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Removal of Pollutants from an AMD from a Coal Mine by Neutralization/Precipitation Followed by "In Vivo" Biosorption Step with the Microalgae *Scenedesmus* sp.

Karine Batista dos Santos, Vítor Otacílio de Almeida, Jéssica Weiler and Ivo André Homrich Schneider *

Laboratório de Tecnologia Mineral e Ambiental, Programa de Pós-Graduação em Engenharia de Minas, Metalúrgica e de Materiais, Escola de Engenharia, Universidade Federal do Rio Grande do Sul, Porto Alegre 90040-060, Brasil; karineebatista@gmail.com (K.B.d.S.); vitor01almeida@hotmail.com (V.O.d.A.); jessica.weiler18@gmail.com (J.W.)
* Correspondence: ivo.andre@ufrgs.br; Tel.: +55–51–3308–7882

Received: 24 June 2020; Accepted: 4 August 2020; Published: 12 August 2020

Abstract: This work evaluates the benefits of a complementary treatment step of acid mine drainage (AMD) using the algae *Scenedesmus sp.* in terms of algae biomass production, residual metal removal, and the toxicity of the discharged water. Conventional treatment by neutralization/precipitation of an AMD from a coal mine in Brazil was conducted with $Ca(OH)_2$ at pH 8.7. Algal growth studies were performed in the treated AMD, with and without a nutrient supply. The raw effluent and treatments were compared in terms of residual concentration of metals and sulfate, conductivity, and toxicity with the *Allium cepa* and *Daphnia magna* test organisms. The results show that the conventional treatment allowed a major metal removal, reduction in the conductivity, and good indices in the toxicological parameters evaluated. The biosorption with in vivo microalgae improved the quality of the effluent for residual metals. No significant toxicity was observed to *Allium cepa* in all treatments performed, while the *Daphnia magna* test indicated a reduction in toxicity after the biosorption step. It was concluded that algae growth can be carried out in treated mine waters, providing algae biomass and helping to achieve the standards for water discharge.

Keywords: acid mine drainage; conventional treatment; biosorption; algae; metal removal; toxicity

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1. Introduction

Active chemical treatment by neutralization has been widely applied by the coal mining industry to prevent the damages caused by acid mine drainage. The process involves the addition of alkaline reagents to increase the pH and precipitate the dissolved metals as hydroxides [1–3]. Recently, Skousen et al. [4] pointed out six chemicals commonly used to treat AMD: limestone—$CaCO_3$, hydrated lime—$Ca(OH)_2$, pebble quicklime—CaO, soda ash—Na_2CO_3, caustic soda—NaOH, and ammonia—NH_3. Considering the acidic mine water treatment plants in Brazil, the most commonly used neutralizing reagent is hydrated lime, raising the pH to 8.7. Calcium chemicals are preferred since they are cheaper and allow the partial removal of the amount of sulfate ions present in the wastewater [5].

This practice was validated by recent studies applying the reagents $Ca(OH)_2$ and NaOH at pH 7.0 and 8.7 to treat acid mine drainage from a coal mine [6,7]. All neutralizing treatments resulted in a satisfactory reduction in the metal concentration, with the best results achieved using $Ca(OH)_2$ at pH 8.7, although Mn and As remained above or near the maximum limits for discharge according to the Brazilian legislation. Additional treatment steps can also be applied for AMD control when greater treatment efficiency is needed [3]. Studies suggested the use of algae to remove residual metals from AMD [7–9], since they can act as metal accumulators through bioaccumulation (active mechanism) and/or biosorption (passive mechanism). Algae species of genera such as *Spirulina*, *Chlorella*, *Scenedesmus*, *Cladophora*, *Oscillatoria*, *Anabaena*, and *Phaeodactylum tricornutum* showed capacity to remove a considerable content of metals from AMD [9]. Algae ponds could also generate a possible biomass use [10] and, in particular, the *Scenedesmus* genera are greatly used for the production of biodiesel, biogas, and proteins [11,12].

Indeed, there is a growing trend in the different uses of treated AMD. Many of the studies aim to integrate AMD treatment systems with the recovery of dissolved metals and the synthesis of products [13–22]. However, there are considerably fewer works concerning the use of discharged water. The main initiatives are for water reuse in the industrial systems [5,23,24], as a resource for drinking water [22], to produce electricity [25], and for algae growth [7,26–28]. We should point out that the study of Pereira et al. [7] was carried out with the *Scenedesmus sp.* strain in treated coal mine water, but not exploring the total algae growth capacity, given that there was a lack of nutrients in the system, mainly in terms of nitrogen and phosphorus.

The objective of this work was to evaluate the benefits of an additional step in AMD treatment by growing the algae *Scenedesmus* sp. in vivo. The water substrate was attained from a coal mine in south Brazil after conventional treatment by neutralization/precipitation with $Ca(OH)_2$ at pH 8.7. The treated effluent was subjected to algal growth both with and without the addition of nutrients. The quality of the treated water was analyzed in terms of conductivity, metals, and sulfate concentration, as well as in terms of toxicological effects for *Allium cepa* and *Daphnia magna*.

2. Materials and Methods

2.1. AMD Sample

The sample of AMD used in this work was provided by a coal mining company located in the municipality of Figueira, northeast of the State of Paraná, Brazil (23°49′15″ S; 50°25′50″ W). The coal seams are explored in the Paraná Basin and are part of the Permian Rio Bonito Formation [29]. Coal waste is highly associated with iron sulfides, with a content of pyrite ranging up to 15%. Reject surface dumps are also associated with toxic pollutants such as Zn, Mn, Pb, and As, which are commonly dissolved in water when the pH decreases [30].

2.2. AMD Treatments

The conventional treatment of AMD was conducted based on the treatment carried out by Brazilian mining companies. First, the pH was raised to 8.7 by adding the alkaline reagent (calcium hydroxide—$Ca(OH)_2$) under stirring and monitoring the pH with a pH meter, followed by precipitation of the metals as hydroxides. The supernatant was filtered and then separated for the additional treatment with algae.

The algae species selected was *Scenedesmus* sp. due to its known metal absorption capacity [9,31], and its use as a biofuel resource [11,12]. Microalgae cultures for inoculation were maintained in 1000 mL Erlenmeyer flasks with the Guillard modified culture medium at 25 °C and 3910 lux and were used when the growth reached the exponential phase. Two scenarios for *Scenedesmus* sp. growth in the treated AMD were observed: (a) without addition of nutrients; and (b) with the addition of 0.1% (volume) of the macronutrients supply of Guillard medium composed of: 36.76 g L^{-1} of $CaCl_2.2H_2O$, 36.97 g L^{-1}, $MgSO_4.7H_2O$, 12.6 g L^{-1} of $NaHCO_3$, 8.71 g L^{-1} of K_2HPO_4, 85.01 g L^{-1} of $NaNO_3$ and 28.42 g L^{-1} of $Na_2SiO_3.7H_2O$ [32]. Erlenmeyer flasks (250 mL) containing AMD and 1% algae inoculum were incubated on an orbital shaker (model CT-712RN Cientec) at 125 rpm, 27.5 °C, with a photoperiod of 16 h/day under illumination with a light intensity of 6200 lux. Dry weight and optical density (OD) were measured daily to determine the microalgal growth and relationship between the dry weight and optical density. OD was measured at 570 nm wavelength (UV1100 spectrophotometer Pro-tools), and the dry matter was measured by filtering the samples through 0.7 μm pre-weighted membranes, which were dried at 60 °C for 24 h.

Thus, the samples (each one in triplicate) were submitted to physical-chemical and toxicological analyses, as illustrated in Figure 1.

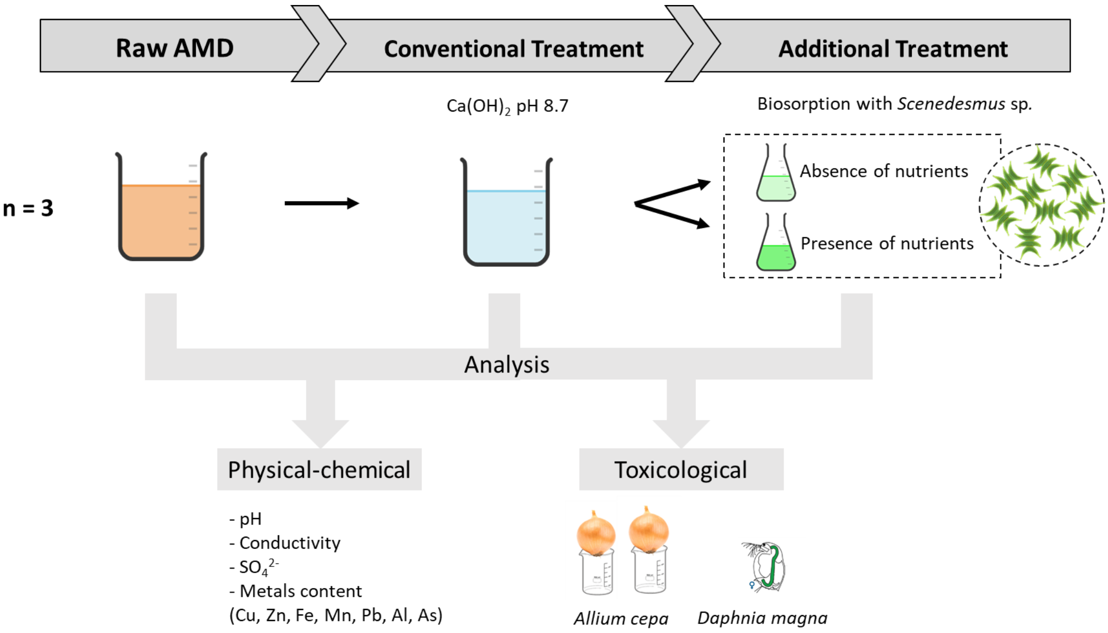

Figure 1. Illustrative scheme of this work, including the treatments and analysis performed.

2.3. *Physical-Chemical and Toxicological Analysis*

Raw AMD and treated waters were analyzed for pH, conductivity and metals content (Cu, Zn, Fe, Mn, Pb, Al, As), sulfate content, and toxicological tests with *Allium cepa* and *Daphnia magna*.

The pH of the suspension was measured using a bench pH meter (AKSO model 86505, São Leopoldo, Brazil). Electrical conductivity was measured using a Zetasizer (Malvern Panalytical, Malvern, UK). Metal analyses were carried out by sample preservation with HNO_3 and by an issuing optical spectrophotometer with inductively coupled plasma (ICP-OES). The sulfate content was measured using the turbidimetric method. All analyses followed the procedures described in the "Standard Methods for Examination of Water and Wastewater" [33].

The *Allium cepa* root growth test was carried out with onions of the same origin and approximate sizes. The outer scales of the onions were removed and the bulb gently scraped. Bulbs were kept in touch with deionized water in beaker glasses for 24 h at room temperature to stimulate root growth. After this period, the bulbs were placed at each test condition and maintained in contact with the same for 72 h under the light and following the procedure of Fiskesjö [34]. The test was performed in triplicate and a control group was adopted using deionized water. The number, as well as the mass and the length of the three largest roots in each bulb, were measured. The results of the different tests conditions were compared with the control in terms of relative growth index (RGI) (Equation (1)), the inhibition index (II) (Equation (2)) and the germination index (GI) (Equation (3)). The phytotoxicity was considered when there was a significant decrease between the test and control groups:

$$RGI = \frac{Lm}{L_0} \tag{1}$$

$$II = \left(1 - \frac{Lm}{L_0}\right) \times 100 \tag{2}$$

$$GI = \frac{G}{G_0} \times RGI \times 100 \quad (3)$$

where Lm is the average length of the roots in the samples, L_0 is the average length of the roots in the control, G is the germination value in the samples, and G_0 is the germination rate in the control.

Acute toxicity tests for *Daphnia magna* microcrustaceans were performed in triplicate and in accordance with the Guideline for Testing of Chemicals using *Daphnia sp.* OECD 202 [35], using the statistical technique EPA 821-R2-012 [36]. This test assesses the immediate toxic effects of a sample on the mobility of this microcrustacean species and is based on the exposure of organisms to a series of dilutions of the sample for 48 h. The effect on organisms is expressed by a loss of mobility. The result is calculated by a statistical procedure and represented by the concentration effect to 50% of the population (EC-50-48 h).

2.4. Statistical Analysis

The results were presented in terms of mean and standard deviation. Results were assessed using analysis of variance (ANOVA) with significance level *p*-value < 0.05 and the Tukey test to compare the differences between averages.

3. Results and Discussion

Chemical parameters of the AMD used in this study, and the results of the AMD treated for each treatment in terms of pH, conductivity, sulfates, and metals (iron, aluminum, manganese, zinc, lead, arsenic, and copper), are compiled in Table 1. These results were compared with the current Brazilian environmental legislation, CONAMA 430/2011 [37].

Table 1. Characterization of acid mine drainage (AMD) after conventional treatment—neutralization/precipitation with $Ca(OH)_2$ at pH 8.7—and additional treatment with growth of *Scenedesmus* sp. algae.

	Raw AMD		**Conventional Treatment $Ca(OH)_2$ pH 8.7**		**Additional Treatment**				**CONAMA 430/2011**
					Algae		**Algae + Macronutrients**		
	Mean	**sd**	**Mean**	**sd**	**Mean**	**sd**	**Mean**	**sd**	
pH	2.33	0.15	8.7	0.0	5.55	0.21	5.75	0.7	
Conductivity (mS cm^{-1})	7.79	0.02	5.28	0.07	4.84	0.08	4.34	0.10	–
SO_4^{2-} (mg L^{-1})	7410.40	36.94	2727.60	19.49	1325.75	5.59	1250.40	3.82	–
Fe (mg L^{-1})	611.38	5.11	0.90	0.02	0.08	0.11	0.05	0.07	15
Al (mg L^{-1})	269.37	11.67	0.08	0.08	0.24	0.33	0.30	0.43	–
Zn (mg L^{-1})	62.65	0.55	0.10	0.10	0.06	0.06	0.08	0.04	5
Mn (mg L^{-1})	37.98	0.36	1.54	0.40	0.46	0.27	0.45	0.11	1
Pb (mg L^{-1})	0.41	0.02	0.15	0.05	0.06	0.08	0.02	0.03	0.5
As (mg L^{-1})	0.85	0.05	0.30	0.31	0.14	0.18	0.12	0.14	0.1
Cu (mg L^{-1})	nd	–	nd	–	nd	–	nd	–	1
Metal content * (mg L^{-1})	982.64	15.16	3.07	0.75	1.03	0.28	1.02	0.73	–

* Sum of the metals Fe, Al, Zn, Mn, Pb, As, and Cu. sd: standard deviation. nd: not detected. Database: Santos [38] and results partially published in Pereira et al. [7].

The conventional treatment was satisfactory considering the pH correction, 60% reduction in the sulfate content, and almost total removal of Fe, Al and Zn. However, for Mn and As, the treatment efficiency was not sufficient to achieve the limits stablished by the legislation. Thereafter, additional treatment with algae was investigated for polishing and removing the remaining metals. The AMD after conventional treatment was submitted to contact with *Scenedesmus* sp. and their growth was monitored over 10 days for each scenario in terms of biomass (Figure 2).

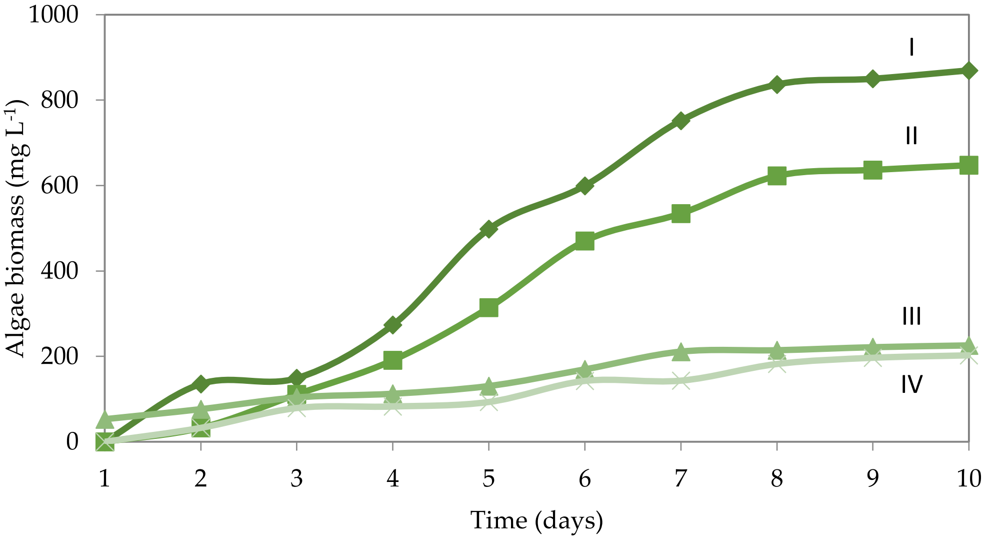

Figure 2. Growth of algae biomass as a function of time for Guillard medium—I; AMD after conventional treatment with nutrients—II; AMD after conventional treatment without nutrients—III; and for the control (water purified by reverse osmosis)—IV.

At the end of ten days of growth, 880 mg L^{-1} of algae biomass was achieved in the Guillard nutrients solution, 650 mg L^{-1} in the treated AMD with nutrient supply, 230 mg L^{-1} in the treated AMD without addition of nutrients, and 200 mg L^{-1} in distilled water. Algal growth in AMD was successful when nutrients were added, reaching values (75%) close to those obtained in an ideal culture medium, in this case the Guillard medium. Without the addition of nutrients, the growth was similar to the control (water only).

After algal growth, the water quality parameters were even more satisfactory for conductivity, sulfates, and the remaining metals. The removal of Mn was effective, reaching values below that established for discharge in water resources in Brazil [37]. There was also a decrease in the concentration of arsenic, but this was still above the limits applied by the legislation.

The differences between the adopted treatment procedures are depicted in Figure 3 in terms of final sulfate concentration and metal content (sum of Fe, Al, Zn, Mn, Pb, As, and Cu). It is possible to observe that the conventional treatment provides a partial removal of sulfates and an almost complete removal of these metals. Regarding sulfates, each treatment step allowed a statistically significant improvement, reaching the greatest efficiency with algae growth with the addition of nutrients. For metals, the same was observed, with the difference being that the addition of nutrients, with an increase in algae biomass production, did not provide a refinement. This may be because the metal removal is already quite high from the conventional treatment. The metal uptake was 8.9 mg g^{-1} of algae (in dry basis) in the case where no nutrients were added, similar to results obtained in other studies [39]. When nutrients were added, the mass of algae increased, but the removal of metals was almost the same, resulting in a metal uptake of 3.15 mg g^{-1} of algae, which is probably below the biosorption accumulation capacity of this *Scenedesmus sp.* The optimal conditions for the metal uptake by microalgae are due to many factors, including pH, metal speciation, the initial concentration of metal ions, and the mass of algae present, with an adsorption limit [40].

In the present case, the approximate values in the residual concentration of metals with and without nutrients may be explained by the low concentration of metal ions in the effluent after the conventional treatment. After this treatment step, the sum of transition metals plus arsenic and aluminum is approximately 3 mg L^{-1}. Considering the amount of algae biomass of 0.65 g L^{-1} in the treated AMD with nutrient supply, 0.23 g L^{-1} in the treated AMD without addition of nutrients, and literature data [40], the adsorption would fits in the beginning of the equilibrium curve plotted in the Cartesian plane, where there is a little variation of the residual pollutant in water solution with the increase in pollutant accumulation onto the biomass-assuming Langmuir adsorption model, which suits for biosorption with *Scenedesmus* and other microalgae. At these low concentrations, the metal ions listed in Table 1 are at a disadvantage to compete with other ions present in the solutions for the metal binding sites. Still, we cannot rule out that the residual elements may be chemically bound to soluble organic molecules excreted by the algae, making them not available for passive adsorption. This set of results indicates that both passive and active mechanism of metal removal may occur by in vivo *Scenedesmus* growth on treated AMD, even though quantitative values were not determined and this would be the subject of investigation on the continuity of this study. Although there was no significant difference in metal removal, the addition of nutrients enhances the biomass growth, which can be interesting from another perspective. The use of nutrients should be considered in scenarios where algae biomass can be used as raw material for the production of biofuel and biofertilizers [28,41,42].

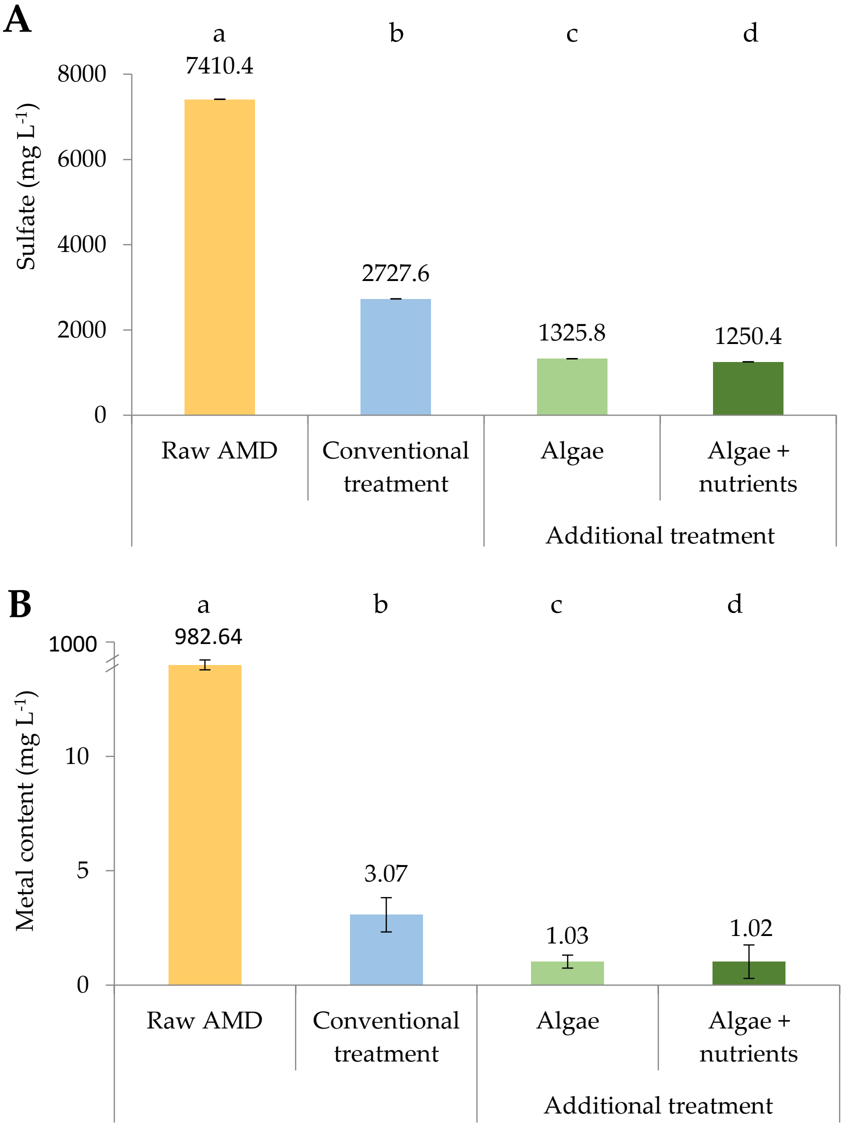

Figure 3. Comparison between raw AMD, conventional and additional treatments in terms of sulfate (**A**) and metal content (**B**). Means that differ significantly (Tukey's test, $p < 0.05$) are indicated by different lowercase letters.

Nevertheless, although physical-chemical analysis is important to attend the legal framework for effluent discharge, they do not predict toxicity to living organisms. The environmental effects and behavior of substances should also be evaluated by biological assays [43,44]. In this specific work, it was carried out with the toxicological bio-indicators *Allium cepa* (onion) and *Daphnia magna*.

The toxicity results with *Allium cepa* were based on relative growth, inhibition, and germination indices of the roots (Table 2). In raw AMD, it was possible to observe the total inhibition in onion root growth, probably due to the high acidity of the medium and high metal concentrations. These values are in accordance with the study of Geremias et al. [45], which also suggested that the phytotoxic effect could be associated with low pH values (pH = 2.5) and the expressive concentration of metals such as iron, aluminum, manganese, zinc, and lead, present in the effluent, that could be incorporated by the plant. After conventional chemical treatment with $Ca(OH)_2$ at pH 8.7, the effluent was non-toxic. There was an adequate root growth, both in length and mass, being in fact superior to the control (negative II values). Similar results were attained for the effluent after the additional treatment step with algae. In other words, the polishing step with algae did not modify the behavior of the onion root growth, either negatively or positively. Regarding the germination index, according to the classification adopted by Zucconi et al. [46], it was also not possible to observe a difference between treatments, which were classified as absent or low toxicity.

Table 2. Relative growth index (RGI), inhibition index (II), germination (G) and germination indices (GI) of *Allium cepa* roots for control (H_2O); raw AMD; AMD after conventional treatment; and AMD after additional treatment with algae without and with nutrients addition.

	RGI	II (%)	Toxicity	G (%)	GI (%)	Toxicity
Raw AMD	0	100	Inhibition	0	0	High
Conventional treatment	1.37	−37	Stimulation	71	97	Absent or low
Additional treatment						
Algae	1.16	−17	No significant effect	74	86	Absent or low
Algae + nutrients	1.32	−32	Stimulation	90	119	Absent or low

Toxicity criterion (II): inhibition—RGI between 0 and 0.8, no significant effect—RGI between 0.8 and 1.2, stimulation—RGI greater than 1.2. Toxicity criterion (GI)—absent or low toxicity—GI greater than 80%, moderate toxicity—GI between 50 and 80%; high toxicity—GI less than 50%.

Table 3 shows the results obtained for the acute toxicity tests for the *Daphnia magna* microcrustaceans exposed to raw AMD, and the respective treatments by conventional and additional treatment with biosorption of algae *Scenedesmus* sp. The toxicity results show that raw acid drainage presented a toxicity factor (TF) of 400 and concentration of effect at 50% of the population (CE-50) of 6.25%, which classified it as an extremely toxic effluent. Similar results were presented by Kang et al. [47], who showed that acid mine drainage is highly toxic to microcrustaceans. This high toxicity may be due to the acidic pH, considerably below the no effect pH range for *Daphnia magna* (pH 4–11.5), and the high concentration of metals [48,49]. After conventional treatment, the TF values improved to 4, and the CE-50 was greater than 100%, classifying the samples as slightly toxic. The remaining toxic effect may be related to the Mn and As concentrations still present after treatment, since the studied species has sensitivity to these metals [50,51]. It may also be related to the conductivity, since the organism is sensitive to conductivity values greater than 2 mS cm^{-1} [52]. After the additional treatment with algae, there was another noticeable decrease in FT, reaching the value of 2 for both situations considered—with and without nutrients addition. The CE-50 was greater than 100%, framing it as slightly toxic.

The results show that microalgae may be suitable candidates for simultaneous remediation and sustainable biomass production in aqueous environments fed by treated acid mine drainage—at least from those of the coal mining industry. These efforts may help to attain the environmental standards that regulate the mining sector [53,54] and to contribute to the existing tendency of the mineral sector to look for sustainable practices, such as zero waste emission or further use of residual materials according to the economic model of the circular economy [55,56].

Table 3. Toxicity assays for *Daphnia Magna* microcrustaceans exposed to raw AMD; AMD after conventional treatment; and AMD after additional treatment with algae, with and without nutrients addition.

	TF	**CE-50 48 h (%)**	**Toxicity**
Raw AMD	400	6.25	Extremely toxic
Conventional treatment	4	>100	Slightly toxic
Additional treatment			
Algae	2	>100	Slightly toxic
Algae + nutrients	2	>100	Slightly toxic

Toxicity criterion: extremely toxic—CE-50 < 25%, highly toxic—CE-50 between 25 and 50%, moderately toxic—CE-50 between 50 and 65%, and slightly toxic—CE-50 > 65% [46].

4. Conclusions

The biosorption process with the in vivo microalgae *Scenedesmus* sp proved to be efficient as a polishing step in AMD treatment systems considering both physical-chemical and toxicological analyses. After 10 days of algal growth, the *Scenedesmus* sp biomass (on a dry basis) concentration can reach 230 mg L^{-1} without the use of nutrients, and 650 mg L^{-1} with the addition of nutrients. Regardless of the use or not of nutrients, the biosorption process contributed significantly to reducing the total metal and sulfate content, as well as the conductivity.

Concerning the toxicological studies, the organisms tested in this work proved to be suitable as bioindicators to evaluate the performance of AMD treatment procedures in coal mining, each one presenting certain particularity. The growth of *Allium cepa* roots presented the advantage of a simple test and allowed a differentiation between treated and untreated AMD, but showed a low sensitivity among the various treatment scenarios. The acute toxicity test with *Daphnia magna* showed a higher sensitivity, allowing the differentiation of results between the raw and treated AMD, as well as between the different types of treatment applied.

Author Contributions: K.B.d.S.: Conceptualization, Investigation, Formal analysis, Writing—original draft. V.O.d.A.: Formal analysis, Investigation. J.W.: Investigation, Writing—original draft, Writing—review & editing. I.A.H.S.: Conceptualization, Formal analysis, Resources, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grant number [88882.345791/2019-01] and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) grant number [310369/2016-9] and [160570/2019-0].

Acknowledgments: This work was supported by the following Brazilian funding agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ). The authors are also grateful to Jean Carlo Salomé dos Santos Menezes for providing AMD samples.

Conflicts of Interest: The authors declare no conflict of interest.

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