Study Of The Toxicity Of Mine Waters: Implications Of Suspended Matter On The Performance Of Tolerant Plants

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Abstract

Systems affected by acid mine drainage are extremely reactive, mainly due to the presence of nanometric particle size. The study investigates the role of suspended matter in mobilizing pollutants and increasing AMD toxicity in plants.

So, tests conducted by tolerant plants and natural acidic solutions rich in sulfate, iron, and aluminum were used in unfiltered and filtered water samples. Physical-chemical monitoring revealed improvements in the sampled effluent. The species showed a favorable vegetative evolution and no signs of senescence.

The research is intended to contribute to optimizing the biological stages of passive systems, demonstrated by the ability of Phragmites sp. for AMD remediation.

Keywords: AMD, toxicity, colloids, Phragmites sp., passive treatment

Introduction

Acid mine drainage (AMD) represents an extreme type of pollution associated with mining wastes. These highly reactive acidic solutions ($pH < 3$), mainly due to the presence of small particles often of nanometric sizes, present high content of sulfate and potentially toxic elements (PTE), such as iron, aluminum, and zinc (Chen *et al*., 2021). They are responsible for environmental degradation at pernicious levels of different environmental compartments (Gomes *et al*., 2014). Aquatic ecosystems are susceptible to anthropogenic activities, especially vulnerable to multistressor effects (Vári *et al*., 2022). So, the surrounding rivers and reservoirs are characterized, from an ecological point of view, by low biodiversity (Gomes *et al*., 2021). In this way, urgent mitigation and remediation measures are necessary to improve and preserve natural resources, in line with the United Nations Sustainability Development Goals (SDG-UN, Agenda, 2030).

Although there is an extensive bibliography on AMD, the toxicity of colloids and other small particles still needs to be better known. Thus, this study intends to understand what impact the content of suspended matter (typical of AMD environments), including nanometric-sized colloids, could have on phytoremediation treatment. The investigation includes using natural solutions collected in an abandoned sulfide mine, in the North of Portugal. In this sense, pilot tests were performed with the plant Phragmites sp to evaluate the remediation potential of the sampled water in two different scenarios. So, filtered water samples with 0.22 µm membranes were used to remove suspended material and unfiltered samples.

Results are intended to contribute to optimizing the biological stages of passive systems from an environmental sustainability perspective.

Methods

Valdarcas mine, exploited for W (NW Portugal), is an abandoned mine closed without environmental rehabilitation (e.g., Valente *et al*. 2007). Constituting a skarn deposit with massive sulfides, mainly pyrrhotite, and pyrite, can neutralize minerals, such as calcite and Ca-silicates. The rejected materials were deposited in waste dumps, responsible for effluent with AMD characteristics.

This mine was classified as having a high degree of danger (Santos Oliveira *et al*., 2002) as it has one of Portugal's most reactive waste dumps. The environmental recovery project occurred between 2005 and 2007 with competent authorities (EDM).

The region has a maritime temperate climate, with an average temperature of 20.5°C, with July and August as the hottest. With 9.5°C, appear January as the coldest month. According to Gomes *et al.* (2014), it represents one of the rainiest regions of Portugal, with annual precipitation of 1470 mm.

Here, a point was chosen (V4w), where approximately 80 L of the natural solution was collected (December 2021) for pilot tests. Measurements of physical-chemical parameters were also carried out. Furthermore, 500 mL of surface water was collected in a sterilized polyethylene container for laboratory analyses. The other 50 mL was filtered with 0.22 µm membrane and acidified with HNO3. Thermo Scientific Orion was used in situ to temperature (°C), pH, electrical conductivity (EC µS/cm, at 25°C), potential redox (Eh), and total dissolved solids (TDS).

The study carried out involved the development of pilot tests that used phytoremediation as an environmental remediation technique using the species Phragmites australis. At the laboratory, 40 L of water was filtered (0.22 µm) to carry out two pilot tests: one with filtered water and another with colloids in suspension (unfiltered water).

A multiparameter HQd Field Case (Hach) was used to monitor daily procedures. This includes evaluating in situ physical-chemical parameters (pH, EC, TDS, dissolved oxygen (DO), and Eh). The follow-up of plants evolution, such as chlorophyll content (measured by spectrophotometry), number of new leaves, and color, are also contemplated. To analyze the growth of the plants during the monitoring, the Adobe Photoshop 2020 program was used to obtain measurements of a random plant from each replicate from the 1st and 11th day of monitoring. For each image, it was necessary to carry out a scale of its own. In the program, the width of the box used to carry out the tests was measured in pixels, and later the size was associated with the size in cm.

A complete hydrochemical characterization (sulfate, metals, and arsenic) took place on the 7th and the 11th day of the experiment period, by turbidimetric method (Standard Methods 4500 E) and inductively coupled plasma-mass spectrometry (ICP/MS), respectively. All the reagents were of analytical grade or Suprapur quality (Merck, Darmstadt, Germany). The standard solutions were Merck AA Certificate. Milli-Q water was used in all the experiments. Metals and arsenic analyses were performed at Activation Laboratory, Lda (Actlabs, Canada), including analysis of duplicate samples and blanks to check.

Results and discussion

Physical-chemical properties

Table 1 represents the summary of parameters evolution during the monitoring time. The results reveal that both tests showed similar behavior for replicates, presenting improved pH characteristics.

Concerning TDS and EC, it shows an immediate reduction on the first day. However, the tests revealed an increase around the 7^{th} day, ending up with very similar EC (2520 and 2476 µS/cm in Filtered and Unfiltered replicates, respectively). The sum of the PTE analyzed showed the same behavior. The evolution of the effluent, namely the dissolution of hydroxysulfates present in the water (filtered and unfiltered), together with the interaction reactions between the effluent, the plants, and the soil, can explain the increase observed.

Regarding DO, the Filtered (RF) and Unfiltered water (RNF) replicates initially show quite different values. The NF sample shows much lower concentrations (3.08 mg/L) when compared to the Filtered sample (7.80 mg/L) right on the 1st day. There is a positive evolution of the increased concentration of this parameter up to the 7th day. However, after this period, the oxygen value tended to decrease, eventually coming close in both experiments (6.91 and 6.44 mg/L, unfiltered and filtered, respectively).

The Eh is used to characterize the reducing or oxidizing system capacity. Its evolution shows that this parameter decreased for the two pilot tests. Despite the final values

***Table 1** Parameters analyzed. Sulfate and PTE are in mg/L; RF = filtered water; RNF = unfiltered water. N.d.= not determined.*

Parameters	V4w	RF			RNF		
		1st day	7th day	11th day	1st day	7th day	11th day
pH	3,19	3,06	3,063	5,003	3,013	3,013	5,28
EC (µS/cm)	2020	1 803	1803	2520	1828	1828	2477
TDS (mg/L)	990	866	866	1517	857	857	1488
DO (mg/L)	6,35	7,80	7,80	6,91	3,07	3,68	6,44
T (°C)	8,0	28,6	33,2	33,1	25,7	34,5	32,0
Sulfate	25209	-	35196	42721	-	35905	42276
Eh (mV)	410	445	455	312	462	462	318
∑(As, Pb, Cu, Zn, Cd, Al, Fe, Mg)	2055224	n.d	113406	175821	n.d.	108022	171338

showing improvements, according to Reddy & DeLaune (2008), they continue to reflect the oxidizing environment in which they are found since they present values between 700 mV and 300 mV.

Vegetative evolution

Phragmites australis is a species used worldwide in treating contaminated water due to its resistance to PTE, presenting characteristics that promote efficient phytoremediation (Rezania *et al*., 2019). In order to understand the evolution of the plant itself in the face of stressful situations, a count was carried out to analyze the development capacity by increasing or not the number of shoots. Table 2 presents these results, showing the number of shoots and compared to the starting number.

The results indicate that both tests showed an increase in the number of shoots at the end of the experiments. These had identical behavior in both pilot tests for Filtered Water and Non-Filtered Water; however, the Filtered Water test had a higher number (average of 35). The number of shoots in the Filtered Water test may be related to decreased suspended colloids. This increase appeared more evidently next to the stems of the larger Phragmites australis.

It is possible to observe, through Figure 1, a replica of the tests, which contains Filtered Water, on the 1st and 11th day of monitoring. The plants, in general, showed good growth and development.

In general, the species used remained healthy, from a visual point of view, and showed no signs of loss of vitality. The leaves, with a yellowish color observed, are the same as at the beginning of the monitoring, with no signs of senescence.

The results (tab. 3) reveal that the Pilot Test of Filtered Water presents a greater growth than the Pilot Test of Unfiltered Water. Shoots measurements were also done using the same program (Adobe Photoshop 2020).

For each replica, a shoot was selected, and through a scale specific to each photograph, the measurements of the 1st day of the test and the 11th day were taken so that it was possible to make a difference and achieve growth, which is recorded in Table 4.

***Table 2** New shoots that emerged during monitoring.*

Quantification of shoots – Phragmites australis				
Replica	Number of shoots		Difference	Mean
	Start	End		
R1F	8	50	42	35
R2F	12	35	23	
R3F	13	53	40	28
R1NF	10	39	29	
R2NF	16	43	27	
R3NF	11	39	28	

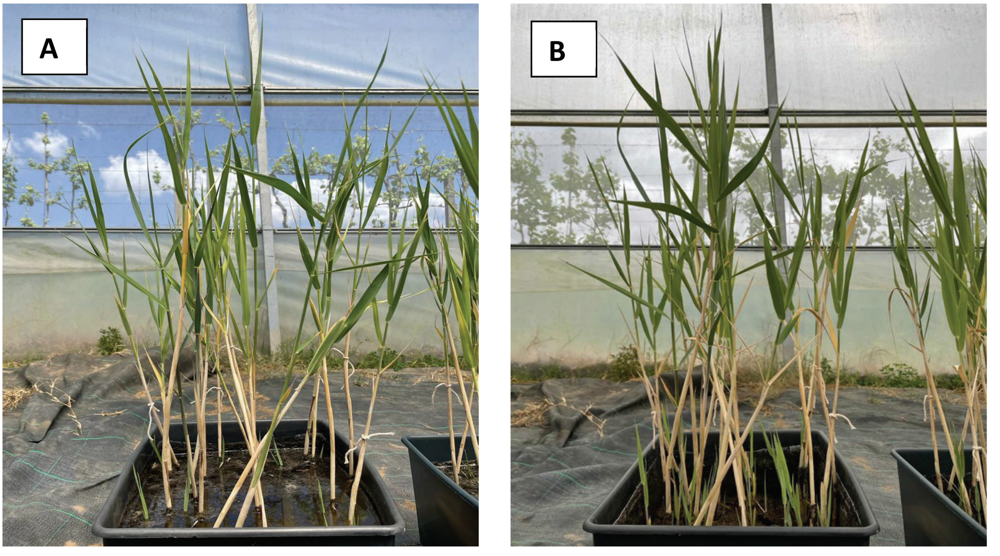

Figure 1 *Replica of the Filtered Water Pilot Test. "A" corresponds to the 1st monitoring day, and "B" corresponds to the last day.*

Table 3 *Phragmites australis growth (in cm), performed using Adobe Photoshop 2020 measurements.*

Measurement	R1F	R2F	R3F	R1NF	R2NF	R3NF
1st Day	17.06	24.56	23.99	22.74	20.87	22.86
11th Day	20.90	28.24	24.63	23.51	26.11	23.48
Difference	3.84	3.68	0.64	0.77	5.24	0.62
Mean	2.72			2.21		

Table 4 *Phragmites australis shots growth (in cm), performed using Adobe Photoshop 2020 measurements*

Measurement	R1F	R2F	R3F	R1NF	R2NF	R3NF
1st Day	4.92	6.27	4	3.50	7.17	8.36
11th Day	11.59	11.48	9.22	10.37	12.13	10.96
Difference	6.67	5.21	5.22	6.87	4.96	2.60
Mean			5.7			4.81

Generally, the Phragmites australis plants from the Filtered Water Pilot Test show better results; however, the difference compared to the Non-Filtered Water Pilot Test is irrelevant. Both tests gave their plants a healthy appearance and increased the number of shoots. Exposure to the effluent had no visible consequences on the *Phragmites australis* used. The high values of EC, sulfate, TDS, and the presence of PTE seem not to have created restrictions for their development, as can be seen by the formation of new shoots. The plants thus demonstrated, in all replicates, a good adaptation and development in the face of the subject conditions.

Photosynthetic pigments: chlorophyll a and b

Figure 2 shows chlorophyll a and b, evaluated at the end of the tests, for both replicates: filtered and unfiltered, as it also considers the control group, plants not subject to AMD. The results indicated that the RNF pilot trial has higher Chl a and Chl b values than the control group and the RF trial. However, the difference between the latter was not relevant. The characteristic elements of this type of water have boosted the plant's development and its chlorophyll levels. The results indicate that the plants used to treat the mining effluent present regular activity even when exposed to several pollutants and a low pH. According to Khaleghi *et al.* (2012), water stress can potentiate a decrease in chlorophyll concentration due to the sensitivity that this pigment has to environmental pressures. However, this fact was not verified. Despite being subjected to high evaporation conditions, the plant used in the phytoremediation tests maintained and increased its concentrations of chlorophyll a and b, showing no signs of senescence. The same results would have already been reported by Gomes *et al.* (2020) in studies related to acidophilic algae, where the authors reported obtaining higher concentrations of chlorophyll in the dry season when evidence of AMD was more intense.

It is believed that Phragmites australis, through the phytoremediation technique, may be resistant to PTE contained in its effluents. As observed by other authors (e.g., Romaní and Sabater, 2000), despite extreme conditions, photosystem II (PSII) is not damaged, most likely due to adaptation mechanisms. Therefore, it is thought that the plants used have stable vital functions, with the amount of chlorophyll not being affected.

Conclusions

The pilot tests demonstrated that, in general, there was a reduction in the contaminating potential of AMD. The plants included in the tests (Phragmites australis) seemed to support high amounts of contaminants, efficiently reducing elements with disruptive characteristics for the environment. Trial monitoring revealed that they maintained their growth rates and healthy development, including forming new shoots. Chlorophyll a and b concentrations were even higher than the control group, showing no signs of senescence. Particles of nanometric dimensions do not influence the toxicity of the evaluated plants. The interaction of these with the substrate may be the key to dynamic phytoremediation processes, and its more in-depth study is critical.

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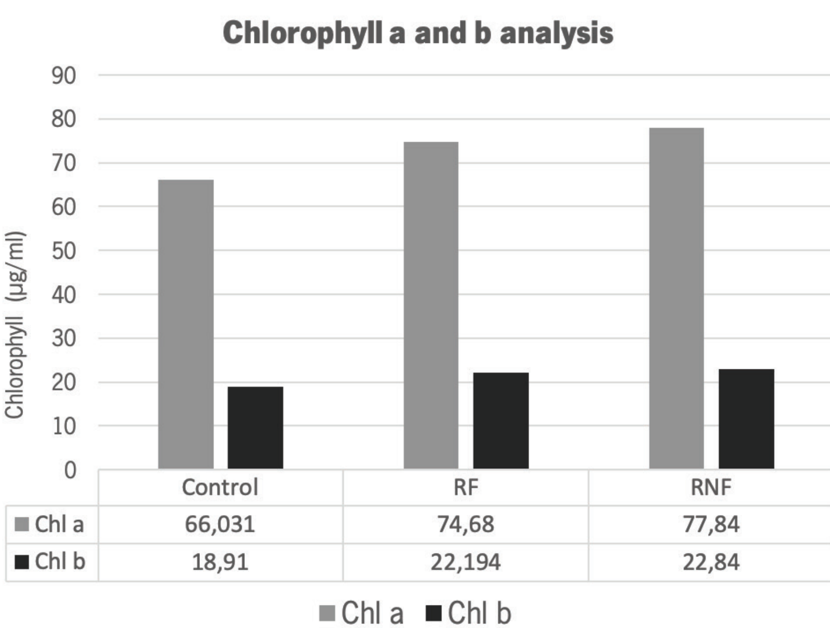

Figure 2 *Concentration of Chl a and Chl b in the tests carried out: RF, RNF, and control.*

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References

Gomes P, Valente T, Pamplona J, Sequeira Braga MA, Pissarra J, Grande Gil J A, de la Torre ML (2014) Metal Uptake by Native Plants and Revegetation Potential of Mining Sulfide-Rich WasteDumps. International Int. J. Phytoremediation, 16(11), 1087–1103. doi:/10.1080/15226514.2013.810586

Gomes P, Valente T, Geraldo D, Ribeiro C (2020). Photosynthetic pigments in acid mine drainage: Seasonal patterns and associations with stressful abiotic characteristics. Chemosphere, 239, 124774, doi: /10.1016/J.CHEMOSPHERE.2019.124774

Gomes, P (2021) Impact of acid mine drainage on the environmental quality and potential accumulation of strategic metals in water dams located in the Iberian Pyrite Belt. University of Minho

Khaleghi E, Arzani K, Moallemi N, Barzegar M (2012) Evaluation of Chlorophyll Content and Chlorophyll Fluorescence Parameters and Relationships between Chlorophyll a , b and Chlorophyll Content Index under Water Stress in Olea europaea cv . Dezful. International Scholarly and Scientific Research & Innovation, 6(8), 2108–2111.

Portuguese Environment Agency (2021) Criteria for the Water Masses Classification. 160. https://apambiente.pt/sites/default/files/_Agua/DRH/ParticipacaoPublica/PGRH/2022- 2027/3_Fase/PGRH_3_SistemasClassificacao.pdf

Reddy KR, DeLaune RD (2008) Biogeochemistry of Wetlands: Science and Applications. CRC Press, doi:/10.1201/9780203491454

Rezania S, Park J, Rupani PF, Darajeh N, Xu X, Shahrokhishahraki R (2019) Phytoremediation potential and control of Phragmites australis as a green phytomass: an overview. Environ. Sci. Pollut. Res. 2019 26:8, 26(8), 7428–7441. doi:/10.1007/S11356-019-04300-4

Romaní A, Sabater S (2000) Influence of Algal Biomass on Extracellular Enzyme Activity in River Biofilms. Microb Ecol 40, 16–24. doi: 10.1007/s002480000041

Santos Oliveira JM, Farinha Ramos J, Matos JX, Ávila P, Rosa C, Canto Machado MJ, Danie, FS, Martins L, Machado Leite MR (2002) Diagnóstico Ambiental das Principais Áreas Mineiras Abandonadas do País. Boletim de Minas, 39(January), 67–85.

Valente T, Gomes C (2007) The role of two acidophilic algae as ecological indicators of acid mine drainage sites. J. Iber. Geol., 33

Vári Á, Podschun SA, Erős T, Hein T, Pataki B, Iojă IC, Adamescu CM, Gerhardt A, Gruber T, Dedić A, Ćirić M, Gavrilović B, Báldi A (2022) Freshwater systems and ecosystem services: Challenges and chances for cross-fertilization of disciplines. Ambio, 51(1), 135–151, doi:/10.1007/s13280-021-01556-4