sup>®</sup>. Diatoms were identified and semi-quantified (400 valves per sample) under a light microscope (Leitz Biomed 20 EB) using a 100X objective (N.A. 1.32). Taxonomy was based on Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Round et al. (1990) and Prygiel and Coste (2000).

### Statistical analysis

For the statistical treatment of the 18 physicochemical (Al, As, Ca, Cd, Cl, Cu, Fe, K, Mg, Mn, Na, Pb, Si, Zn, total N, pH, conductivity, SO<sub>4</sub><sup>2-</sup>) and two biotic (*N<sup>a</sup>* species and %*P. alj.*) variables, a data matrix was developed (20 variables 9 18 sites). Then, a pre- analysis of the physicochemical parameters was done, integrating data in an X–Y Excel graph. A simple numerical factor adjustment was done; each variable was adjusted in a range of 500 to display the data in the same graph (first, the concentration units were homogenized to mg/L) in order to see the different concentrations of the physicochemical parameters in each sampling site.

The statistical summary of variables, as well as the linear cluster of the hydrochemical–biological variables and the linear cluster of the sampling points, were obtained with nonstandardized/adjusted data (just homogenized to mg/L). When applied to a set of variables, cluster analysis orders and classifies them in the most homogeneous groups possible based on the similarity of the variables themselves (Grande et al. 2003) and then, to propose a hydrochemical operation model, based on the grouping of variables in different subclusters, which are related with the physicochemical characteristics of the waters.

Data processing, including statistical summary, was done with the software package Statgraphics Centurion XVI, by applying the Ward method or ‘second-order central moment’, which is a hierarchical method that calculates the mean of all the variables for each cluster; after, it calculates the Euclidean distance between each factor and the average of its group and then adds the distances from each case. In each step, the clusters obtained are those that yield the smallest increment in the total sum of the intracluster distances (Bisquerra 1989; Grande et al. 2003). The application of this technique, in this study, helped to classify the variables into different ‘categories’ as follows.

Figures 3 and 6 were plotted using AUTOCAD vs. 2013.

## Results and discussion

### Spatial distribution of the environmental variables

The statistical summary of 18 physicochemical variables, number of diatom species and relative abundance (in %) of *P. aljustrellica* at the 18 sites is shown in Table 1. In general, the statistical summary clearly evidences the existence of a complex water system expressed by the high variability of either physicochemical and biological parameters in the analyzed waters (see standard deviation and variance) that are much higher than would be expected for typical continental waters occurring in similar geo-climatic domains. These abrupt variations point out the existence of severe cases of contamination in some sites, highlighted by the high concentrations of heavy metals (e.g., Al, As, Cu, Fe, Mn, Pb, Zn), SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup> and electrical conductivity (Cond), with an evident high pH range (1.9–8.4).

In order to better understand the spatial data variability, a projection of the physicochemical and biotic parameters (20 variables) was done according to the 18 sampling sites (Fig. 2). Two main types of waters can be distinguished:

*Type 1:* Acid–metal–sulfate waters: extremely low values of pH (between 1.9 and 4.9), very high electrical conductivity (highest values above 17,000  $\mu\text{S}/\text{cm}$ ), as well as extremely high contents in sulfate (up to 28,239 mg/L), and in dissolved heavy metals (up to 6173 mg/L of Fe, and 348 mg/L of Cu). Diatoms' diversity is low, having species as *Pinnularia acidophila*, which normally occurs in pH <7 (discovered in an opencast low pH mining lake by Krammer 2000); or as *P. aljustrellica*, first discovered by Luís et al. (2012) showing an ecological pH preference of 2–3 (Luís et al. 2009, 2011, 2016). The values assessed and the geological mining context of the sampling area, allow to link this condition to the process of AMD generated by the Aljustrel mine (Chambel and Almeida 1998; Luís et al. 2009, 2016)

(Fig. 3). The stream sites MR and BE, and the DC dam, all situated in Água Forte stream, are the most contaminated and also the most representative of this water type.

*Type 2:* Circumneutral–Na–Cl waters: In this case, the surface waters not affected by the mining activity, presented a circumneutral to alkaline pH and high electrical conductivity values for inland waters, with maxima above 2200  $\mu\text{S}/\text{cm}$  and minimum never lower than 570  $\mu\text{S}/\text{cm}$ . These values are related to the high chloride ( $\text{Cl}^-$ ) concentration with a maximum of 1295 mg/L. These high values of Cl and therefore of electrical conductivity are the water response to the dissolution process of salts precipitated on the Cenozoic sediments, probably due to the existence of disseminated chlorides precipitated in these sediments during extremely dry periods (Schermerhorn et al. 1987) (Fig. 3). These conditions are also responsible for the presence of brackish to marine species as *Entomoneis alata* (Luís et al. 2016).

Table 1 Statistical summary of the 20 variables analyzed in the 18 sampling sites (= 18 samples) in spring (all concentrations are in mg/L except for Al, As, Cd, Cu, Fe, Mn, Pb, Zn, Si that are in  $\mu\text{g}/\text{L}$ )

Variables	Average	SD	% Variance	Minimum	Maximum
Al	189,730	512,966	270	1	1,816,839
As	3944	12,243	310	2	48,507
Ca	236	213	90	35	727
Cd	328	704	215	b.d.l.	2487
Cl	403	293	73	82	1295
Cu	36,619	96,915	265	2	347,780
Fe	641,158	1,742,080	272	10	6,173,473
K	561	1615	288	1	5000
Mg	243	316	130	22	1083
Mn	34,460	66,565	193	b.d.l.	203,603
Na	170	90	53	59	442
NT	b.d.l.	1	220	b.d.l.	2
Pb	243	976	402	b.d.l.	4152
Si	20,334	25,347	125	261	88,736
Zn	159,143	346,903	218	1.7	1,201,542
pH	5.8	2.3	40	1.9	8.4
$\text{SO}_4^{2-}$	3809	7829	206	27	28,239
Cond	3996	4335	108	570	17,140
Nº species	18	12	71	0	41
% <i>P. alj.</i>	15	32	223	0	95

*b.d.l.* Below detection limits, *NT* Nitrogen (Total),

*Cond* Conductivity ( $\mu\text{S}/\text{cm}$ ), *Nº species* Number of species, %*P. alj.* Percentage of *Pinnularia aljustrellica*

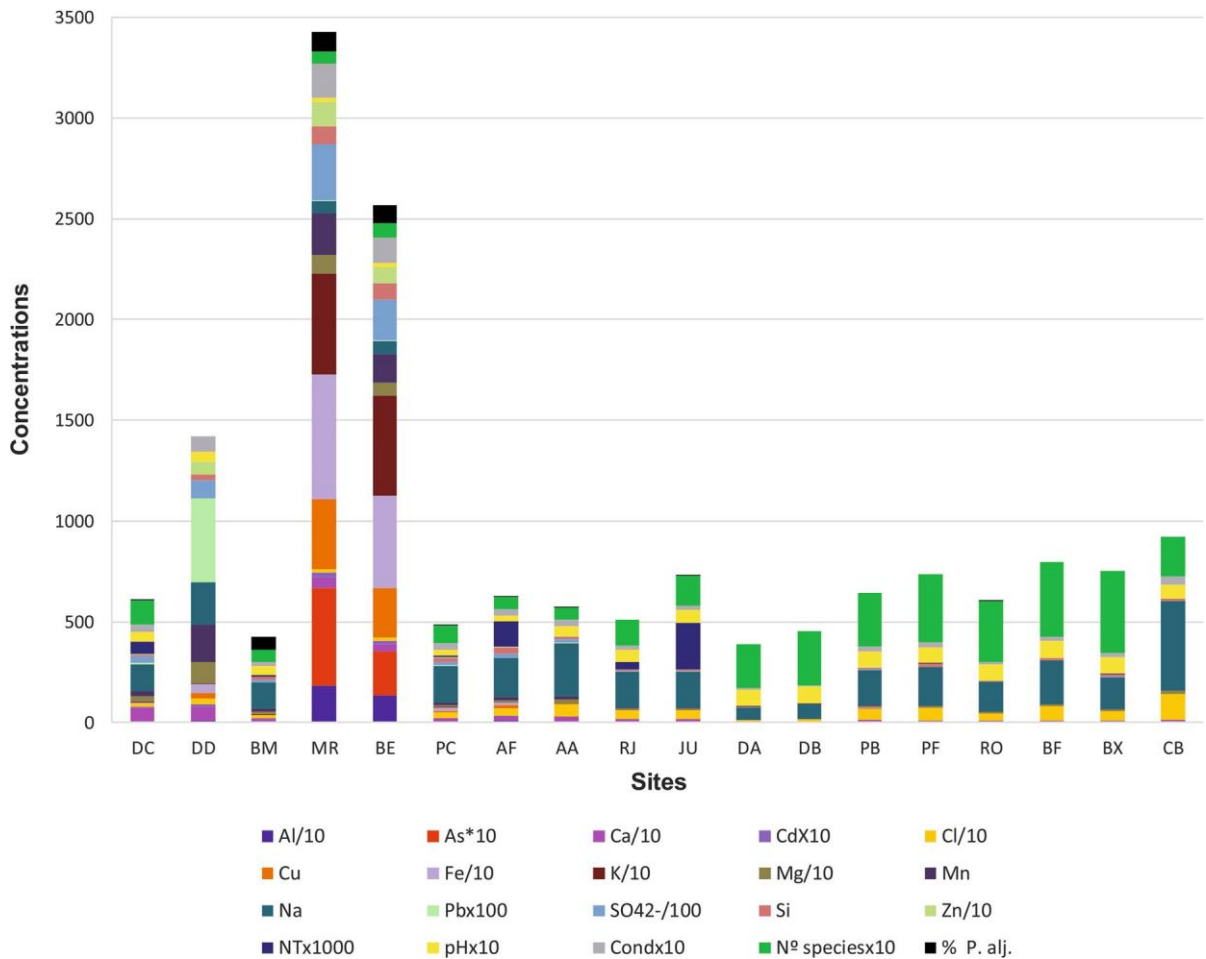


Fig. 2 Spatial evolution of the physicochemical and biological parameters in each sampling point (on the graph the, sites are assigned in relation to mine contamination: *Contaminated sites*—DC Mine dam, DD Este reis DAM, BM Barranco Morgado, MR Monte Ruas, BE Porto Beja, PC Ponte Curval, AF Água Forte, AA Água Azeda, *Intermediate sites*—RJ Roxo Jusante, JU Jungeiros, *Uncontaminated sites*—DA Águas Industriais dam, DB Monte Ruas dam, PB Pero Bonito, PF Porto Ferreira, RO Roxo, BF Barranco Farrobo, BX Barranco Xacafre, CB Canal Barrada)

## Biogeochemical characterization of physicochemical and biological parameters from cluster analysis

The dendrograms corresponding to the spring sampling campaign are presented in Figs. 4 and 5. In general, an evident grouping of the variables in subclusters suggests different causality contamination processes of the hydrological environment.

### *Circumneutral-Na-Cl waters*

The dendrogram of Fig. 4 shows two main clusters. The first one (SC1), at the far-right end, grouping together Cl, Na, pH and the number of species revealing the close relationship between these variables, i.e., the association of Na and Cl, indicating the salts dissolution (halite) that occurs in the Cenozoic sediments of Sado Basin (Schermerhorn et al. 1987), with higher concentrations in the most alkaline waters.

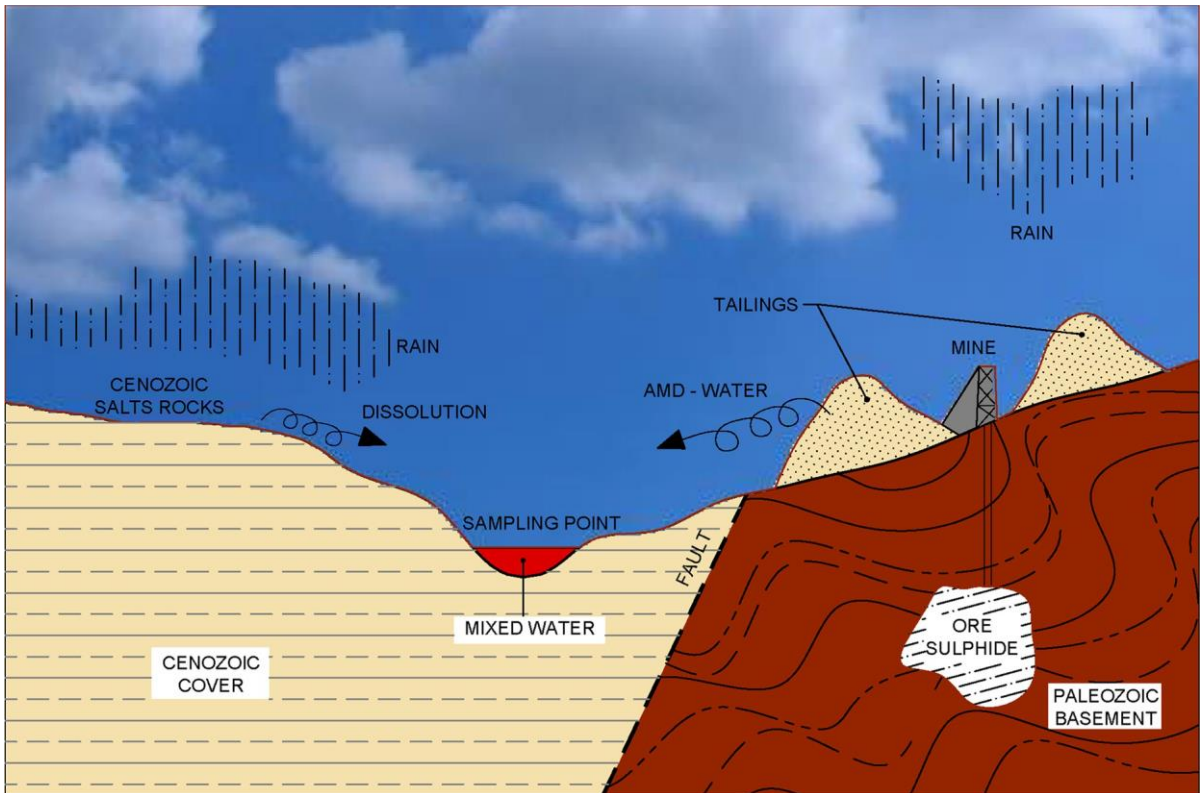


Fig. 3 Dissolution process of precipitated salts on the Cenozoic sediments, due to the existence of natural disseminated chlorides precipitated on these sediments during extreme dry periods, combined with the AMD water, resulting in mixed waters from both processes

pH here indicates a closer relationship with the diversity of diatom communities ( $> \text{pH} > N^{\circ}$  species). This fact can indicate a relationship of dependence between the high number of diatom species and the high chlorinity of the medium under study, pointed out on the dbRDA analysis in Luís et al. (2016). In fact, pH is the parameter that most directly influences the number of species present in the waters of the sampling points, as evidenced by Pearson's proximity ratio in the dendrogram extracted from the correlation matrix. This fact, described by Luís et al. (2016) in the same scenario, is now better supported by the application of cluster analysis as a widely applied tool for the analysis of AMD-affected waters (Grande et al. 2010a, b, 2015).

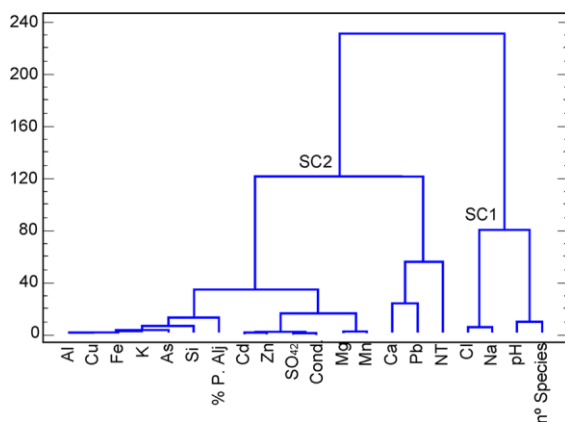


Fig. 4 Dendrogram resulting from the cluster of the physicochemical and biological variables using the Ward, Euclidean Square Method

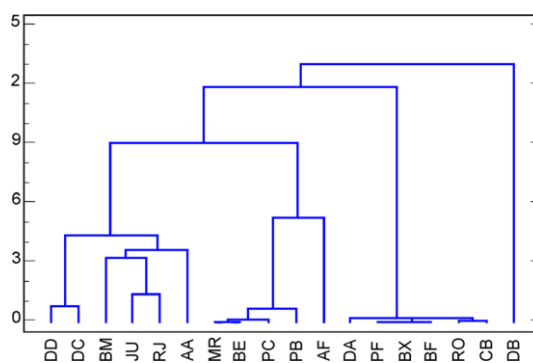


Fig. 5 Dendrogram resulting from the cluster of the sampling sites using the Ward, Euclidean Square Method

### Acid–metal–sulfate waters

The second cluster (SC2) is composed by the remaining parameters considered in this analysis, showing very high proximity ratios (distance close to zero in the dendrogram—Fig. 4). Basically, this cluster reflects the water contamination by AMD, characterized by high metal(loid) and sulfate contents, and materialized by very high electrical conductivities, since sulfuric acid generation by oxidation of the existing sulfides in the mining tailings, causing the immediate decrease in pH to values close to 2, as well as the progressive dissolution of the minerals from the ore and the hosting rocks.

On the left part of SC2, a subgroup consisting of the variables Al, Cu, Fe, K and As, closely associated with Si, represents either the oxidation of paragenesis sulfides as pyrite, chalcopyrite and arsenopyrite from the mineralization (Andrade and Schermerhorn 1971; Gaspar 1996), or the dissolution of aluminosilicates (e.g., feldspars) from the hosting and country rocks that contact with the acid waters. An important aspect of this group is the association of metal(loid)s with *P. aljustrellica*, a well-adapted species to this type of contaminated waters. Also the association of this species with Si explains the great success of *P. aljustrellica* to survive in this type of environments through a reinforcement of the siliceous frustule, as hypothesized previously by Luís et al. (2016).

On the right side of this previous subgroup, another one composed by the variables Cd, Zn, SO<sub>4</sub>, electrical conductivity (Cond.), Mg and Mn reflects the typical characteristics of AMD media. In fact, sulfate is grouped with conductivity, as expected since this anion is the main contributor to the high ionic content of this type of waters (Nordstrom and Alpers 1999; Grande et al. 2010b). The association of Cd and Zn indicates their similar provenance from the ore paragenesis (i.e., sphalerite). Despite the chemical similarities of Zn and Cu, as well as the high concentrations of both metals in these waters (Table 1), they are not grouped in the same subcluster,

which can be due to the preferential partitioning of Cu to melanterite solid solution during its precipitation when drier periods prevail (Alpers et al. 1994a). In turn, Cu and Fe are within the same group reinforcing this interpretation. On the other hand, the main sources of Mn and Mg are rocks from the VSC, particularly the Mn–Fe jaspers and the tuffs for Mn and the ferromagnesian minerals of the volcanic rocks. Magnesium and Fe, which are two of the most important cations in this type of acid mine waters (e.g., Durães et al. 2017), are located in separated subclusters. For similar reasons, as pointed out for Cu and Zn, also Mg and Fe show different behavior in relation to sulfate precipitation; since at very low pH, the prevalence of  $\text{Fe}^{2+}$  promotes the precipitation of melanterite, whereas with  $\text{Fe}^{2+}$  depletion,  $\text{Fe}^{3+}$  favors the precipitation of Fe-oxyhydroxides, and the precipitation of Mg sulfates is facilitated due to the relative increase in Mg in solution (Alpers et al. 1994b; Romero et al. 2006). In addition, Mg sulfates show higher affinity for the incorporation of Zn and Cd than Fe sulfates (Romero et al. 2006), explaining their association in the same subcluster.

*Lime-treated waters* A third subgroup that associates the variables Ca and Pb is not so easily justified, but the most reasonable explanation is the addition of lime/limestones to the acid waters of dams that receive underground mine water, as well as the runoff tailing waters. In fact, the highest values of Pb and Ca were determined in DC and DD dams, where the pH values were low and where Ca compounds were added, thus explaining this association in the dendrogram. The sporadic addition of these materials is not enough for facing the continuous discharge of acid waters to the dam and therefore to reach the neutralization. Thus, the persistent low pH allows metals (e.g., Pb) to remain in solution, as well as the solubilization of Ca from carbonates. Within this group, at greater distance from the variables associated with the mining processes, total N was found.

Thus, the integration of both biotic and abiotic parameters of waters collected in the same region enabled the distinction of two types of hydric contaminations (Fig. 4). On one hand, the Na-Cl type, in response to a geogenic source. Worthy of notice, are the higher Cl and Na values (1295 mg/L for Cl and 442 mg/L for Na—Table 1) in comparison with the values of these elements in underground waters of the region (Schermerhorn et al. 1987), where the period of water–rock interaction is higher than in surface waters, or even by comparison with AMD-contaminated waters from the IPB (e.g., Dures et al. 2017), where concentrations of all elements are usually extremely high due to the very low pH values. On the other hand, the acid – metal – sulfate type, resulting from the weathering of unrestored mining tailings.

#### Biogeochemical characterization of sites from cluster analysis

In the dendrogram of Fig. 5, the sampling sites were taken as variables, where two main groups of samples were achieved, i.e., the sites affected by AMD (DD, DC, BM, JU, RJ, AA, MR, BE, PC, AF and PB) and the uncontaminated sites (DA, PF, BX, BF, RO, CB). This separation is in accordance with the previous distinction of AMD-contaminated and uncontaminated sites (Luís et al. 2016). Site DB, which is projected as an independent variable, may reflect the geological contribution, since this site and DA are uncontaminated dams located in the Paleozoic basement. However, DA is a waterproof/confined dam where the water–rock interaction is negligible. Site PB showed an anomalous behavior here, apart from the other sampling sites (see Luís et al. 2016), located close to sites with higher metal(loid) concentrations.

#### *Contaminated sites*

In the cluster grouping the AMD-contaminated sites, two subclusters are evidenced, being the severity of the contamination process the main distinction: the first subcluster, encompassing DD, DC, BM, JU, RJ and AA sites with pH values ranging from 4.3 to 6.4. The DC–DD site association should be of notice, since these are two contaminated dams where lime/lime-stone treatments are performed. The association of JU and RJ, located in Roxo stream, is justified due to their lower AMD contamination levels compared to the remaining sites of this subcluster. The second subcluster, comprising MR, BE, PC and AF, shows pH values ranging from 1.9 to 2.9, with diatom communities composed mostly by *P. aljustrelica*. All these four sampling sites are located in Água Forte stream, and their position order in the dendrogram (Fig. 5) highlights the increase in the mine distance (the contamination source), and respectively, the reduction in the contamination (Fig. 6).

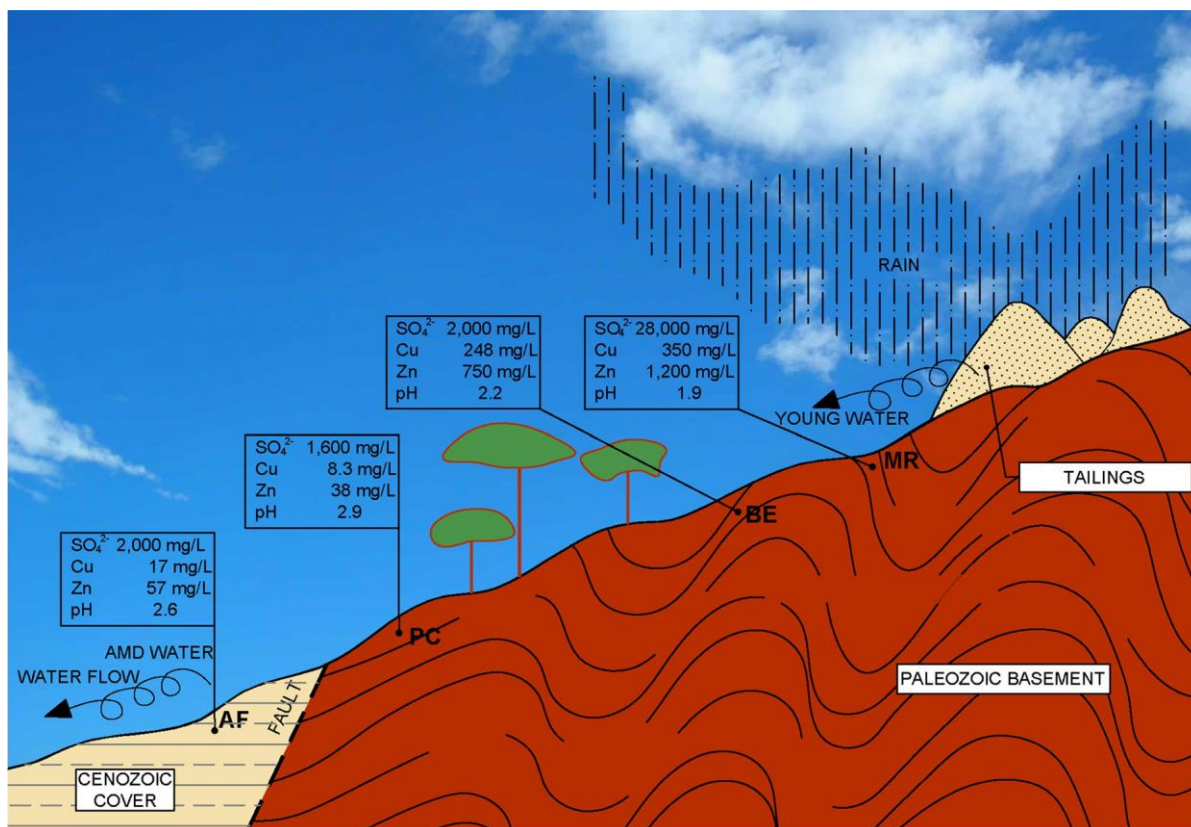


Fig. 6 Schematic representation of the evolution of most important physicochemical parameters (metals: Cu, Zn),  $\text{SO}_4^{2-}$  and pH) in the most AMD-contaminated sites of A'gua Forte stream: MR → BE → PC → AF. → increasing distance from the mine (see Fig. 1)

Água Forte stream evolves downstream to lower values of sulfate and metallic load and higher values of pH. However, in the most downstream site (AF), lower pH values were found again. This phenomenon is explained by the processes of re-dissolution with the hydrolysis of the new precipitated salts between these last two sites. The transformation of ferrous to ferric iron, using the oxygen of the water, occurs with the release of hydrogen ions; thus, more protons are incorporated in the water, decreasing the pH. However,  $\text{Fe}^{3+}$  is a strong oxidant, which may produce even more acidity than by  $\text{O}_2$  mediation (Singer and Stumm 1970). The presence of acidophilic bacteria also causes the acceleration of  $\text{Fe}^{2+}$  oxidation ( $\text{pH} < 3$ ), where  $\text{Fe}^{3+}$  is the only effective oxidizer of pyrite (Singer and Stumm 1970).

#### Uncontaminated sites

The cluster composed by sites DA, PF, BX, BF, RO and CB represents the waters with circumneutral to alkaline pH values (6.8–8.3) and high Cl concentrations (400–1295 mg/L), which is due to the response of the hydrological medium to the natural dissolution of the Cenozoic salt crusts (Schermerhorn et al. 1987). The saline conditions of the waters from this group allowed the colonization by brackish to marine diatom species as for example *E. alata* (Lu'is et al. 2016), typical from marine environments.

#### Conclusions

In mining areas, particularly with high amounts of exposed sulfide minerals, the geological system is greatly disturbed, due to AMD production. The extremely low pH of mine waters increases the dissolution ability of

minerals in a short period of time, even the most weathering resistant minerals. In addition, the dry and rainy season cycles have a great influence in the water geochemistry due to evaporation–concentration–precipitation during dry periods and due to dissolution–concentration after rainfall events. Therefore, sampling was always performed during spring in order to avoid differences in results due to seasonality alone.

Multivariate analysis, especially cluster analysis, has been confirmed as a useful tool for characterizing AMD processes by grouping physicochemical variables, according to each element's influence over the others and revealing the influence of the AMD process on them. In this study, biological parameters were also considered. The results obtained allowed to confirm and validate previous considerations (Luís et al. 2016), and better discriminate some elements' associations in a complex hydrological system composed by distinct types of waters, with direct influence on the diatoms communities. Cluster analysis also allowed to point out the behavior of the elements' association in consequence of mineral precipitation/incorporation processes or, of dissolution processes in AMD/chlorinated waters.

The most relevant conclusion was the distinction of two main and marked situations in this region: (1) the circumneutral-Cl–Na water type, expressing the geological contributions of the Cenozoic sediments, with greater biotic diversity regarding the diatom communities; and (2) the acid–metal–sulfated water type, expressing the AMD effects of sulfides' oxidation and the dissolution of other minerals (e.g., silicates) from the hosting rocks, showing less diatom diversity but with specific bioindicators of acid and metal-contaminated waters. The coexistence of these two processes configures a particular scenario, very different from those described by numerous authors in all the Iberian Pyritic Belt, where the Cenozoic salt cover is inexistent.

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