EVALUATION OF ACID MINE DRAINAGE TREATMENT USING *Artemia sp.* AND *Allium cepa* AS BIOINDICATORS OF TOXICITY AND GENOTOXICITY[1].

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Abstract. Coal mining produces residues containing high levels of heavy metals and other components that contaminate surface and ground water. This research evaluated the efficiency of physical, chemical, and biological treatment of acid mine drainage using alternative biological indicators *Artemia* sp. (brine shrimp) and *Allium cepa* L. (onion). Samples were collected at four stations that are located at specific treatment system: 1) pH control and precipitation, 2) biological damping pond outlet, 3) wetland inlet, and 4) wetland outlet. Acute toxicity analysis using *Artemia* sp. was performed at sample concentrations of 25, 50, 80, 90, and 100%. Toxicity in *A. cepa* was observed as root growth inhibition after a seven-day exposure period at 100% effluent concentration. Genotoxicity was observed in *A. cepa* meristematic cells using the comet assay that evaluates DNA strand breaks in single cells. *Artemia* sp. test results indicated a reduction of lethality in station 4 (less than 30%) when compared to station 3 (100%), indicating that the wetland is effective at reducing residue toxicity for these organisms. The high lethality at Station 3 was investigated and resulted in the discovery that contaminated ground water from residue leachate was entering the treatment system between Stations 2 and 3. Root growth inhibition showed no significant results, indicating that the effluent has no activity to *A. cepa* considering this parameter. The comet assay results indicate that stations 1 and 2 are genotoxic and a significant reduction can be observed after the biological processes, particularly at the wetland output. Thus, the results obtained indicate that *Artemia* sp. is a suitable bioindicator for toxicity assays of this effluent and that *A. cepa* is only suitable for evaluating genotoxicity. We can also observe that the treatment process is efficient at eliminating effluent toxicity and reducing genotoxicity.

Additional Key Words: Coal, Wetland, Bioremediation, DNA Damage.

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Introduction

The Catarinense coal mining basin is located in the southeastern region of the State of Santa Catarina and occupies an area of 1850km^2 (715 mi^2), in a strip measuring 95km (56mi) in length by 20km (12.5mi) in width between the parallels 28º48'25" and 28º23'54" and meridians 49º33'38" and 49º15'11" (Horbach et al., 1986). Mechanized coal exploration in this region began around 1940 (CETEM, 2001) and since then, has provoked physical, chemical and biological alterations in the local ecosystems, directly compromising water, soil, and biota resources (Zocche, 2005, 2008) over an area that varies from 2000 to 6000 ha (Alexandre, 1999; CETEM, 2001).

Acid mine drainage (AMD) generated by the pyrite residue deposits in the Catarinense coal mining basin is heavily contaminated with dissolved heavy metals and sulfate ions (Table 1); thus, it should be treated prior to being discarded into natural drainage.

Table 1. Solution obtained after infiltration in a pyrite residue pile (leaching solution), in the Catarinense Coal Mining Basin (Madeira et al., 2005).

Parameter	Leaching solution
pH	2.89
SO_4^{-2} (mg/L)	14,920.0
Al (mg/L)	800.0
Fe (mg/L)	4,537.0
Cu (mg/L)	73.0
Zn (mg/L)	0.01
Mn (mg/L)	155.0

Several acid mining drainage treatment methodologies have been used; however, commonly used neutralization methods are efficient at correcting pH and metal precipitation, while sulfate remains soluble (Madeira et al., 2005).

In the last few years, the use of passive acid mining drainage treatments has increased; these make use of natural ecosystem structures and functions and constitute an economically attractive alternative, as well as aggregating additional value, such as landscape restoration and the maintenance of biodiversity (Lacki et al., 1990; Brenner and Hofius, 1990; Mota Marques et al., 2000; Ji et al., 2004).

Research aimed at evaluating the adequacy and efficiency of natural and/or constructed wetlands for the removal of pollutants and toxic characteristics of effluents has been performed

at real scale and at micro and mesocosmic scales, and macrophytes have been considered fundamental (Taylor and Crowder, 1982, 1983a, 1983b; Jamison and Rauch, 1990; Calabrese et al., 1990; Nawrot and Klimistra; 1990; Mota Marques et al., 2000; Ji et al., 2004).

Evaluation of the efficiency of such systems is commonly achieved by physical and chemical monitoring of the effluent; however, the use of biological indicators has increased. Depending on the approach adopted, quick or slow answers highly relevant to ecological or toxicological status can be obtained. Indices of richness, diversity, trophic structure, and ecological succession provide slow, though highly ecologically relevant information, while molecular, physiological, and histopathological approaches provide quick answers with high toxicological relevance. Over the last few years, the use of bioindicators has continually increased, both at an organism level (toxicity) and molecular level (genotoxicity).

Bioindicator is a term used to describe an organism that can respond to a variety of environmental alterations in which it lives in a particular way, thus providing physiological, biochemical, genetic and behavioral information, among others (Shugart, 1994).

To perform environmental toxicological analysis, bioindicator organisms are used with the aim of testing their survival; for this purpose, the acute toxicity test has been indicated on the microcrustacean *Artemia salina*, as well as other organisms (Svensson et al., 2005).

In contrast, genotoxicity tests study the potential of chemical agents to induce mutations in somatic cells or those that can be transmitted to future generations (Da Silva et al., 2003). To evaluate DNA damage, the comet assay is used; this is a rapid, sensitive technique used to quantify lesions and detect the effects of DNA repair in individual mammalian cells (Singh et al., 1988; Fairbairn et al., 1995). This test presents certain advantages over biochemical and cytogenetic tests, including the requirement of only a few cells that do not have to present cell division. The cells are immersed in gel on a slide and are submitted to an electrical current that forces free DNA segments (resulting from breaks) to migrate from within the nucleus. After electrophoresis, cells that present a round nucleus are identified as normal, with no recognizable DNA damage, while lesioned cells are visually identified by a kind of tail, like a comet, formed of DNA fragments that migrate from the nucleus. These fragments occur in different sizes and are associated with the nucleus by a simple chain (Fairbairn et al., 1995). According to some authors, comet size is proportional to the damage caused (McKelvey-Martin et al., 1993), but current consensus only fully accepts that visualization of the comet implies some level of DNA

damage, such as simple or double strand breaks and/or alkali-label lesions (Fairbairn et al., 1995; Tice, 1995).

The objective of the present study was to evaluate the efficiency of a constructed wetland at biopolishing acid mine drainage previously treated by conventional physical and chemical processes, using the alternative bioindicators *Artemia* sp. and *Allium cepa* L.

Material and Methods

Location and Description of the Study Area

The study was realized at Mining Unit II, which is owned by Carbonífera Criciúma S.A, and located at 28º47'19'' S and 49º26'32'' W, in the municipality of Forquilhinha, Santa Catarina, Brazil (Fig. 1). Mining Unit II occupies an area of 135 ha, within which two pits are located, together with an inclined plane to access the coal layer, a coal improvement plant, workshops, refectories, offices, coal storage patios, improvement residue deposits, stabilization ponds and an effluent treatment plant.

All of the AMD, from the subterranean drainage, surface runoff from the storage patios, and leachates generated in the residue piles is captured by a system of dikes and canals designed to channel the AMD through a primary treatment plant, where the water is neutralized for use in the coal improvement process (1,100m^3/h; 38,850ft^3/h), and then passes through stabilization ponds, that promote precipitation of oxidized metals together with the fine coal residue discarded during the coal cleaning process.

These ponds occupy four hectares, where the processes of suspended particle decantation occur concomitantly with metal precipitation, resulting in a neutral supernatant free from suspended solids and together with an alkalinity, which favors the spontaneous establishment of vegetation mass, formed by aquatic macrophytes, such as *Typha domingensis* (Pers), *Eleocharis acutangula* Schult. and *Eleocharis interstincta* R.Br. The first collection station for this study was installed at the supernatant outlet point of these stabilization ponds (Fig.2).

From this station, the supernatant is captured by pipes responsible for directing the flow to two small damping ponds (6,000m^2), which contain dense vegetation growth of *Eleocharis acutangula* and *Typha domingensis*. The second effluent collection station was installed at the outlet of the second damping pond (Fig.2), from which effluent flow is conducted by an open canal to a constructed wetland, where two more collection stations were installed, positioned at

the inlet and outlet of this wetland (Fig. 2). After the wetland, the effluent flows into the River Sangão.

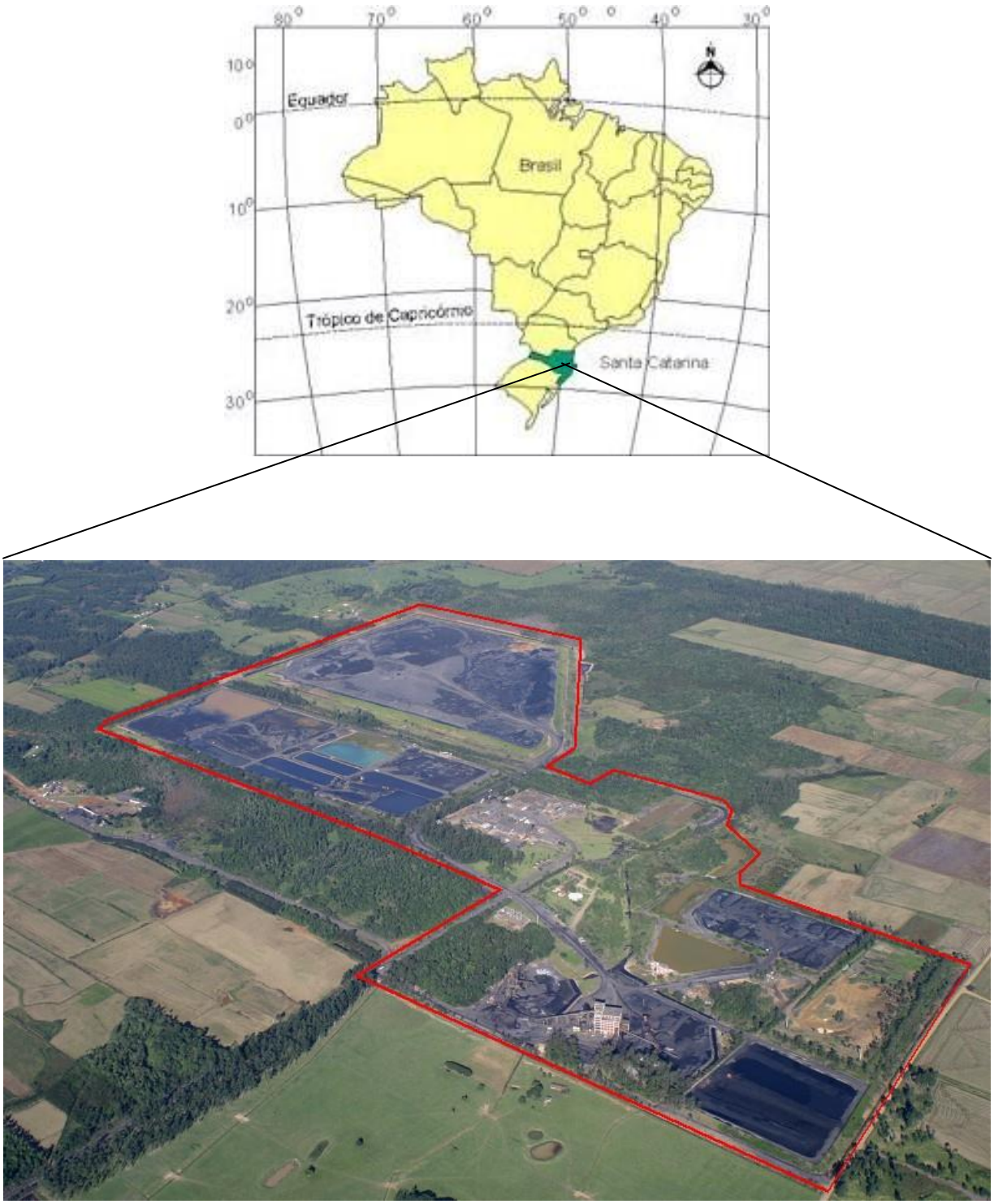

Figure 1. Location of Mining Unit II of Carbonífera Criciúma S.A, located at 28º47'19'' S and 49º26'32'' W, municipality of Forquilhinha, Santa Catarina, Brazil. A red line indicates the property limits.

Figure 2. Locations of sample collection stations 1 (pH control and precipitation), 2 (second damping pond outlet), 3 (wetland inlet), and 4 (wetland outlet), marked with yellow circles. (Source: Image base captured from Google Earth).

The wetland was constructed in January 2007 and comprises a circuit formed by 12 chicanes installed to forced the passage of the effluent through an area of approximately 32,000m² (160 x 200m) (344,450ft²; 525ft x 655ft), forming a layer of water 0.30 to 0.50m (1 to 1ft 8in) deep, corresponding to a continuous flow of about 520,000L/h (137,000Gal/h US). Spontaneously developed vegetation covering consists of numerous aquatic and amphibious macrophytes, principally Poaceae, Cyiperaceae, Juncaceae, and Typhaceae.

Effluent Collection

Samples of effluent were collected monthly from September 2007 to August 2008, though only analyses of the results obtained in January 2008 (summer) are presented in this study, since this was when the constructed wetland showed the greatest development of vegetation covering during the period studied.

Effluent collection was performed manually using a 5L polyethylene vessel, all on the same day and time at stations: 1, pH control and precipitation; 2, second damping pond outlet; 3,

constructed wetland inlet; and 4, constructed wetland outlet (Fig.2). All samples were refrigerated until being exposed to the organisms, when they were removed from the refrigerator and left standing on a laboratory bench to stabilize at room temperature (25^{o}C).

pH, Total Iron and Soluble Manganese Analysis.

Physical and chemical analysis data was obtained from Carbonífera Criciúma S.A. Samples were analyzed each 3 days during the study and data are presented as mean values with standard deviation.

Acute Toxicity in *Artemia* sp.

The acute toxicity assays were performed according to the method described by Guerra (2001), with minor modifications. *Artemia* sp. cysts were incubated for 24h in a synthetic sea water solution (30g.L^{-1}) under constant aeration and illumination. After eclosion, individual microcrustaceans (n=10) were incubated in multiwell plates for 24 h at 25ºC in the absence of light in 2mL of effluent sample collected in the respective collection stations described above and diluted in synthetic sea water solution. Salinity of the dilutions was corrected at 30g.L^{-1}. The acute toxicity of the effluent of each of the four collection stations was tested in serial concentrations to best determine the LC_{50}. For each concentration tested, four replicas of 10 individual microcrustaceans were performed. A negative control (0% concentration) was conducted in parallel using only synthetic sea water solution.

After incubation for 24 h, the microcrustaceans were considered dead if they exhibited no movement for 20 sec while under observation. The LC_{50} was calculated by the Trimmed Spearman-Karber mathematical method (Hamilton et al., 1977), based on the dead microcrustacean count. The LC_{50} is defined as the concentration at which 50% lethality occurs in the bioindicator microorganisms, when exposed to the effluents under study (Svensson et al., 2005).

Subacute Toxicity in *Allium cepa* L.

Evaluation of subacute toxicity in *Allium cepa* was performed by the root growth inhibition test, according to the method described by Fiskesjö (1985), with minor modifications. Individual *A. cepa* seeds were exposed for 7 days to 50mL of effluent (100% concentration) collected from each of the four collection stations described above at room temperature of 25^{o}C and in a dark room. Commercial mineral water (0% concentration) was used for negative controls. Every 24

h, the effluent samples or water were renewed. At the end of the exposure period, root length was measured and the CR_{50} was determined, defined as the concentration at which root growth length was inhibited by 50% of the respective negative control growth.

Genotoxicity in *Allium cepa* L. using the Comet Assay

DNA damage in *Allium cepa* exposed to the same conditions described above in the root growth inhibition assay was evaluated by the comet assay, as proposed by Singh et al. (1988). After the exposure period, the apical portion of the root of individual *A. cepa* seeds (n=6), exposed to effluents from each of the respective collection stations described above, was removed using a scalpel and stored in eppendorf deepwell plates. The roots were macerated with a nucleus extraction solution until the formation of a homogenate. Next, the homogenate was dissolved in low melting point agarose (0.75%) and then plated on a slide precoated in 1.5% agarose. The slides were immersed in lysis solution (2.5M NaCl; 100mM EDTA; 10mM TRIS; 1% Triton X-100; 10mL DMSO) at 4ºC for 2 h and then submitted to horizontal alkaline electrophoresis (10N NaOH; 200mM EDTA; pH>13) for 20 min at 300mA and 25V. Next, the slides were washed in neutralizing buffer (0.4M TRIS; pH 7.4), fixed in ethanol, stained with silver nitrate and observed under an optical microscope.

For each group of six individual roots exposed to the effluents from each collection station, 100 nuclei images were randomly analyzed (50 nuclei per slide, in duplicate), with the size of the comet (nuclear region plus tail) visually classified into one of five classes of damage: zero (comet with no damage) to 4 (comet with maximum damage), according to the methodology proposed by Collins (2004), followed by calculation of the number of damaged cells (damage frequency, DF) and the total damage presented by these cells (damage index, DI).

Statistical Analyses

Statistical analyses of the data obtained for subacute toxicity and genotoxicity in *Allium cepa* exposed to effluent from the collection stations was realized by one-way analysis of variance (ANOVA 1) (Zar, 1985). When a significant difference occurred by ANOVA, *post hoc* analysis was realized using the Student-Newman Keulls (SNK) and Bonferroni tests. The software GraphPad Prism 5.0 (Graphpad Inc., USA), was used for these analyses and significance was determined at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$. All the results were expressed as the mean ± standard deviation (SD).

Results

The physical and chemical data are presented in Table 2. The pH measured at the four sample collection stations showed minimal variation. The other values indicate a significant increase in total Fe and soluble Mg^{+2} on sampling station 3 and the ability of the wetland to reduce its levels until sampling station 4.

The acute toxicity tests involving the microcrustacean *Artemia* sp. (Fig. 3) revealed that the first and second collection stations (pH control and precipitation; biological damping pond outlet) presented no indication of toxicity, such that 100% of the organisms survived at all the concentrations tested.

Table 2. pH, Fe and Mn mean values* at each of four effluent sample collection stations defined in the study.

Sample collection station	pH	Total Fe (mg/L)	Mn^{+2} (mg/L)
Station 1 (pH control and precipitation)	6.38±0.28	0.20±0.22	0.31±0.11
Station 2 (Second damping pond outlet)	6.36±0.30	0.20±0.22	0.31±0.10
Station 3 (Wetland inlet)	6.30±0.29	0.95±1.05	0.84±0.28
Station 4 (Wetland outlet)	6.42±0.30	0.39±0.76	0.32±0.07

*Mean values based on monthly lectures in January 2008.

At the constructed wetland inlet (collection station 3), the indication of toxicity began at 50% concentration and caused total lethality at 100% concentration. The LC_{50} of the station was calculated as 81.24%, which means that the effluent collected at this location was considered toxic at this concentration. In contrast, at the constructed wetland outlet (collection station 4), the effluent shows some indication of lethality at 90% concentration, compromising the survival of approximately 30% of the microcrustaceans at 100% concentration; it was not possible to calculate the LC_{50} for this station.

In the phytotoxicity test using effluent at 100% concentration (Fig. 4), observation verified indications of inhibition in *A. cepa* root growth; however, this inhibition was insufficient to register a statistically significant difference between the collection station sample and negative control (mineral water) and, consequently, the effluents were considered nontoxic. Despite this, a tendency for increased toxicity at the constructed wetland inlet was observed that returned to lower values at the outlet.

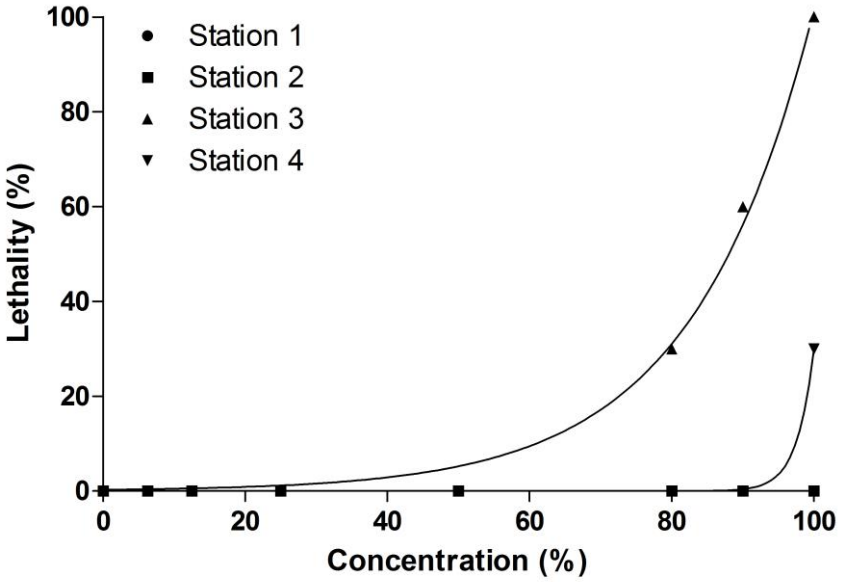

Figure 3. Effluent lethality for *Artemia* sp. at diverse concentrations at the four collection stations (1. pH control and precipitation, 2. second damping pond outlet, 3. wetland inlet, and 4. wetland outlet). An LC_{50} was observed only at station 3 at 81.24% concentration.

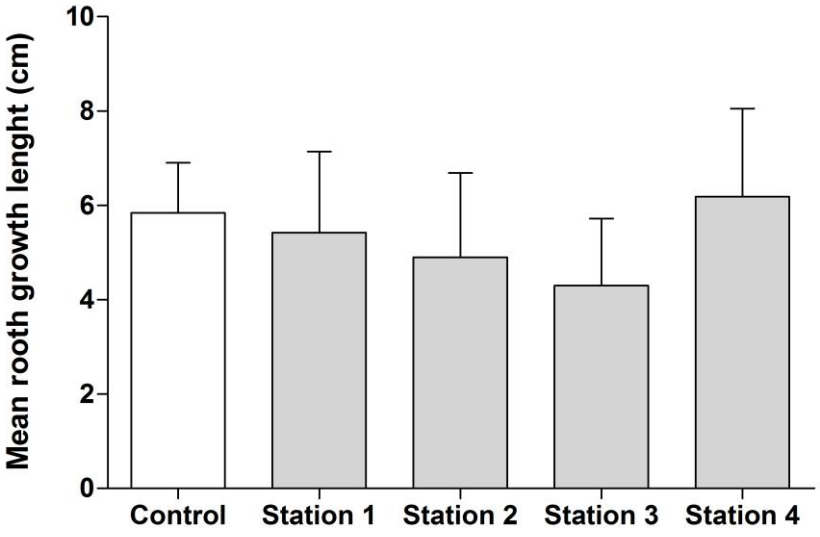

Figure 4. Variation of mean root growth length of *Allium cepa* exposed to effluent from the four collection stations (1. pH control and precipitation, 2. second damping pond outlet, 3. wetland inlet, and 4. wetland outlet) at 100% concentration. No significant difference was observed compared to the negative control (mineral water).

The genotoxicity of the effluent evaluated by comet assay on meristematic cells of *Allium cepa* expressed as the damage index (DI) (Fig. 5) and damage frequency (DF) (Fig.6), revealed significantly different results for collection stations 1 and 2 (pH control and precipitation and

second damping pond outlet, respectively) when compared to controls, while the effluents collected at stations 3 and 4 (constructed wetland inlet and outlet, respectively), which were considered toxic in the acute toxicity tests, showed no evidence of genotoxicity.

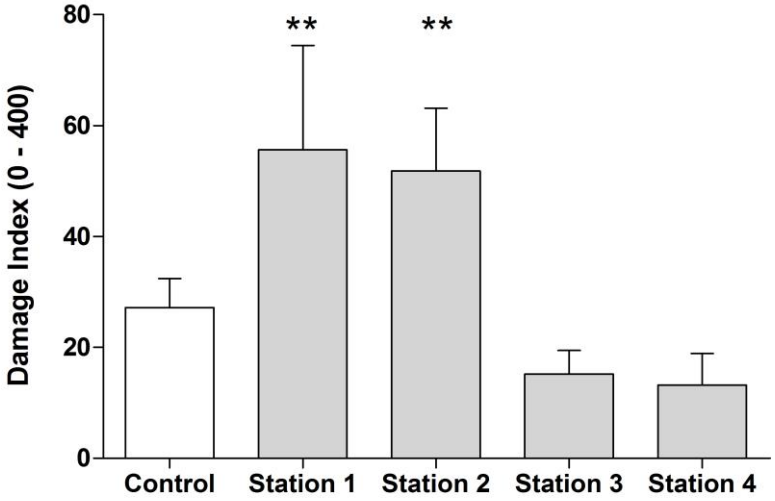

Figure 5. Results of the comet assay expressed as the index of DNA damage to meristematic cells of *Allium cepa* roots exposed to effluent from the four collection stations (1. pH control and precipitation, 2. second damping pond outlet, 3. wetland inlet, and 4. wetland outlet) at 100% concentration compared to the negative control (mineral water). ** indicates $p \leq 0.01$.

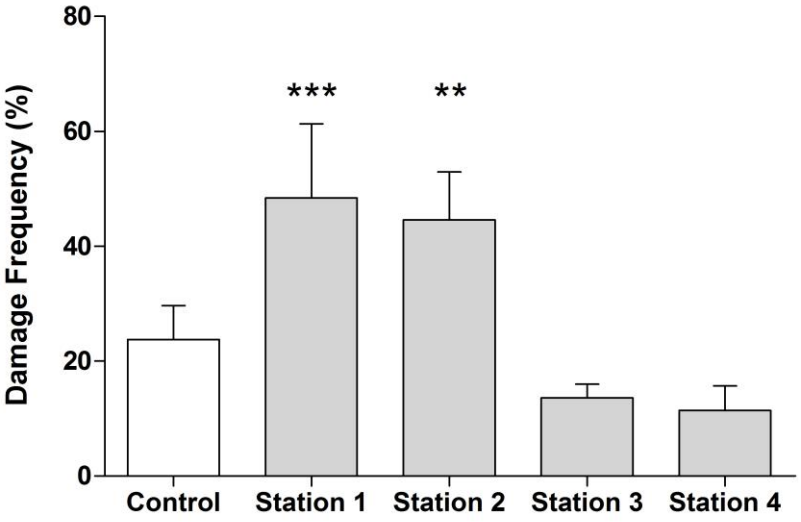

Figure 6. Results of the comet assay expressed as the frequency of DNA damage to meristematic cells of *Allium cepa* roots exposed to effluent from the four collection stations (1. pH control and precipitation, 2. second damping pond outlet, 3. wetland inlet, and 4. wetland outlet) at 100% concentration compared to the negative control (mineral water). ** indicates $p \leq 0.01$ and *** indicates $p \leq 0.001$.

Discussion

The use of passive treatment for acid mine drainage, involving wet areas and/or constructed wetlands, constitutes an attractive alternative from an economic point of view, since besides reducing financial costs, this process also exhibits additional values, such as landscape restoration and the maintenance of biodiversity (Lacki et al., 1990; Brenner and Hofius, 1990; Mota Marques et al., 2000; Ji, 2004). Such passive treatment is based on bioremediation techniques and involves aquatic living forms; specially algae, bacteria, and macrophytes, that stabilize, absorb, or chemically alter the potentially toxic contaminants to environmentally safe forms (Cunningham and Berti, 1993).

Evaluation of adequacy and the efficiency of natural and/or constructed wetlands to remove pollutants and toxic characteristics of effluents are of considerable importance in the development of such methods and their continued improvement and normally involve physical and chemical monitoring of the quality of the effluent that passes through the system. Different responses are obtained depending on the approach adopted, including information relevant to ecological and toxicological status. The use of bioindicators has shown a marked increase over the last few years, both at an organism level (toxicity) and molecular level (genotoxicity) and new low cost strategies are of great interest, but the toxicity of heavy metals for aquatic organisms is not easy, even when only considering the effects of acute toxicity, since lethal doses vary enormously between biologically close species (Bastos and Freitas, 2000) and a variety of organisms is usually needed.

In this study, four different stages of a treatment processing plant were analyzed for toxicity, root growth inhibition and genotoxicity parameters using two biological models. The results obtained in both models indicated low effluent toxicity in practically all the stages of the process and permitted the detection of interference during processing, caused by infiltration of AMD originating from the piles of deposited residues.

The mean pH at the four sample collection stations, which normally present very low values in untreated AMD, were only slightly acidic, revealing a small increase in acidification resulting from the transition from the physical and chemical treatments to the biological treatment adopted by Carbonífera Criciúma S.A, demonstrating the efficiency of the processes of effluent treatment in relation to this parameter. Total Fe and soluble Mn presented a significant increase on

sampling station 3 probably due to the AMD infiltrations along the treatment system as mentioned above.

In the *Artemia* sp. model, sample collection station 3 located at the constructed wetland inlet, presented positive results for toxicity, with a LC_{50} of 81.24%, corroborating the results of root growth inhibition test on *Allium cepa*, in which the roots presented diminished growth that was not statistically significant. These results led to a more detailed examination of the unit where the collection station had been installed and the identification of contamination of the treatment system by ground water that originated from the pile of residue deposits. We would like to emphasize that the results of the tests also verified the capacity of the constructed wetland to correct, at least in part, the toxicity generated by this interference in the treatment system, thus proving its efficiency, as observed by the reduction in the toxicity of the effluents collected at the constructed wetland outlet in relation to the inlet of the same. Rank and Nielsen (1998) found a good correlation between the degree of and the concentration of heavy metals in three leachate samples, demonstrating that the greatest growth inhibition occurred in samples the had the highest concentration of these substances. These results also indicate that these models are able to detect their presence in amounts that can affect the environment.

The affinity of heavy metals for solid particles, which tend to sediment in the stabilization ponds, controls the time of residence and concentration of these elements in the water (Stumm and Morgan, 1996). The pH also has a strong influence on the behavior of these contaminants in the stabilization ponds, as it does in a soil environment, since the concentration of most cations decreases as the pH increases, and precipitated and absorbed forms are favored by alkaline pH values (Brooks, 1983).

Once flocculated, the trace element can precipitate or be deposited as sediment at the bottom of the water column. It returns to the dissolved form by mineralization of the biota, desorption, or resolubilization. Close to the water-sediment interface, an anoxic zone of oxygen depletion can occur, where reactions involving Fe(III) and Mn(IV) oxides can liberate metal cations (e.g., Cd^{+2}, Cu^{+2}, Pb^{+2}, Zn^{+2}) or oxyanions (e.g., AsO_3^{-3}), which are absorbed by these oxides. The dissolution of these oxides leads to the liberation of Fe^{+2} and Mn^{+2} which, once they reach the oxic zone close to the surface, undergo oxidation and reprecipitation in the presence of oxygen, leading to formation of Fe(III) and Mn(IV) oxides that can absorb dissolved trace elements and

eventually undergo sedimentation at the bottom of the body of water, where they are subject to further cycles of reduction and dissolution (Stumm and Morgan, 1996).

The importance of the macrophytes in the process of liberation, sorption, and absorption of toxic elements should be recognized. Plants that tolerate the presence of heavy metals at high concentrations contribute to the stabilization of ecological systems, favoring the establishment of less tolerant species, which accelerates the ecological succession. These plants also help filter suspended particles from the water, and affect the redox conditions of their environment.

The experiment in which the genotoxicity test was applied, analyzed by DNA damage in *Allium cepa* root cells, showed that effluent samples collected at stations 1 and 2 had considerable genotoxic potential, both in the damage index and the frequency index, when compared to the results obtained in the negative control. The high indices of genetic damage could be derived from the persistence of non-precipitated substances or heavy metals that interact with other solutes, forming complexes, and/or remain in the dissolved form and possess a strong potential to attack biological molecules, especially nucleic acids (Stohs and Baghi, 1995; Rank and Nielsen, 1998; White and Claxton, 2004). In sampling station 3 a reduction o genotoxicity was observed probably due to the increase of toxicity indicated by an increase of apoptotic cells (data not shown) caused by the infiltrations of AMD on the treatment system. When the effluent passed through the passive treatment in the constructed wetland, from sample collection stations 3 to 4, the genotoxic potential of the effluent was significantly reduced with results that were not statistically different from those obtained for the negative control, and no apoptotic cells were observed demonstrating the applicability of this treatment in improving the quality of the AMD effluent treated by conventional physical and chemical processes.

Our results lead to the possibility of using *A. cepa* to detect genotoxicity cause by AMD and to evaluate its ability to reduce it as already used on testing the genotoxic potential of landfill leachates, that present a great diversity of substances, and significant results were obtained (Cabrera and Rodriguez, 1999; Chandra et al., 2005; Srivastava et al., 2005).

At the inlet and outlet of the constructed wetland, a clear reduction in genotoxicity was observed in the model tested, revealing that either the treatment was effective at removing the genotoxic potential or, more likely, that the lethality provoked by the interference cited above masked the results of genetic damage by events like apoptosis or tissue necrosis.

Conclusions

The acute toxicity results obtained using the *Artemia* sp. model indicated an increase in toxicity during the process between sample collection stations 2 and 3. This increase could have been due to AMD infiltration, detected visually and later proven by chemical monitoring executed by Carbonifera Criciúma S.A., or to oxidation of iron present in the effluent as a consequence of the increased oxidation potential of the effluent. The increased toxicity was largely corrected by bioabsorption and biopolishing realized in the constructed wetland.

Subacute toxicity detected by root growth inhibition revealed that the effluents do not possess the toxic potential for inhibition, since the roots exposed to effluent collected at the four sample collection stations presented virtually normal growth compared to the negative control, demonstrating the removal and/or immobilization of an expressive proportion of the contaminant metals that normally interfere in *Allium cepa* root growth.

The genotoxicity tests indicated that the physical and chemical treatments adopted by the company, consisting of the control of acidity and precipitation of toxic elements, are efficient at reducing the toxicity but not the genotoxicity of this effluent, as indicated by the results obtained for effluent collected at stations 1 and 2. The genetic damage observed at sample collection stations 1 and 2 could be due to the presence of dissolved chemical agents or heavy metals that were not removed by the conventional physical and chemical treatments.

The constructed wetland proved to be a viable alternative for biopolishing of the AMD effluent treated by conventional physical and chemical processes. Similarly, the bioindicators used were efficient at detecting contamination in the effluents submitted to conventional treatments; therefore, these could be used in programs that monitor the quality of effluents from coal exploration. Further studies should be conduct after correcting the infiltration detected by the toxicity tests, in order to corroborate the conclusions and hypotheses raised in this work.

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