Biogeochemical indicators (waters/diatoms) of acid mine drainage pollution in the Odiel river (Iberian Pyritic Belt, SW Spain)

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Abstract

Odiel river basin is located in the Iberian Pyritic Belt (IPB) and mostly of its tributaries are severely affected by acid mine drainage (AMD). It is originated when pyritic minerals from abandoned mines, especially mineral residues from waste rock dams, get in contact with air and water. Fifteen sampling points were chosen to analyze interactions between diatom communities and water hydrogeochemistry. Considering physicochemical characteristics, sampling points were assigned as highly, moderately, and unpolluted by AMD. No correlation was observed between ecological diversity indexes and physico-chemical parameters. However, a dependency relationship between diatom species distribution and specific pH, conductivity, redox potential, sulfate, and metal concentrations was observed. Cluster analysis based on Pearson correlation and *rs* values of the non-parametric Spearman correlation allowed to identify *Pinnularia acidophila*, *Pinnularia subcapitata* var. *elongata*, and *Eunotia exigua* as the main bioindicators of AMD-polluted Odiel streams. Finally, a principal component analysis led to associate the most abundant diatoms species to specific physico-chemical parameters.

Keywords: Diatoms, Ecology, Acid mine drainage, Hydrogeochemistry, Odiel river basin, Multivariate analysis, Diversity indexes

Introduction

The Iberian Pyritic Belt (IPB) is probably the most important massive sulfide region in the world. IPB is located in the SW of the Iberian Peninsula and has about 200 km long and 40 km wide. This mining region run from the northwest of Seville (Spain) to the Lousal area near Grandola (Portugal) (Saez et al. 1996; Tornos et al. 2009; Inverno et al. 2015). From antiquity (about 3000 BC), IPB has been exploited for its deposits of copper, gold, silver, and other metals (Leblanc et al. 2000; Olias and Nieto 2015). More than 80 mines are distributed in the region, although most of them were abandoned (Grande 2016). Over the years, especially from midnineteenth century to end-twentieth century, mining activity has dramatically altered the landscape of the region, today covered by open pits, waste rock dams, and abandoned mine galleries. This situation has produced a strong environmental problem, namely acid mine drainage (AMD), because of the exposition of reduced sulfide to the air (oxygen) and water (Grande et al. 2011; 2018).

In such a scenery, old mine activities exposed and released fragmented minerals facilitating the growth of on and/or sulfide/sulfur-oxidizing bacteria, as *Acidithiobacillusferrooxidans*, *Leptospirillum ferrooxidans*, and *Thiobacillus thioxidans*, which accelerate AMD processes and mineral biolixiviation in the rivers crossing the IPB (Lopez-Arcilla and Amils 1999; Lopez-Arcilla et al. 2001). Thus, Tinto and Odiel rivers, the main rivers crossing IPB and running towards the Atlantic Ocean, have a pH of 2‑4 and a high conductivity and oxidant redox potential, due to the huge amount of sulfate and metals (Fe, Zn, Al, Mn, Cd, As, etc.) transported in their waters (Nieto et al. 2007; 2013).

The hydrogeochemistry and microbial ecology of the Tinto river are very well known after the many reports from Dr. Ricardo Amils (Center of Astrobiology of Madrid) and other researchers (Zettler et al. 2002; Amils et al. 2007; Grande et al. 2000; 2011; Aguilera 2013; Nieto et al. 2013; Quatrini and Johnson 2018; Tejada et al. 2020). However, the Odiel river basin is extraordinarily complex, since many old mine rejects as well as AMD-polluted waters run to its course, also receiving non-polluted waters from a diversity of clean streams. Thus, 37% of the total length of the Odiel watershed is affected by AMD (Sarmiento et al. 2009), and the river transports a huge quantity of metals towards the marshes at its mouth, close to Tinto river (Grande et al. 2003; Olias et al. 2006; Nieto et al. 2007). Moreover, climatic conditions, especially rainfall, are the most important external controlling factors of the type of mining pollution. The study area is characterized by a semi-arid climate from Mediterranean type. A noticeable mark from the Odiel river basin is the negative hydric balance, since average evapotranspiration is around 900 mm/year, while the average annual rainfall is around 750 mm/year. This condition explains the existence of ephemeral streams which only transport water during rains. Consequently, the levels of suspended or dissolved metals and the value of pH in these polluted waters depends on the weather (Olias et al. 2004; Canovas et al. 2008; Sarmiento et al. 2009). Adapted species must be able to cope with these harsh conditions and, consequently, be considered as bioindicators of these limiting and unstable environments.

In such a condition, the diversity of life is severely restricted. The AMD effects on aquatic ecosystems are twofold: (a) impacted communities experience lethal levels of pH and metals, which lead to a decrease in algal species richness and diversity (i.e., Mulholland et al. 1986; Verb and Vis 2001); (b) communities are restricted to tolerant organisms, which are able to survive in these conditions. However, diatoms, the most cosmopolitan algae group, are poorly investigated in the Odiel river basin, despite their relevant role as bioindicator of water quality as it is written in the Water Framework Directive: diatoms are used to assess the ecological quality of rivers (European Union 2000; Battarbee et al. 2010; Lobo et al. 2016).

Benthic algae as diatoms are the main biologic components of aquatic ecosystems, since they are in the forefront of food web serving as food for other organisms. They act as environmental indicators to assess physical, chemical, or biological perturbations. Spanish normative for environmental quality (RD 817/2015) recommends a minimum of one diatom sampling per year in the rivers to complete the surveillance control program.

The main goal of this work was to analyze the diatom communities in a wide representative sampling network of the Odiel river basin, including both non-polluted waters and differentially AMD-affected streams. Multivariate statistics and diatom’s diversity indexes were used to verify diatoms’ utility as bioindicators of AMD-affected streams and the interactions/relationships among them and/or the other parameters.

Materials and methods

To achieve the described objective, water samples were sampled to be characterized hydrogeochemically and biologically.

Sites selection, sampling, and physical–chemical analysis

Representative sampling sites were meticulously chosen to guarantee that cover a wide pH range (each point is georeferenced in Fig. 1). The sampling took place in February and March of 2019 (corresponding to Spring in this Mediterranean climates), at the end of the rainy season when all the described sub-basins carry water (Fig. 2).

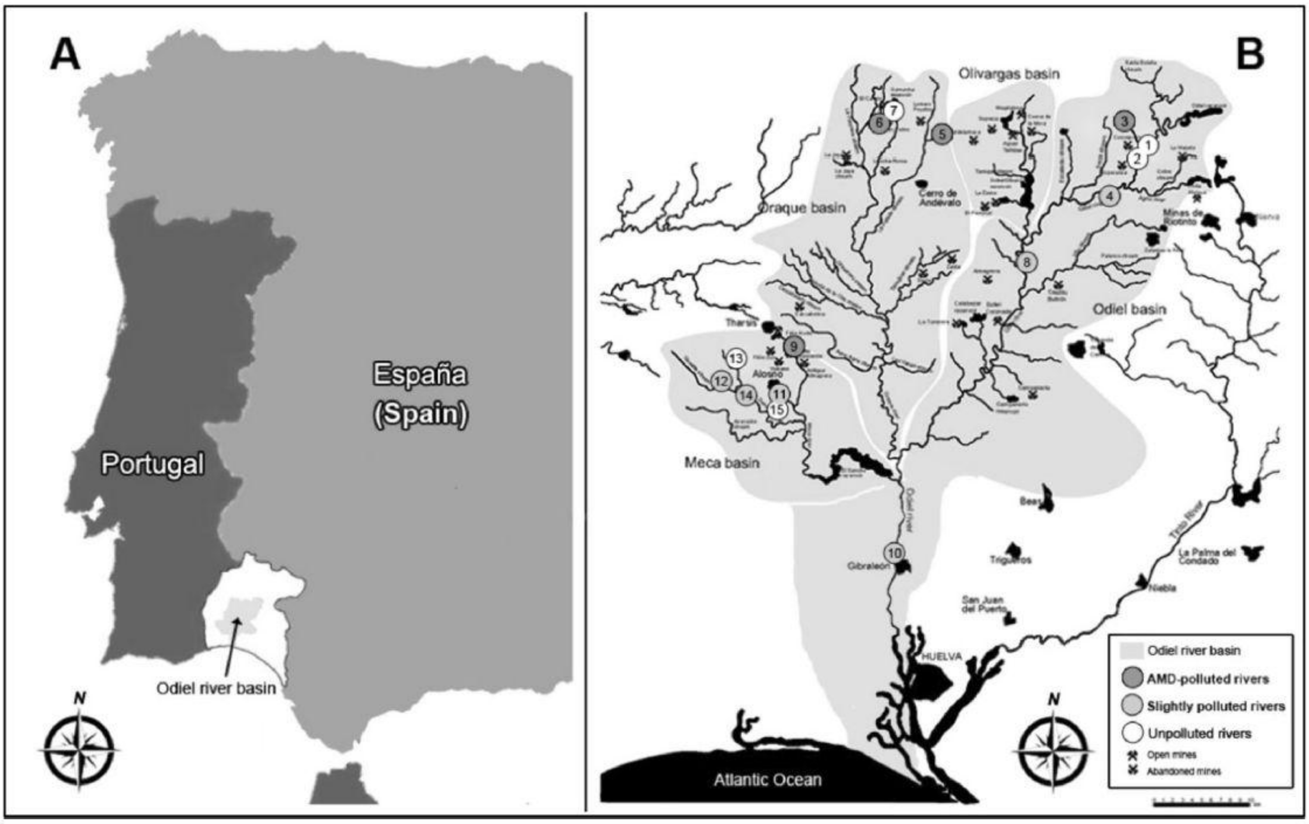

Fig.1. Location of the sampling points. A The location of the Odiel river basin in SW Spain, close to Portugal, is shown. B Sampling points and the pollution degree are indicated by circles

The main stream (Odiel river) corresponds to sites 1, 2, 8, and 10. Site 1 is Odiel before being contaminated and 10, the last one, before the tidal influence; the remaining sites 2, 4, and 8 are downstream sites. The rest of them are not located in the same stream. They go from zero contamination degree: regional background (site 7) and sites 13 and 15, also unaffected sites. The remaining sites are shown in Fig. 1 regarding their increasing contamination degree, slightly to AMD-polluted sites.

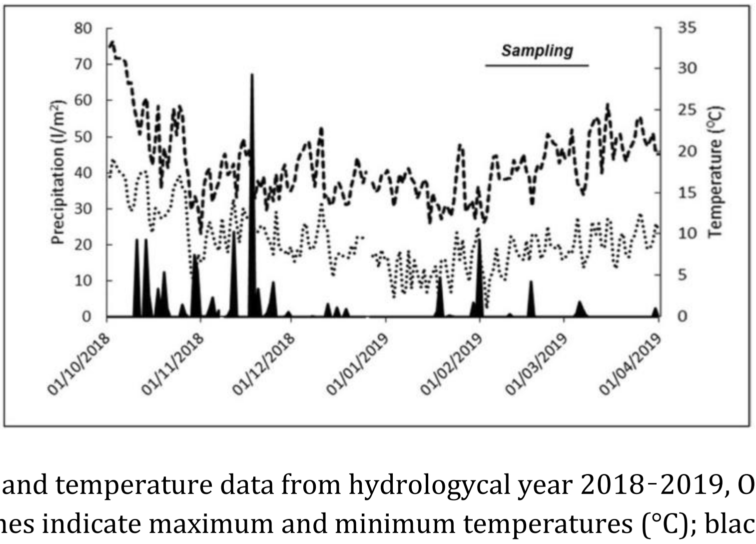

Fig. 2. Pluviometry and temperature data from hydrologycal year 2018‑2019, October 1 to April 1. Discontinuous lines indicate maximum and minimum temperatures (°C); black triangles show precipitations (l/m$_2$). Black horizontal line indicates sampling period. Data are from the Spanish Agency of Meteorology-Station of Thasis-Alosno

At the time of sampling, pH, temperature, electrical conductivity, and the total of dissolved solids were measured in situ using a CRISON-MM 40 + , multiparametric meter, and a redox potential (ORP) measured in the field using a Hanna portable instrument. The ephemerality of many of these streams do not always make possible to have a seasonal sampling per hydrological year, in all sites, since this is a region subject to a negative hydric balance, where some of the rivers only carry water during the rainy season. Spring is a convenient period to collect water accumulated during winter in waste rock dams since its discharge in pounds is continuous during this season. Also for diatoms, spring, after the rainy season, is the best moment to collect, because the communities are more stable and diverse (Luis et al. 2019).

At each sampling point, a sample was taken to analyze sulfate and metals, in which 2% nitric acid Suprapur was added until reaching a pH < 2 in order to keep metals dissolved during the transport to the laboratory. This was carried out in 100 ml PVC bottles and stored in a portable refrigerator at 4 °C. Water samples were filtered immediately in the field through 0.45-μm Millipore filters fitted on Sartorius polycarbonate filter holders. Metal concentrations in water samples were analyzed by ICP-OES (Jobin Yvon Ultima 2) at the CIDERTA lab of the Huelva University (Spain). The precision of all analyses was always within 5%. All reagents were of suprapure quality (Merck, Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used in all the experiments, for the dilutions, when they were needed.

Diatom's preparation and identification

Fifteen epipsammic diatom samples were obtained by removing the top 5 mm layer of the superficial sediment with a syringe, under a 10‑20 cm water column, always in sunny and not stagnant waters (Prygiel and Coste 2000). This substrate was elected due to its ubiquity in the study area. Collected samples were immediately transferred to sterile 100-ml containers and preserved with 70% ethanol until being processed in the lab.

Diatom samples collected in the field were transported to the lab and stored at 4 °C. For definitive preparations, according to the standard norm UNE-EN 13, 946:2014, diatom samples were firstly centrifuged at 1500 rpm and repeatedly washed to remove ethanol, resuspended in distilled water, and then 30% hydrogen peroxide were added (4:1 v/v) to each sample in crystal commercial tubes, following UNE-EN 14,407:2015. Every tube was heated at 95 °C by 1.5‑2 h in a thermostatic bath to remove organic compounds. After cooling to room temperature, tubes were three times centrifuged and washed to remove H_2O_2. Pellets were resuspended in distilled water and dried over a slide in a hot plate (ca. 40 °C) following by the addition of a high refractive resin (Melmount, RI 1.74). These stable preparations were observed at × 400 and × 1000 to identify and count diatoms in all samples under light and phase contrast microscopes with digital cameras and dedicated software (from Leica and Nikon). Identification was based mainly on Krammer and Lange-Bertalot (1985‑1991); Round et al. (1990); Prygiel and Coste (2000); Coste and Rosebery (2011); and Luis et al. (2012).

Data analysis

The data measured in the field, those obtained from the chemical analyses and the different ecological indexes, were integrated into a matrix for further analyses.

Several diversity indexes were calculated directly by using the Past 3.25 software (Oyvind et al. 2001): richness (number of species, *S*), Shannon's diversity ($-\Sigma p_i \ln(p_i)$, *H'*), Pielou's evenness (H'/lnS, *J*), Berger-Parker index of dominance (Nmax/N, *d*) (Morris et al. 2014; Kim et al. 2017; Thukral 2017).

Statistical analyses were performed with Past 3.25 and Minitab 17. All data were subjected to normality test (Anderson‑Darling), correlation tests (Pearson and Spearman; matrix 15 samples × 26 variables), and principal component analysis (PCA). When indicated, cluster analysis (based on a matrix of correlation) was performed.

Results

Physical and chemical parameters

In all sampling points, pH, ORP, EC, TDS, and metals were recorded and analyzed as indicated in the "Materials and methods” section. To simplify, TDS was not shown because of the high correlation with EC (Pearson’s *r* > 0.99) and the most abundant metals were grouped as the Ficklin summatory (i.e., Zn + Cu + Cd + Pb + Co + Ni) (Plumlee et al. 1999). When compared by pairs, all considered metals show a Pearson correlation value higher than 0.986. As shown in Table 1, EC, sulfate, and Ficklin summatory of metals were very significantly correlated. pH was negatively correlated with these parameters and interestingly the highest correlation value was observed for ORP. However, ORP was moderately correlated with EC, sulfate, and Ficklin summatory of metals.

	pH	EC	ORP	Sulfate	∑Ficklin
pH		0.007	$2.03\cdot10^{-6}$	0.045	0.073
EC	-0.663		0.007	$3.87\cdot10^{-9}$	$5.39\cdot10^{-8}$
ORP	-0.913	0.661		0.048	0.079
Sulfate	-0.523	0.967	0.518		$8.62\cdot10^{-15}$
∑Ficklin	-0.476	0.951	0.468	0.996	

Table 1. Pearson correlation of physico-chemical variables. Pearson's *r* values are shown below the diagonal and *p*-values are shown in italic letters above the diagonal

The obtained results are summarized in Fig. 3. Data profile follows “saw teeth” showing that points with the lowest pH have the highest sulfate concentration, EC, ORP, and Ficklin summatory and vice versa. In general, the lowest pH corresponds to the highest values for the other parameters which is typical in AMD-affected sites.

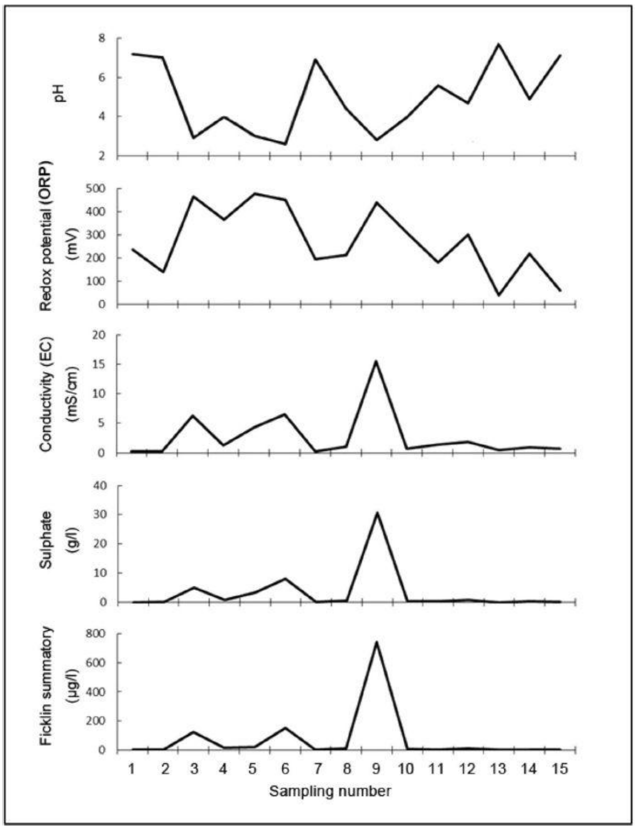

Fig.3. Physico-chemical parameters of waters from the sampling points; pH, redox potential (ORP), decimal logarithm of conductivity (log EC), sulfate concentration, and Σ Ficklin and the most abundant metals in AMD-polluted rivers

A principal components analysis (PCA) was performed using all data. Results are shown in Fig. 4 with the component 1 explaining 99.99% of the variance. Sampling points were grouped by discontinuous lines based on analytical results of physico-chemical parameters as show in Fig. 1. It groups sites 3, 5, 6, and 9 as highly polluted, sites 4, 8, 10,11, 12, and 14 as moderately polluted, and sites 1, 2, 7, 13, and 15 as unpolluted.

Diatom analysis

In all sampling points, epipsammic diatoms were collected. A total of 100 species were identified as shown in Table 1. More than 400 valves per sample were counted. However, diatom distribution did not fit to normality, since most species were represented by a small number of individuals (Online Resource 1). The mean for relative abundance was 1% and the 10 most abundant species include 72% of all the counted individuals. These most abundant species in overall sampling points were as follows: *Achnanthidium minutissimum* (Kutzing) Czarnecki > *Pinnularia acidophila* Hofmann & Krammer in Krammer > *Navicula reichardtiana* Lange-Bertalot var. *reichardtiana* > *P. subcapitata* Gregory var. *elongata* Krammer > *Planothidium lanceolatum* Brebisson ex Kutzing Lange-Bertalot > *Eunotia exigua* (Brebisson ex Kutzing) Rabenhorst > *Brachysira vitrea* Grunow Ross in Hartley > *Navicula gregaria* Donkin > *Staurosira venter* (Ehr.) Cleve & Muller > *Staurosirella pinnata* (Ehr.) Williams & Round.

Diversity indexes

Several diversity indexes were calculated to describe diatoms communities in the different sampling points. For each sample, S, H', J, and d were calculated. Then, results were grouped as in Fig. 4, i.e., non-polluted (samples 1, 2, 7, 13, and 15), moderately polluted (4, 8, 10, 11, 12, 14), and highly polluted (3, 5, 6, 9). In each group, the mean and standard error were calculated and expressed as a percentage to facilitate comparisons. Results are indicated in Fig. 5. In all cases, richness (S), diversity (H’), and evenness (J) decrease as pollution increased. Aversely, dominance (d) increased in response to AMD-pollution mainly in the samples highly polluted.

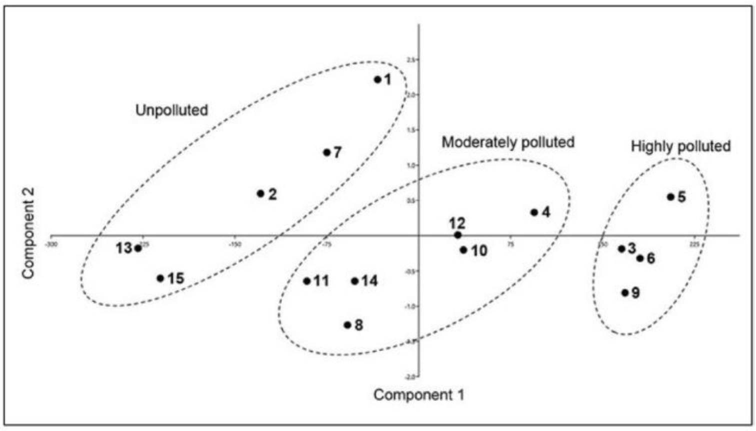

Fig. 4 Principal components analysis (PCA) of sampling points based on physico-chemical parameters. PCA was based on a variance‐covariance matrix of physico-chemical parameters. Each dot represents a sampling point. The component 1 explains the 99.99% of the variance and the component 2 the 0.004%. Discontinuous circles were drawn to group sampling points according to their AMDpollution degree

To analyze the possible relationship between physicochemical parameters and diversity indexes, the correlation of Pearson was calculated to compare all variables. All diversity indexes were significantly correlated among them ($p < 0.05$). However, no correlation was observed between physico-chemical parameters and diversity indexes. Only EC was negatively correlated ($p < 0.05$) with richness (S).

Once many diatoms species from the 15 sampling sites were poorly represented, the most abundant diatoms (relative abundance > 1% in overall samples, 17 species) were chosen.

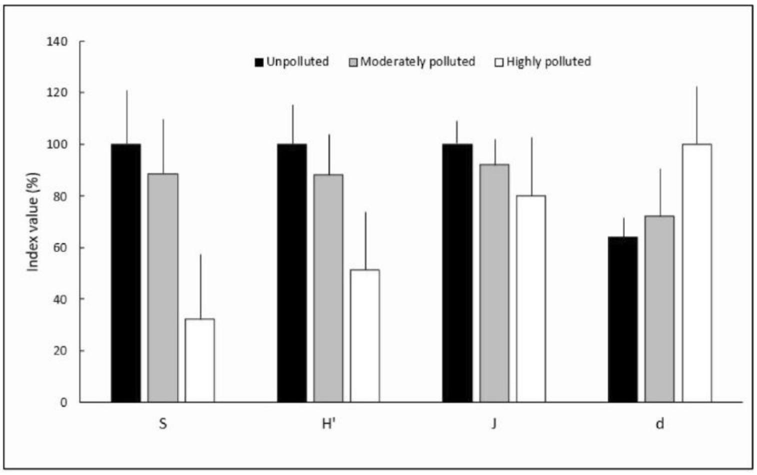

Fig. 5 Diatomologic diversity indexes. Indexes were grouped as in Fig. 4 and expressed as percentage. Data represents mean + standard error. A total of 100% correspond to 28 (S), 2.25 (H'), 0.69 (J), and 0.62 (d). The selected indexes are as follows: (S) richness, (H') Shannon, (J) evenness or Pielou index, and (d) dominance or Berger-Parker

Two procedures were used to ascertain which taxa could be considered as bioindicator of AMD-polluted streams, once many diatom species could also be recognized in moderately polluted waters. First, a cluster analysis using the Pearson correlation (Fig. 6) shows an interesting isolated subcluster grouping typical species of AMD-polluted rivers: *Eunotia exigua*, *Pinnularia acidophila*, and *P. subcapitata* var. *elongata*, while the rest of the clusters grouping other species. Second, the coefficient *rs* of the Spearman correlation was calculated considering individuals/species number and physicochemical parameters of each sampling point. The Spearman correlation was chosen because it is a nonparametric test used to measure the relationship between two variables when the normality assumption of the values distribution is not fulfilled. Results are shown in Fig. 7 where data were ordered according to the *rs* value in respect to pH. Diatoms living in clean, AMD-unpolluted waters are located in the left side of the figure, while adapted species to AMDpolluted waters are located on the right side of the figure. These diatoms are the same differentiated in Fig. 6 and show negative correlation with pH and positive correlation with EC, ORP, sulfate levels, and Ficklin summatory of metals. Contrariwise, diatoms of unpolluted waters show negative correlation with EC, ORP, sulfates, and Ficklin summatory and positive correlation with pH.

Finally, all data were grouped to perform a PCA including physico-chemical parameters (pH, ORP, EC, sulfate, and Ficklin summatory), some diversity indexes (richness, Shannon, dominance, and evenness), and the 17 more abundant diatom species chosen before.

As shown in Fig. 8, Shannon (H’) diversity index, evenness (J), richness(S), and most of the diatom species are linked to the pH arrow and explained positively by the first component. As expected, EC, ORP, sulfate, and Ficklin summatory arrows are explained by the first component, projected together, and opposite to pH. *Eunotia exigua*, *Pinnularia acidophila*, and *P. subcapitata* var. *elongata* are isolated again, negatively explained by the first and second components and strongly correlated with d (dominance of Berger-Parker).

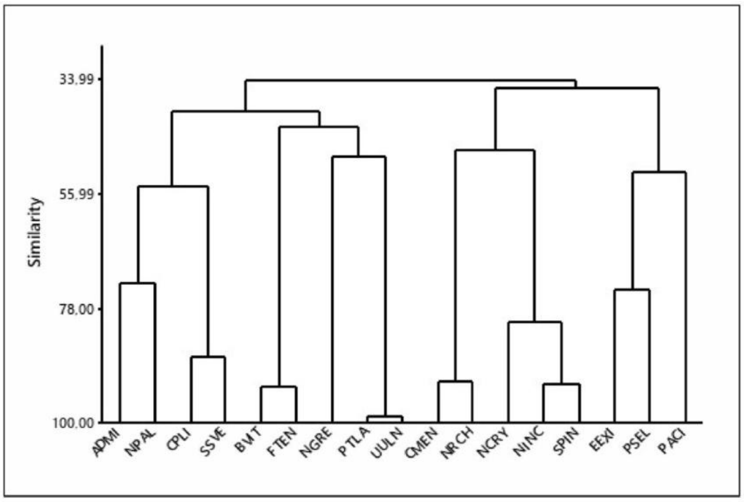

Fig. 6 Cluster analysis of the 17 most abundant diatom species in the sampling points. Cluster analysis based on Pearson correlation. Diatom species are indicated in the horizontal axis and the index of similarity is shown in the vertical axis

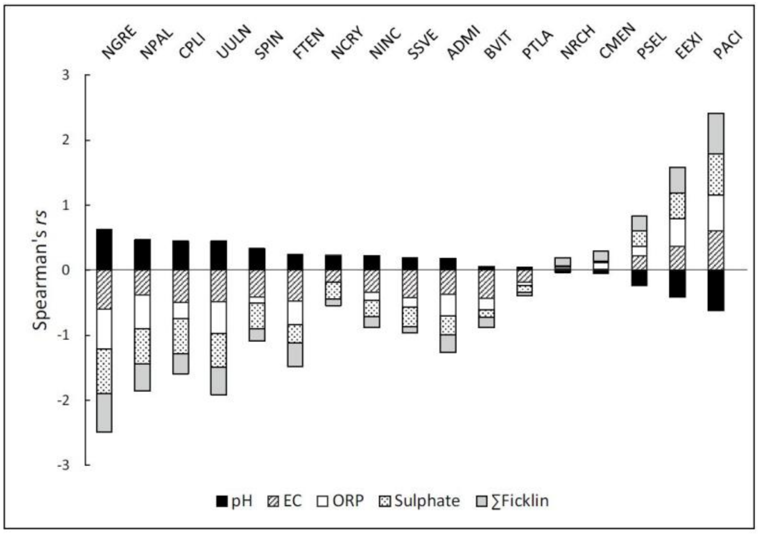

Fig. 7 Spearman correlation of diatoms species vs. physicochemical parameters. Data was sorted from the high to the low value of Spearman's rs for pH. Accumulated data considering all rs values for the other physicochemical parameters were included

Discussion

The magnitude of water contamination crossing the Iberian Pyrite Belt (or from other AMD-polluted regions in the world) mining areas have been referred in numerous occasions. Causes and consequences of AMD pollution are relatively well known and widely debated in numerous scientific publications (Younger et al. 2002; Nieto et al. 2007; Sanchez-Espana et al. 2007; Sarmiento et al. 2011; Blowes et al. 2014; Pearce et al. 2016).

The results of this study were focused in the Odiel river basin, whose algae (e.g., diatoms) are not so well known as those from the Tinto river, thus, our huge interest in this high and complex basin with the confluence of many streams more or less AMD-polluted.

The results confirm the close relationship between pH, conductivity, redox potential, and the metal concentrations. These parameters easily differentiate AMD-pollution degree investigated in this paper. Interestingly, a very significant and inverse correlation between pH and redox potential (measured as

ORP) is demonstrated, as expected after the study of Dold (2014) about the biogeochemical iron cycling at the sulfide oxidation front.

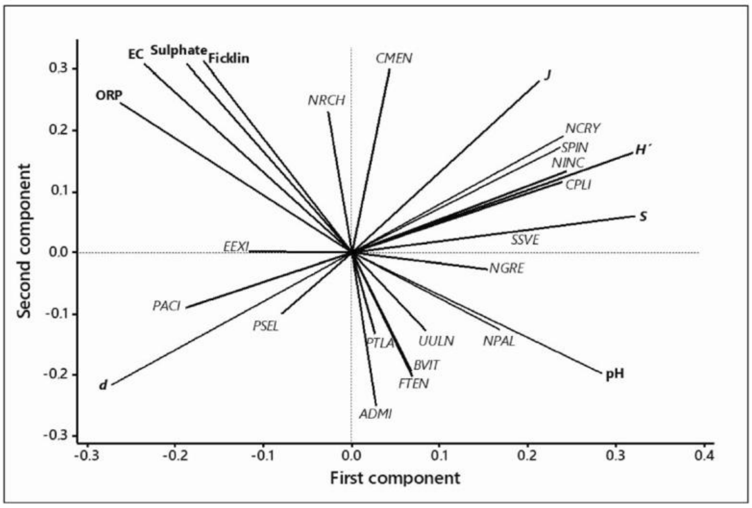

Fig. 8 Principal components analysis (PCA) of 17 most abundant species vs. diversity indexes and physico-chemical parameters as variables. PCA was based on the Pearson´s correlation and integrates data from physico-chemical parameters (pH, ORP, EC, sulfate concentration, and Ficklin summatory), diversity indexes (richness, Shannon, dominance, and evenness), and the 17 most abundant diatoms species according to their relative abundance in all sampling points; matrix 15 × 26). S richness, H' Shannon index, d dominance of Berger-Parker, and J evenness or Pielou index

The analysis of more abundant diatom species gives highly significant results: three species define the AMDpolluted sites, once their high Pearson correlation with physico-chemical parameters of AMD-polluted waters, i.e., low pH and high EC, ORP, sulfate, and Ficklin summatory, were demonstrated by cluster analysis. In this paper, Spearman correlation, an unusual tool to analyze the relationships among diatoms and physico-chemical parameters, was used. Data, sorted by their correlation with pH and ordered from the highest to the lowest *rs* value, allows to distinguish easily two groups of diatoms: those living in non-polluted waters from those that thrive in AMD-polluted waters. These species, *Pinnularia acidophila*, *P. subcapitata var. elongata*, and *Eunotia exigua*, and others from genus *Pinnularia* as *P. acoricola* and *P. aljustrelica*, have been previously identified as bioindicators of AMD-polluted waters both in the IPB (Urrea-Clos and Sabater 2009; Valente et al. 2015; Luis et al. 2009; 2011; 2013; 2019; Rivera et al. 2019) and other similar mining areas (in New Zealand, Schowe et al. 2013; in Korea, Kim et al. 2008) These diatoms are present also in a hot acid-sulfate‐chloride, metal-enriched spring from Yellowstone Park in USA (Hobbs et al. 2009).

Our results further support that pH may be the limiting factor explaining diatom survival. In this regard, DeNicola (2000) suggested a breaking point between pH 3.5 and 4.5 where many diatoms are not able to survive. The same author concluded, after a review of 28 studies, that *E. exigua* or *P. subcapitata var. elongata* and others *Pinnularia* spp. may be considered as acidophilic (pH ≤ 3.5). However, pH is not the unique factor to consider. For instance, *Achnanthidium minutissimum*, the diatom with the highest relative abundance in our study, was observed in most of the samples (from pH 2.6 to 7.7) that was previously identified in metalpolluted waters at circumneutral pH (Lavoie et al. 2012; Tolotti et al. 2019).

However, neither metal contamination nor conductivity and redox potential are currently included in the quality index of water based on diatoms (Stevenson et al. 2010; Morin et al. 2016; Pandey et al. 2017) despite the toxicity of some common metals (mainly As, Cd) in AMD-polluted rivers and the synergic effect of acidity in their solubility and speciation (Morin et al. 2012; McCuen and Synder 1986). Recently, Fernandez et al. (2018) proposed a metal pollution index useful for AMDcontaminated environments and was validated in rivers from the Iberian Pyritic Belt. This index drifted from a pre-existent one and needs a considerable development in terms of choosing diatom species and the type of diatom features. Considering that Tinto and Odiel rivers transport huge quantities of toxic metals and flow through agricultural fields and even through dams that store water for urban supply and irrigation (Rivera et al. 2019), human health must be evaluated too, due to the metal bioaccumulation in the trophic chain and their deleterious effects on the human quality of life (e.g., Baghaie and Fereydoni 2019; Shen et al. 2019; Ma et al. 2020). This study highlights that pH cannot be the only one explaining the obtained results on the distribution, richness, diversity, and dominance of diatom populations, but yes on the interactions between pH and metals and other parameters depending on them. In this sense, and considering the temporal and spatial heterogeneity of environmental variables, Verb and Vis (2001) recommend enhancing the number, location, periodicity, and type of substrates for microalgae sampling to access their diversity. In other sense, the spatial ecology and the turnover rate of the diatom communities in the environment (Soininen and Teittinen, 2019) would allow to assess the state of AMD polluted streams, after a (bio)remediation strategy rendering unpolluted waters by using diatom indexes including metals/ pH interactions and also on water balance criteria, once the water recovery would allow a change to more stable diatom populations.

In this study, several diversity indexes were measured. As expected, in more polluted waters, lesser richness and diversity were observed and also more dominance, once only few species are able to thrive in this polluted environment; hence, diatom communities are quite uneven. However, it is noteworthy that diversity indexes did not correlate with physicochemical parameters, which supports the suggestion made by Blanco et al. (2012), discouraging the use of diatom diversity indexes in biological quality monitoring protocols in inland waters after analyzing 934 diatom taxa in 640 stations because of poor linear correlations of the diversity indexes with environmental factors.

Results of this work support the idea that better than quality (Luis 2007; Luis et al. 2012, 2016) or diversity indexes, *Eunotia exigua*, *Pinnularia acidophila*, and *P. subcapitata* var.*elongata* are currently the best bioindicators of AMD contamination in the IPB, once no good indexes were done yet to assess inorganic (metal/pH) contamination.

Conclusions

The use of a variety of statistical and ecological techniques for diatom taxa analysis allowed the identification of diatom species linked to the most polluted AMD streams from the Odiel river basin, a river crossing many abandoned mines at the IPB, whose eucaryotic microbiology is much less well known than that of Tinto river. However, typical ecological diversity indexes have to be considered with caution in freshwater samples from sulfidic mining areas, because they are defined by a set of very heterogeneous variables. Thus, more research is needed to develop diatom indexes based on metal-polluted waters with very acidic pH, once metal interactions in such environmental conditions are relevant to understand tolerance of diatom taxa. Moreover, water balance is necessary to understand changes leading to increase/decrease of pH, conductivity, redox potential, and metals, because of the ephemerality of many small streams from the Iberian Pyritic Belt. Also, the strong evapotranspiration in summer drives to metal salt precipitation on the polluted river bed.

The results obtained are coincident with those presented by other authors in similar AMD-polluted scenarios. However, in this work, the cause-effect relationships between physico-chemical parameters and diatoms were defined in a whole watershed (Odiel River), for quite different pH ranges, in one of the most AMD-polluted rivers in the world due to secular mining activity.

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Author contribution

Conceptualization JAG; formal analysis ML, MS; investigation AMS; project administration JCF; resources JMD; software OAB; supervision FC, AL; validation JAG; writing–original draft FC; writing–review and editing FC, ATL. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate. Not applicable.
Consent for publication. Not applicable.
Conflict of interest. The authors declare no competing of interests.

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