Ecotoxicity with *Allium cepa* to determine the efficiency of conventional ARD treatment by neutralization/ precipitation from a brazilian coal mine

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Abstract

ARD (acid rock drainage) represents a major problem in the mining industry worldwide due to the risk of water and soil pollution. The active treatment of ARD involves the addition of alkaline reagents to increase the pH and precipitate the dissolved metals as hydroxides. The ARD treatment plants in Brazil mostly use sodium hydroxide or lime for neutralization. However, even after the treatment the water contain substantial amounts of dissolved ions. The aim of this work was to assess the chemical and physical characteristics of the treated effluent and evaluate the toxicity using *Allium cepa* (onion) as the organism test. Onion as a bioindicator has been widely used in tests to examine effects caused by toxic metals present in wastewaters. The ARD studied came from coal mine located in Brazil, highly associated with pyrite. This wastewater is concentrated in iron, sulfate ions with the presence of aluminum, manganese, zinc and small amounts of lead and arsenic. The following treatment condition were tested accordingly the alkaline chemical reagent and neutralization pH: NaOH - pH 7.0 +/0.1; NaOH - pH 8.7 +/-0.1; $Ca(OH)_2$ - pH 7.0 +/0.1; $Ca(OH)_2$ - pH 8.7 +/- 0.1. All treatment conditions resulted in a considerable reduction of the concentration of the metals, although Mn an As still remained above or very near the maximum limits for discharge accordingly to the Brazilian legislation. Best results in terms of overall metal removal, including manganese and sulfate removal and final conductivity were achieved with $Ca(OH)_2$ at pH 8.7. Phytotoxicity tests were performed with *Allium cepa* in order to verify the efficiency of the adopted processes. The results showed that the raw ARD drainage was toxic, since it was able to cause total inhibition of root growth in the phytotoxicity assay. On the other hand, the treatments generated clarified water that does not present toxicity. There were no inhibition nor a delay in the growth of *Allium cepa* roots when compared to the control sample. Treatment $Ca(OH)_2$ at pH 8.7 presented a little bit better performance in terms of roots growth compare to the other conditions applied.

Introduction

Control of ARD (acid rock drainage) continues to be a major subject of interest in mining. Although new approaches are in development, conventional active treatment of acidic waters still remains as an efficient way to avoid water and soil pollution (Kefene et al., 2017; Masindi et al., 2018; Neculita e Rosa, 2019; Naidu et al., 2019; Skousen et al., 2019).

The active treatment of ARD involves the addition of alkaline reagents to increase the pH and precipitate the dissolved metals as hydroxides (Skousen et al., 1996; Kontopoulos, 1998; Matlock et al., 2002; Johnson and Hallberg, 2005). The choose of the best pH depends on the dissolved metals to be remove. Considering the acidic waters treatment plants in coal mining in Brazil, some prefer a circumneutral pH and others pH 8.7 when necessary remove manganese along with other metals. The reagents that are commonly used are the calcium hydroxide

($Ca(OH)2$) and caustic soda ($NaOH$). Calcium hydroxide is preferred since it is cheaper and allows partial removal of the amount of sulphate ions present in the wastewater (Silveira et al., 2009).

Assessment of the toxic potential of mining wastewater is very relevant. Test organisms such as *Daphnia magna, Lactuva sativa* (lettuce) and *Allium cepa* (onion) has been references in ecotoxicological studies of ARD. Sivula et al. (2018) evaluated the toxicity associated with an ARD by using the organism *Daphnia magna*, due to high sensitivity of the organism to environmental changes, especially regarding the toxicity of metals and variation of acidity. Steyn et al. (2019) has chosen to evaluate the effectiveness of treatment of ARD using organisms *Lactuva sativa* and *Allium cepa,* as they are quick and simple methods to evaluate phytotoxicity of substances based on seed germination and growth inhibition root, respectively. Geremias et al. (2012) evaluated the effectiveness of treatment of ARD using the organism *Allium cepa*, and justified your use highlighting advantages such as low cost, high sensitivity, reproducibility and high productivity. A series of other studies described the advantages of *Allium cepa* as bioindicator in detail, such as the works of Fiskesjö (1985), Rankand Nielsen (1994), Maluszynska and Juchimiuk (2005), and Arraes and Longhin (2012).

The aim of this work was to assess the chemical and toxicological characteristics of the treated effluent and evaluate the toxicity using *Allium cepa* (onion) as the organism test. The ARD studied came from coal mine located in Brazil, highly associated with pyrite. This wastewater is concentrated in iron, sulfate ions with the presence of aluminum, manganese, zinc and small amounts of lead and arsenic. The main variable studied were the alkaline chemical reagent ($Ca(OH)_2$ or $NaOH$) and pH (pH 7.0 +/0.1 pH 8.7 +/-0.1).

Materials and methods

The sample of Acidic Drainage used in the development of this work was provided by Companhia Carbonífera do Cambuí LTDA, located in the municipality of Figueira, northeast of the State of Paraná, in the area of the Paraná sedimentary basin.

This work involves the treatment of acid rock drainage by means of the method of neutralization. The chemical and toxicological characteristics of the raw and treated effluent were analysed as illustrated in Figure 1.

The neutralization of the ARD through the addition of two gross reagents alkalizing ($NaOH$ and $Ca(OH)_2$) in two situations different pH (pH 7.0 +/0.1 pH 8.7 +/-0.1) under constant stirring. The slime formed by precipitation of metals was separated from the solution through filtration. Samples of ARD and the samples after neutralization were analyzed for pH, conductivity and concentration of the metals of Cu, Zn, Fe, Mn, Pb, Al and by issuing optical spectofotometer with Inductively Coupled Plasma (ICP-OES). The sulfate content was measured using the turbidimetric method. All analysis followed the procedures described in the "Standard Methods for Examination of Water and Wastewater" (Eaton et al., 2005)

Eighteen units of *Allium cepa* of the same origin of approximate diameters were selected. The experimentation were carried out in triplicate for each condition. After scraping shallow, this bulb was kept in touch with deionized water for 24 hours at room temperature (in all groups of samples.) After this period, the bulb was transferred to test substance and maintained contact with the same for 72 hours, adopting a control group in deionized water. The test was performed under the light and following the procedure of Fiskesjö (1985). For analysis of toxicity

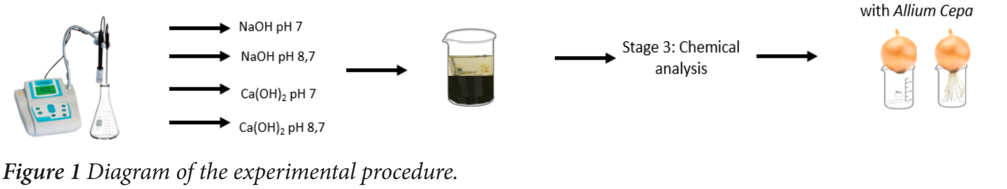


***Figure 1** Diagram of the experimental procedure.*

was measured number, mass and the length of the three largest roots in each bulb. The results of the test substances were compared with the control. The growth inhibition (phytotoxicity) was considered when there was a significant decrease between the test and control groups. Results of were assessed using analysis of variance (ANOVA) with significance level $p \leq 5\%$ and Tukey Test to compare the differences between averages.

Results and discussion

Table 1 presents the results of the analysis of metals, conductivity and sulfate in raw and treated acid rock drainage, compared to the standard for discharge of wastewater in Brazil (CONAMA Nº 430 of 2011 of the Ministry of Environment – Brazil).

It can be observed that raw acid drainage has a low pH and a high concentration of metals and sulfate. Considering the treated water, it is possible to see that both reagents (NaOH and $Ca(OH)_2$) were equally effective in terms of metal removal, but Ca(OH)2 allowed a higher removal of sulfate ions. Adjustment of pH to 8,7 allowed a higher removal of Fe, which can be explained by the presence of Fe^{2+}. At this pH the removal of Mn was also more effective, however not attending yet the concentration for discharge in water courses in Brazil. Concerning the concentration of As, the procedures of neutralization/precipitation was also not effective to reduce the concentration for discharge. All this results are in according with the expected in terms of acid mine drainage chemistry (Skousen et al., 1996; Kontopoulos, 1998)

Table 2 shows the average results of growth of the roots of *Allium cepa* and Figure 2 depicts the bulbs of *Allium cepa* after the phytotoxicity test. Direct exposure of *A. cepa* bulbs to raw ARD configured in a complete inhibition of roots growth. However, after treatment, regardless of the reagent applied or the final pH adjustment, it was observed a good roots development with values close to that achieved in the control condition. It should be noted that the difference in length and by weight does not exceed 25% between the control condition and ARD after treatment.

The results statistical analysis is shown in Figure 3. There is a significant difference between the raw and the treated effluents. The control (contact made with deionized water) showed no significant difference with any treatments carried out. It should be mentioned that the analysis of variance (ANOVA) was applied for a 95% confidence level.

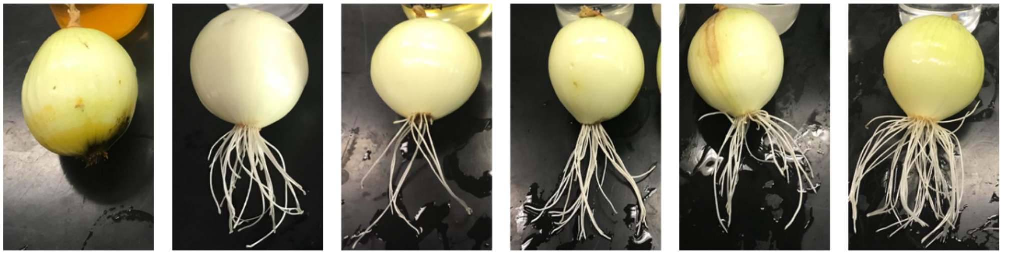
It is evident from the results that untreated acid rock drainage promoted phytotoxicity on plants, since it was capable of causing total root growth inhibition, when compared to the control sample (deionized water). Geremias et al. (2012) suggested that the phytotoxic effect would be associated with the low values of pH and a substantial concentration of (semi)-metals such as iron, aluminum, manganese, zinc, lead, and arsenic present in the effluent.

Table 1 *Physical chemical characteristics of raw and treated ARD considering the alkaline reagent NaOH or $Ca(OH_2)$ and the final pH adjustment (pH 7.0 +/0.1 pH 8.7 +/-0.1).*

Parameter	Raw ARD	Treated ARD				CONAMA 430
		NaOH pH 7	NaOH pH 8,7	$Ca(OH)_2$ pH 7	$Ca(OH)_2$ pH 8,7	
pH	2.49	7.00	8.70	7.00	8.70	
Cu	0.00	0.01	0.01	0.01	0,00	1
Zn	62.03	0.18	0.03	0.05	0.02	5
Fe	605.6	13.9	1.73	5.77	0.92	15
Mn	37.6	14.6	2.93	10.2	1.88	1
Pb	0.44	0.13	0.13	0.20	0.26	0.50
Al	262.1	0.07	0.13	0,00	0.15	-
As	0.90	0.67	0.53	0.71	0.62	0.10
SO4	7410.2	6443.7	5985.2	3124.8	2726.2	-
Conductivity (mS)	7.80	9.90	10.10	5.10	5.30	-
Sludge mass (g)	-	7.80	7.40	9.20	10.90	-

Table 2 *Average values (n = 3) of* Allium cepa *roots growth in raw and treated ARD considering the alkaline reagent NaOH or Ca(OH$_2$) and the final pH adjustment (pH 7.0 +/0.1 pH 8.7 +/-0.1).*

	Length (cm)	Number of roots	Mass (g)
H_2O	5.1	28	0.7
Raw ARD	0.0	0	0.0
Treated with NaOH at pH 7.0	6.4	20	0.5
Treated with NaOH at pH 8,7	6.6	22	0.6
Treated with $Ca(OH)_2$ at pH 7.0	6.5	25	0.5
Treated with $Ca(OH)_2$ at pH 8,7	6.9	20	0.6



(a) ARD (b) H_2O (c) NaOH pH7 (d) NaOH pH8,7 (e) $Ca(OH)_2$ pH 7 (f) $Ca(OH)_2$ pH 8,7

Figure 2 *Bulbs of Allium cepa after the phytotoxicity test.*

The effect of low pH is corroborated by the work of Fiskesjo (1985), which states that *Allium cepa* is not very sensitive to pH. Acceptable growth in water were found in the pH range between 3.5 and 11.0, since the roots of this species are able to change, to a certain extent, the pH to a level that allows its development. However, the very low pH of ARD of 2.49 falls outside of this range, which explain the absence of roots growth.

Treatment of the effluent allowed the pH adjustment and removal in great part the presence of metals. Despite of high conductivity of the treated water, *Allium cepa* showed a good development. This ecotoxicological did not showed differences between the chemical reagent applied and the differences of conductivity derived. Neither showed differences between the pH of metal precipitation - pH 7.0 or 8.7.

Considering the metals, copper and lead are indicated by Fiskejö (1985) as the main responsible for changes in the development of Allium cepa. However, these elements are present in low concentration in the effluent studied. Concentration manganese and arsenic, their concentration remains superior to the standards established by CONAMA 430. It is known that manganese concentrations above 18 mg L-1 can cause effects on the growth of roots (Fiskesjö, 1985) and, based on this information, it is important to choose pH 8.7 at ARD treatment plants. The toxicity for arsenic is even greater than manganese, chronical effects are associated by the presence of this metal gives a well a decrease in mitotic index and generates chromosomal (Patra, 2004). The process of neutralization/precipitation applied in the conditions of this work was not suitable to remove this element to Brazilian standards of wastewater discharge, being a subject of future attention.

Conclusion

Acid rock drainage used in this work presented a pH of 2.5 and a high concentration of metals. Treatment through of neutralization/precipitation proved efficient, with removal of most metals. Even after treatment, the presence of Mn and As were above the standards established for discharge in water bodies in Brazil. The results of the tests of phytotoxicity with *Allium Cepa* showed that the raw ARD has a high degree of phytotoxicity, which caused complete inhibition of growth of the roots. Such inhibition was not evidenced in the post-treatment carried out, demonstrating no phytotoxicity roots growth for *Allium*

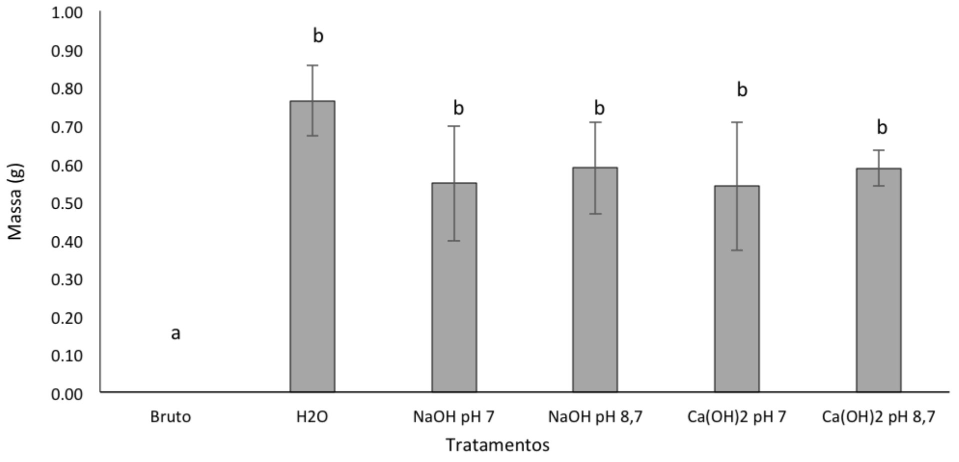


Figure 3 *Mean of the roots mass of Allium cepa and the results of statistical evaluatian with a 95% confidence level.*

cepa. The statistical evaluation of the results indicated that there is no significant difference for this toxicological teste applied for anyone of the treated effluents (considering NaOH and Ca(OH)2 as reagents or precipitation pH at 7.0 or 8.7) compared to the control, proving the effectiveness of the treatment.

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