Toxicological Evaluation of Biological and Electrochemical Treatments of Coal Mine-impacted Water (MIW) on Duckweed Landoltia Punctata

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Research Article

Keywords: Acid mine drainage (AMD), mine-impacted water (MIW), electrocoagulation, biostimulation, duckweed, toxicity assay

Posted Date: February 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1232787/v1

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Abbreviations: AMD: acid mine drainage; DF: dilution factor; DO: dissolved oxygen; elC: electrocoagulation; EC_{50}: 50% effect concentration; I_r: inhibition rate; MAV: maximum allowable value; MIW: mine-impacted water; r: growth rate; SC: Santa Catarina state; SRB: sulfate-reducing bacteria.

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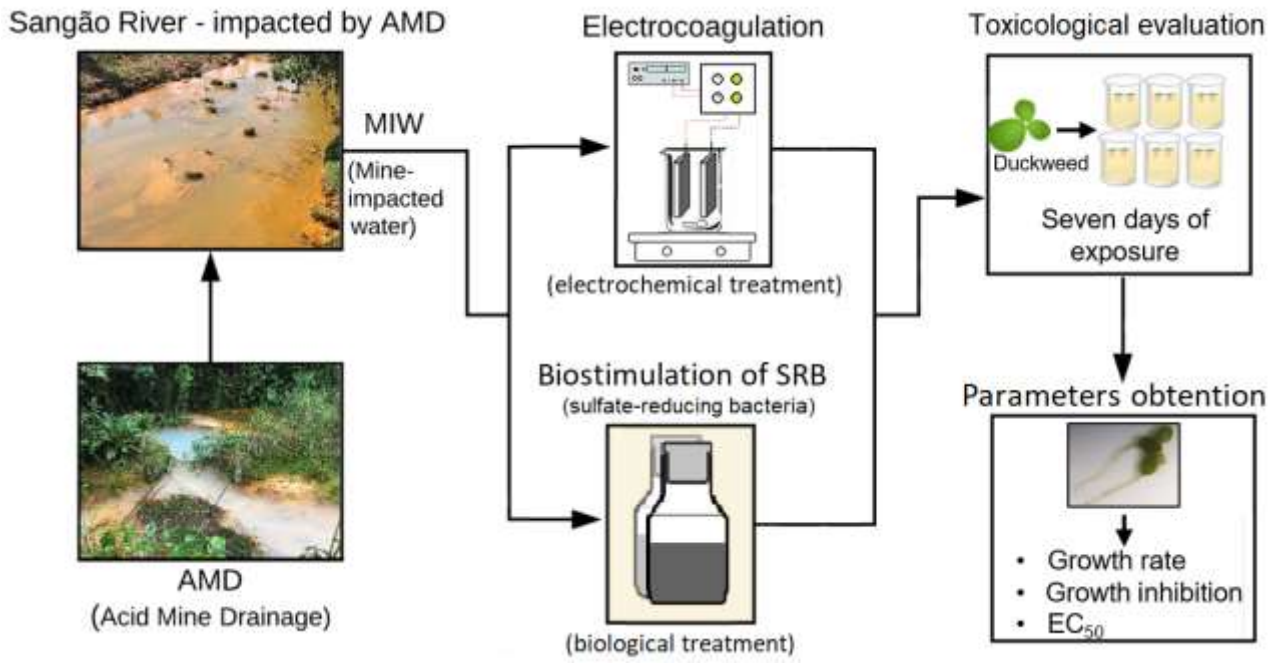
GRAPHICAL ABSTRACT



ABSTRACT

Two different coal mine-impacted water (MIW) treatments (biological via biostimulation of sulfate-reducing bacteria (SRB), and electrocoagulation (elC)) were proposed, reaching efficiencies of up to 99.79% in relation to SO_4^{2-}, Fe, Mn, and Al ions, as well as acidity removals. Thus, toxicological assays with duckweed *Landoltia punctata* were performed, in order to verify the safeness and usability of the two treated waters. Therefore, duckweeds were exposed to different dilutions (0, 25, 50, 75, and 100% of samples) of the two treated waters, and the growth (r) and inhibition of growth (I_r) rates were calculated, based on 50% effect concentration (EC_{50}). The water from the biological

23 treatment (microcosm assay) presented the highest toxicity (EC_{50} = 33.42%), even higher when
24 compared to the raw MIW (EC_{50} = 42.78%), probably due to the hydrogen sulfide, that even after a
25 purge removal, remained in solution. The results showed that this water, despite being within the
26 standards in physicochemical terms, demonstrated risks in terms of toxicity. The water from
27 electrocoagulation (elC) treatment, in the opposite way, showed much less toxicity, even lower than
28 the control, and therefore not reaching EC_{50}, also suggesting a possible nutrient function of the treated
29 water. Consequently, the treated water by elC could, for example, have a non-potable use. The study
30 made it possible to prove the efficiency of elC treatment, the importance of post-treatment
31 toxicological assessments, and the potential of the duckweeds as an option for a test organism in these
32 types of evaluations.
33

34 **1. Introduction**

35 Mining operations can cause severe environmental impacts, because of acid mine drainage
36 (AMD) formation and release. As an example in Brazil, the Carboniferous Basin of Santa Catarina
37 (SC) State region is highly impacted (Núñez-Gómez et al. 2019; Rodrigues et al. 2019). The coal
38 AMD formation results from pyrite oxidation, through several chemical and biological processes that
39 generate an effluent which is highly acidic (pH 2-3), with high sulfate (SO_4^{2-}) and metallic ion (e.g.,
40 Fe, Al, Mn, Zn, Cu, etc.) concentrations (Sánchez-Andrea et al. 2014). This type of effluent, highly
41 toxic and corrosive, continuously contaminates the surface and groundwater, creating water known
42 as mine-impacted water (MIW) (Mamelkina et al. 2017).
43 The sulfate concentrations in coal MIW may range from hundreds to thousands of $mg \cdot L^{-1}$
44 (Nariyan et al. 2017), and previous studies (Rodrigues et al. 2019, 2020b) treated it successfully,
45 yielding high removals of sulfate and metallic ions, as well as pH alkalization, by biostimulating
46 sulfate-reducing bacteria (SRB), using shrimp shell waste as substrate. Electrocoagulation (elC) has
47 also been tested, achieving sulfate removal efficiencies of up to 70.95%, as well as pH neutralization
48 (Rodrigues et al. 2020a).
49 The biota in aquatic environments with lower pH than its tolerance levels can die due to
50 respiratory and osmoregulatory disorders, compromising the food chain (Netto et al. 2013). Thus,
51 post-treatment toxicological assays are essential, since with them it is possible to identify the risks
52 involving test-organisms to this exposure, such as physiological, morphological, and metabolic
53 changes (Lalau et al. 2015). Additionally, these assays can ensure safe levels of exposure to the
54 adverse and the harmful effects (Lee et al. 2018), that traditional physicochemical analysis cannot
55 identify (Costa et al. 2008).
56 Plants are organisms that play a key role within the aquatic ecosystems as a food source,

participating in the biogeochemical cycles through the production of oxygen and nutrients (Stegemeier et al. 2017). However, the exposure of these organisms to extreme environments, such as a high concentration of metallic ions and low pH, can cause phytotoxicity and several other effects (Shanker et al. 2005; Tamás et al. 2006). Also, as plants belong to the basal level of the food chain, they can bioamplify the toxic effects to higher trophic levels (Martins 2014; Lalau et al. 2015).

Duckweed (*Landoltia punctata*) is a group of small floating macrophytes that grows in lentic freshwater environments, offering several advantages as bioindicators, including small size, direct absorption of contaminants by the leaves, rapid growth, and simple crop requirements (Lalau et al. 2020). These macrophytes have been successfully used in wastewater treatment and toxicity testing in recent years (Perreault et al. 2010, 2013; Zezulka et al. 2013; Lalau et al. 2015, 2020; Ziegler et al. 2016, 2019; Pereira et al. 2018). Also widely used to determine the impacts on an extensive range of substances released into the environment (Wang 1990), duckweed is being used in various international guidelines for ecotoxicological risk assessment (OECD 2006; ISO/DIS 20079 2010). Although these organisms are standardized as a toxicological model in several countries, assessments with their use are not yet standardized in Brazil (Lalau et al. 2015).

The purpose of this study was to evaluate the toxicology, through a 50% effect concentration (EC_{50}) and growth rate, of the two MIW treatments proposed: electrochemical (through electrocoagulation) and biological (microcosm biostimulating SRB) treatment on the duckweed, since both proposed treatments provided high removals of sulfate and metallic ions. Thus, this treated effluent could possibly present potential for non-potable use (garden watering, washing sidewalks or crop irrigation), thereby reducing the demand for quality water for population supply.

2. Materials and methods

2.1. Collection and characterization of mine-impacted water (MIW)

The MIW used for this study was obtained from the Sangão River, located inside the carboniferous basin of the southern State of SC, Brazil (28°45′38.7″S 49°25′58.1″W). The samples were collected in non-sterile polypropylene bottles with no headspace, and kept at 4 °C until the start of the analyses (APHA 2017). They were filtered under vacuum using a 0.45-µm pore membrane, and characterized (in terms of pH, and Fe, Al, Mn, SO_4^{2-} ions) on the same day of the collection and after both treatments, at the Water Reuse Laboratory (LaRA), at UFSC. Table 1 shows the methodology used for each analysis.

Table 1. Analytical methods used for characterization of samples

Parameter	Method	Range	Equipment
Fe	Ferrover[a]	0.02-3.00 mg·L^{-1}	Spectrophotometer HACH DR 5000
Mn	Periodate oxidation[a]	0.1-20.0 mg·L^{-1}	
Al	Aluminum[a]	0.008-0.800 mg·L^{-1}	
SO_4^{2-}	Sulfaver[a]	2-70 mg·L^{-1}	
S^{2-} [b]	Methylene blue[a]	5-800 µg·L^{-1}	
pH	pHmeter lecture	1-14	pHmeter Thermo Scientific
DO[c]	Oximeter lecture	-	Optical probe YSI ProODO

[a] Adapted from *Standard Methods for the Examination of Water and Wastewater* (2017).
[b] Analysis comprises the solved forms of sulfide: H_2S, HS^-, and S^{2-}.
[c] DO: Dissolved oxygen.

2.2. SRB biostimulation setup and sulfide removal

The biological treatment experiments were built up as performed by Rodrigues et al. (2020b) to biostimulate the SRB: microcosms glass flasks (500 mL of capacity) containing 260 mL of MIW together with 2.6 g of shrimp shell (10 g·L^{-1}) as a carbon source. In preparation, the shrimp shell waste was washed, dried, and pulverized, as described by Núñez-Gómez et al. (2017). Before being added to the flasks, the MIW was submitted to a N_2 purge, until it reached anoxia (DO ≤ 0.5 mg·L^{-1}, monitored with an oximeter reading), then it was added to the flasks with the aid of a peristaltic pump (to avoid oxygenation). The microcosms flasks were purged with N_2 before and after the MIW was inserted, sealed with a silicone stopper, kept in a dark room at 20 ± 1 °C (controlled with a wall thermometer) for 41 days of incubation, being shaken manually once a day (to ensure homogeneity of the flask contents).

As a result of the sulfate reduction by the SRB, the hydrogen sulfide (H_2S) started to accumulate, and to remove it as gas, at the end of the microcosm period of incubation, an N_2 purge system was assembled. The H_2S is a weak diprotic acid, and its form depends directly on the pH (H_2S, HS^-, and S^{2-}), its neutral form being partially soluble in water and toxic gas. Inside a fume hood, the flasks were purged with N_2, and the outgoing gas flow (H_2S + N_2) was bubbled into a NaOH solution, generating sodium sulfide (Eq. 1), thus avoiding leakage of the toxic gas to the outside. Each flask was purged for 30 minutes (four flasks per time, with a four-way manifold splitter, Fig. 1). After this sulfide removal process, the microcosms contents were filtered (also inside a fume hood), characterized, and submitted to toxicological assay.

$$H_2S_{(g)} + 2\,NaOH_{(aq)} \rightarrow Na_2S_{(aq)} + 2\,H_2O \qquad \text{Eq.1}$$

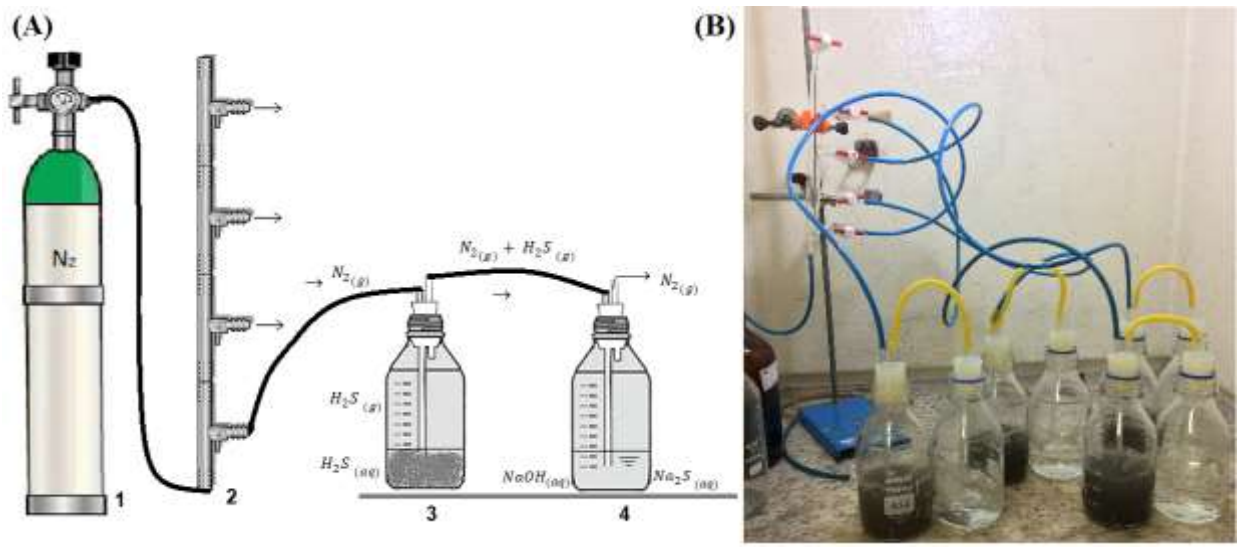


Fig. 1 (A) Scheme and **(B)** picture for the hydrogen sulfide removal apparatus: (1) Nitrogen cylinder; (2) four-way manifold gas line splitter; (3) Microcosm flask after 41 days incubation; (4) NaOH solution flask. The manifold splitter allowed to purge four microcosms per time.

2.3. Electrocoagulation (elC) assay for MIW treatment

An electrochemical system to treat MIW was carried out in bench-scale, as performed previously (Rodrigues et al. 2020a). The system consisted of duplicates of reactors (1-L plastic beaker), in which flat plate electrodes of Al (anode) and stainless steel (cathode) were immersed, spaced 5 cm from each other. The electrodes had the following dimension: 5.65 x 13.9 cm, with a useful area of 28.76 cm^2 (anode). Magnetic stirrers were used during the process to homogenize, since a chemical species concentration gradient naturally occurs. A control panel regulated the electric current from the power supply (PS-A305D), providing a 65 $A \cdot m^{-2}$ current density, in continuous mode of exposure (of electric current) that goes into each elC reactor (Fig. 2). In this batch assay, 1 L of MIW was inserted in each beaker, the room temperature was controlled and kept at 23 ± 1 °C, and the total electric current time was 5 hours. After this period, the content was filtered for characterization, and submitted to toxicological assay.

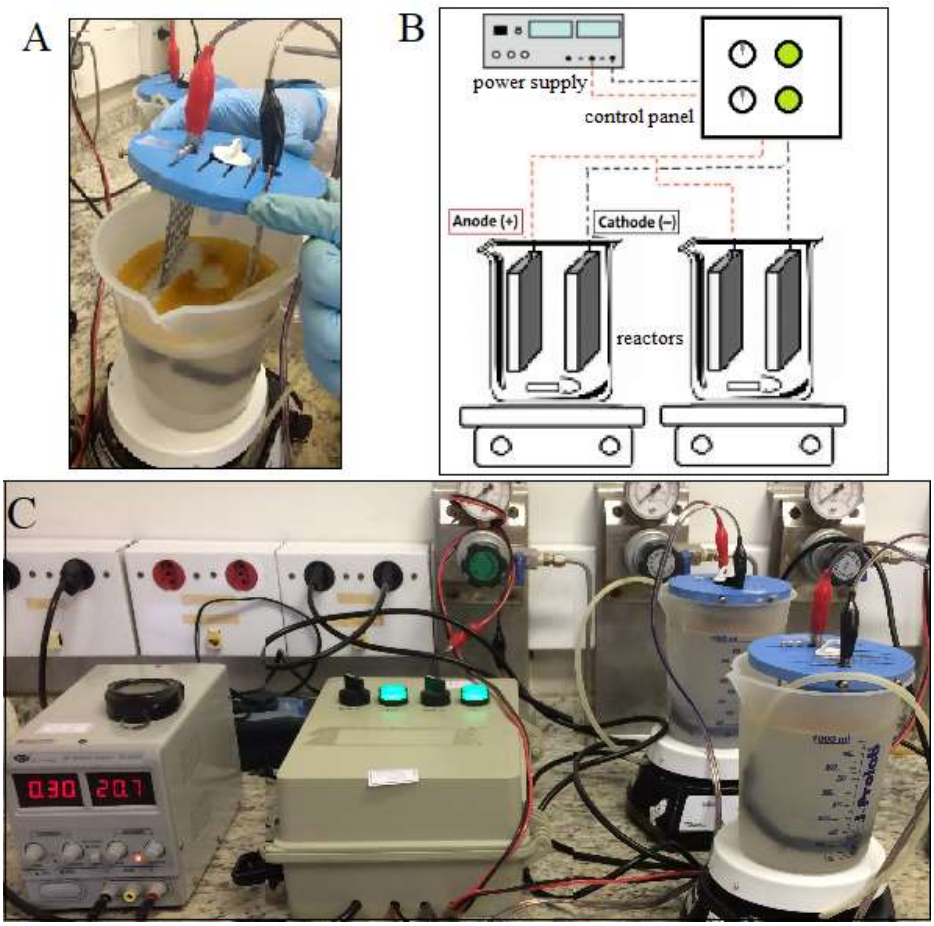


Fig. 2 (A) elC reactor profile along with its immersed electrodes (red anode and black cathode); **(B)** An illustrative scheme **(C)** Photograph of the elC bench apparatus. Adapted from (Rodrigues et al. 2020a).

2.4. Toxicological assay

The toxicological tests were carried out at the Laboratory of Environmental Toxicology (LABTOX), at UFSC. The duckweeds were collected from the natural environment and adapted to laboratory conditions according to international standards (OECD 2006; ISO/DIS 20079 2010). The inoculation procedure of the plant and culture medium were elaborated according to international standards (OECD 2006; ISO/DIS 20079 2010), and as described by Lalau et al. (2015, 2020). The culture medium composition was: $MgSO_4.7H_2O$ (15 $g{\cdot}L^{-1}$), $NaNO_3$ (8.5 $g{\cdot}L^{-1}$), Na_2CO_3 (4 $g{\cdot}L^{-1}$), $CaCl_2.2H_2O$ (7.2 $g{\cdot}L^{-1}$), KH_2PO_3 (1.34 $g{\cdot}L^{-1}$), H_3BO_3 (1 $g{\cdot}L^{-1}$), $MnCl_2.4H_2O$ (0.2 $g{\cdot}L^{-1}$), $Na_2MoO_4.2H_2O$ (0.01 $g{\cdot}L^{-1}$), $ZnSO_4.7H_2O$ (0.05 $g{\cdot}L^{-1}$), $CuSO_4.5H_2O$ (0.005 $g{\cdot}L^{-1}$), $Co(NO_3)_2.6H_2O$ (0.01 $g{\cdot}L^{-1}$), Na_2EDTA (0.28 $g{\cdot}L^{-1}$), and $FeCl_3.6H_2O$ (0.168 $g{\cdot}L^{-1}$).

Four different assays were performed for comparative purposes: MIW after biostimulation, MIW after electrocoagulation, raw MIW, and raw MIW with corrected pH (with NaOH solution until pH=7). The latter assay was included because the MIW pH is acid, and duckweed needs a pH between 5-9.

The MIW samples were diluted with culture medium, and for the analysis of toxic effects, dilution factor (DF) was used, being 0% (control - only culture medium), 25% (1:4, i.e., 1 part of crude sample diluted in 3 parts of culture medium), 50% (1:2), 75% (1:1.333), and 100% (gross sample – 1:1) (Iatrou et al. 2015), as detailed in Table 2, with a total of six replicates for each dilution. The total volume was 100 mL in all cases.

Table 2. Different dilutions applied in *Landoltia punctata* for the growth rate experiments.

	MIW parts:total parts (v/v)	**DF (%)**	**MIW volume (mL)**	**Medium volume (mL)**
Control	0:1	0	0	100
	1:4	25	25	75
	1:2	50	50	50
	1:1.333	75	75	25
Gross sample	1:1	100	100	0

All experiments were conducted in 100 mL-beakers, and each one was inoculated with duckweed and incubated in a temperature-controlled incubator (25 ± 2 °C) under an 18h-continuous illumination with fluorescent lamps (photoperiod). The pH was adjusted to the range of 6.5 to 7, using HCl or NaOH, except for raw MIW. The test started (t_0) with a total of ten healthy fronds (FN_0) for each dilution and lasted seven days. At the end of the test (t_1) the frond number (FN_1) was counted and the growth rate (r) was calculated according to Eq. 2. The inhibition rate (I_r) of the specific growth rate (%) was calculated according to Eq. 3 (OECD 2006; ISO/DIS 20079 2010):

$$r = \frac{\ln(FN_1) - \ln(FN_0)}{t_1 - t_0} \quad \text{Eq. 2}$$

$$I_r = \frac{r_c - r_t}{r_c} x100 \quad \text{Eq. 3}$$

Where r_c is the average specific growth rate of the control, and r_t the average specific treatment growth rate to each DF tested. For the EC_{50} determination, the I_r values were plotted against DF, and the regression of this concentration-response curve was performed. EC_{50} is defined as the sample concentration where 50% of effect is observed, when compared to the control. In this case, the effect is the growth inhibition (I_r).

The values of the parameters (growth rate and growth inhibition) were calculated through mean and standard deviations. The significant differences between the means of treatments and control samples were obtained from analysis of variance according to the Tukey test, and performed through *Statistica* (v.10, 2011) software.

3. Results and discussion

3.1. MIW characterization and treatments

In the biological treatment (SRB biostimulation), where there was sulfate reduction activity, sulfate and acidity were removed, as well as metallic ions (Fe, Al, and Mn). Similarly, for the elC treatment, the same parameters were also removed, and Table 3 shows those values from MIW before and after treatments, as well as Brazilian guidelines for comparison. The maximum allowable values (MAV) were based on Resolution CONAMA 357/2005 (Brazil 2005), which provides parameters for secondary non-potable reuse. Resolution CONAMA 430/2011 (Brazil 2011), environmental legislation for effluent releases, was evidenced as a complimentary guideline.

Table 3. Physicochemical characterization of MIW for SRB biostimulation and elC treatments.

Treatm. \ Analyte		pH	SO_4^{2-} (mg·L^{-1})	Fe (mg·L^{-1})	Mn (mg·L^{-1})	Al (mg·L^{-1})
Before treatment	SRB biostimulation	3.20	180	8.40	1.60	5.00
After treatment		7.19	2.00	0.31	0.10	0.14
Removal efficiency		-	98.89%	96.31%	93.75%	97.20%
Before treatment	elC	3.84	300	29.2	2.0	10.84
After treatment		8.94	84	0.06	0.30	1.37
Removal efficiency		-	72.00%	99.79%	85.00%	87.36%
CONAMA 357 (MAV)[a]	Legislation	6-9	250	5	0.5	0.2
CONAMA 430[b]		5-9	-	15	1.0	-

[a] Maximum allowable values for Class III water, adequate for non-potable reuse (Brazil 2005).
[b] Brazilian conditions and standards of effluent releases (Brazil 2011).

For the biological treatment, although sulfate was already in line with the MAV, it presented a 98.89% removal, restating the efficiency of this process. The metallic ions also presented a 93.75% or greater removal, and reached the MAV, at the end of 41 days of treatment. The pH increased considerably, reaching neutrality due to the natural presence of carbonates in the shrimp shell. It is likely the alkaline pH helped in the removal process of metallic ions, or it was precipitation in the form of hydroxides (OH^-), bicarbonates (HCO_3^-), carbonates (CO_3^{2-}), and sulfides (S^{2-}).

For the elC treatment, equally reasonable efficiencies were reached, besides a much more alkaline pH and a 5 hour treatment length. The sulfate, that was removed via several aluminum sulfate complexes (Rodrigues et al. 2020a), presented a 72% removal efficiency, but this was still within the MAV. The metallic ions yielded efficiencies between 85% and 99.79%, and except for Al, they were in line with the MAV. For Al, even though it can be precipitated in a pH equal or higher than 6 (Falagán et al. 2017), in a pH near 9 it may re-solubilize as an aluminate ion ($Al(OH)_4^-$) (Kaur et al. 2018), likely being the reason that its concentration was almost seven times above the MAV (Table

3). It is worth mentioning that the aluminum is released continuously by the anode as a coagulant agent. In this assay, for operation in a non-reducing atmosphere (differently from the microcosms assay), no sulfide was formed, thus, the precipitation is caused by hydroxides, (bi)carbonates, and complexed sulfates (Rodrigues et al. 2020a).

Subsequently, the MIW from microcosm and elC treatments were submitted to toxicological assay, in order to ensure safeness and quality of the treated effluent.

3.2. Toxicological assay for growth and inhibition rate

Fig. 3 exposes the growth rates of duckweed for the four experiments carried out: effluents from both treatments (biological and elC), raw MIW, and corrected pH MIW. From these results it is possible to observe that the MIW after biostimulation (Fig. 3A), raw MIW (Fig. 3C), and corrected pH MIW (Fig. 3D) presented the same pattern: significant different (and lower) growth rates compared to the control (0 DF), evidencing therefore, that they have considerable toxicity, since growth decreased as the concentration (DF) increased. For specific cases of SBR biostimulation, the growth is zero from 50% of DF. The differences in the growth rate for the four controls are attributed to small differences in the medium composition, as the four experiments were carried out on different days with freshly prepared solutions.

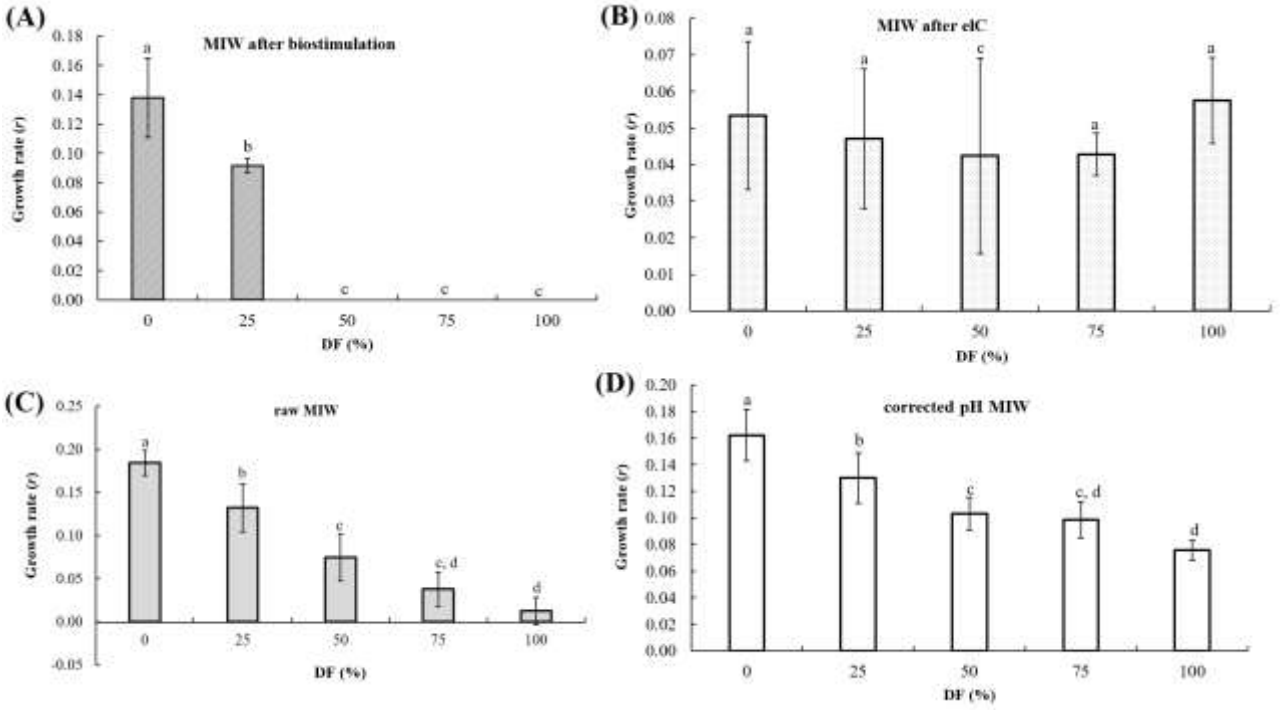


Fig. 3 Growth rate of *Landoltia punctata* after 7 days exposed to different effluents and dilution percentages: **(A)** MIW after biostimulation treatment, **(B)** MIW elC treatment, **(C)** raw MIW and **(D)** corrected pH MIW. Letters indicate significant differences according to *one-way* ANOVA and posterior Tukey test ($p \leq 0.05$). The data points represent the average values and the error bars represent the standard deviation.

Otherwise, in relation to the elC treatment results (Fig. 3B), for all concentrations (except for the 50% DF), they presented no significant difference from the control, as shown by the same letter "a" from the ANOVA study (Fig. 3B), therefore low toxicity and good quality of this effluent is inferred. It is important to note a slight increase in growth rate for the elC effluent in the 100% DF (gross sample). The hypothesis that this sample has some element that may have stimulated the growth of duckweed is raised. The ability of an Mn ion to act like a micronutrient when in adequate concentrations, stimulating the chlorophyll formation, and intervening in the production of enzymes is well known. Enzymes play an important role in protein metabolism and cellular division (Chatzistathis et al. 2011; Soiltech 2021).

In relation to the inhibition of growth rate, most of the results (Fig. 4A, C and D) showed the same toxicological trend: as the DF increased, the inhibition also increased, especially for the SRB biostimulation treatment (Fig. 4A), which increased at a higher rate than the other samples.

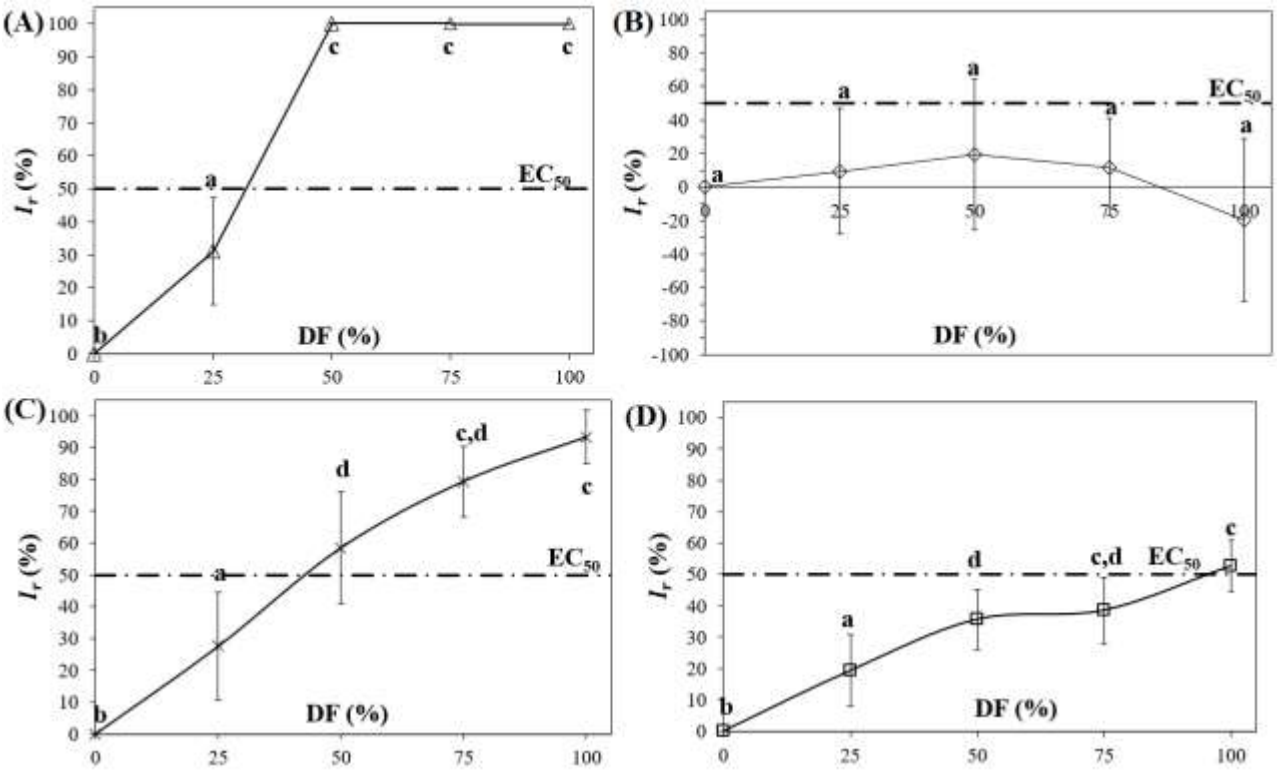


Fig. 4 Response curve after 7-days exposure: the results were based on the % inhibition rate (I_r) *versus* dilution factor (DF) to **(A)** SRB biostimulation treatment, **(B)** elC treatment, **(C)** raw MIW and **(D)** corrected pH MIW. Letters indicate significant differences according to *one-way* ANOVA and posterior Tukey test ($p \leq 0.05$). The data points represent the average values and the error bars represent the standard deviation.

The raw MIW (Fig. 4C) also presented considerable toxicity as the concentration increased, with significant difference between the inhibition rates at the different DF. In the case of corrected pH MIW (Fig. 4D), its inhibition rate was also raised at a higher DF, but in a smoother way. Between

these two last treatment results (raw MIW and corrected pH MIW), the effect of a simple pH correction (to 7) in toxicity is highlighted, revealed by their EC_{50} value (42.78% and 92.37% of DF, Table 4). This is coherent with the ideal conditions for duckweed development (minimum pH of 6.5) (OECD 2006; ISO/DIS 20079 2010). The main factor associated with toxicity was evidenced by pH correction. This fact was already expected, as coal mines in the region impact water resources.

Table 4. DF values for each test reach the EC_{50}, listed in descending order of toxicity.

Type of treatment	DF (%) for EC_{50}	Toxicity level
SRB biostimulation	33.42	▲ more toxic
Raw MIW	42.78	
Corrected pH MIW	92.37	less toxic
elC	-	▼ atoxic

In addition, it should be taken into account that in acid medium there are also potentially dissolved metallic ions. Studies (Chamorro et al. 2018) state that the metallic ions in AMD are largely responsible for its toxicity, and Lattuada et al. (2009) reach the same conclusion.The toxicity of raw MIW is corroborated by an analogous study (Nagy et al. 2020), that also observed toxic effects in duckweed (*Lemna minor*) when exposed to AMD.

In the opposite way, for the elC toxicological experiment (Fig. 4B), no statistical differences were observed in the inhibition of growth rates with increasing DF, even presenting a slightly negative inhibition of growth rate for the gross sample (100% of DF), corroborating the earlier graph (Fig. 3). As it did not reach a 50% inhibition rate, it was not possible to determine the 50% effect concentration (EC_{50}). This type of result is in agreement with that found in the literature: Radić et al. (2014) also obtained a decrease in the toxicity when evaluating AMD treatment using a combined CaO/elC process, using the organisms *Daphnia magna* and *Lemna minor*.

In the present study, since a slight stimulus in the growth of plants exposed to the effluent from the elC treatment was observed, a sufficient removal of metal ions ceasing to be toxic and behaviour like nutrients can be inferred. As macrophytes need nutrients for their development (Teles et al. 2017), the results suggest that the treated effluent may have a nutrient function for the duckweed.

The SRB biostimulation treatment showed toxicity in *Landoltia punctata*, reaching 100% inhibition with only 50% of DF (Fig. 4A) and the lowest EC_{50} value (33.42 of DF, Table 4) in spite of the neutral pH. The highest toxicity from SRB biostimulation treatment is probably due to the presence of residual sulfide, which even after the purge removal process, still showed a 200 $\mu g \cdot L^{-1}$ concentration. This residual concentration can be explained by pka_1, which is shown by Eq. 4 (Atkins et al. 2018):

$$H_2S_{(aq)} \rightleftharpoons H^+{}_{(aq)} + HS^-{}_{(aq)} \ (pka_1 = 6.9) \quad \text{(Eq. 4)}$$

$$H_2S_{(aq)} \rightleftharpoons H_2S_{(g)} \quad \text{(Eq. 5)}$$

Due to the pH of the microcosm (7.19) being close to the pka_1(6.9), the $H_2S_{(aq)}$ remained partly in solution in equilibrium with $H^+{}_{(aq)} + HS^-{}_{(aq)}$ (Eq. 4), making its escape to the gaseous phase difficult (Eq. 5) even with the use of N_2 as a purge gas.

Conventional treatment using electrocoagulation proved to be efficient in reducing toxicity, and the treatment performed by biostimulation, under the conditions of the experiment, proved to be ineffective. However, the correction of MIW pH proved to be efficient in reducing toxicity. Despite the low efficiency of biostimulation, the results show that it presents a good opportunity for studies and that further research can be carried out to improve the method.

Duckweed species are sensitive to extreme environments (Wang 1990), and the results obtained in the present study demonstrate this, probably due to the presence of hydrogen sulfide, known to be toxic even to human beings (APHA 2017). It should also be noted that the odor resulting from this treated effluent is very pungent, detracting therefore from a non-potable secondary reuse application, since it would be impracticable to use it (for example, for garden irrigation, sidewalk washing, etc).

4. Conclusions

According to the toxicological evaluation performed, among the proposed MIW treatments, the BRS biostimulation (microcosm) and the elC, the latter evidenced no toxicity, even presenting nutrient potential for duckweed. However, the effluent from biological treatment, which in physicochemical terms showed even greater removal of sulfate and metallic ions, presented high toxicity, even higher than raw MIW. This is probably due to the residual hydrogen sulfide that remained, even after purging, because of the pH. In this sense, the quality of MIW treated in the elC assay was superior to the assay treated by biostimulation. This supported the fact that the elC treated effluent does not present odor and requires treatment of only a few hours, showing its potential for non-potable use purposes, conferring a use for a water initially highly polluted, for instance for irrigation, due to its suggested nutrient function. The findings also led to the conclusion that despite the physicochemical parameters of the effluent from biological treatment having met the requirements of the legislation for the evaluated parameters, the effluent does not meet the toxicity standards, which reveals the importance of these assessments. In addition, the macrophytes proved to be an interesting organism in these types of evaluations.

Furthermore, in future tests, scanning electron microscopy (SEM) and transmission electron

microscopy (TEM) images from the duckweed organelles tissues will be carried out, in order to visualize possible damage from the exposure, and complement the toxicological evaluation, providing more information about the mechanism of the toxicity observed.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: Not applicable.
Consent to participate: Not applicable.
Consent to publish: Not applicable.
Availability of data and materials: Not applicable.

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Acknowledgements

The funds for this research were provided by the National Council for Scientific and Technological Development (CNPq) and scholarship from the Coordination of Improvement of Higher Education Personnel (CAPES) – finance code 001.

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