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

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Abstract. Coal mining produces amounts of residues containing high levels of chemical elements that contaminate surface and ground water. In the last several decades, constructed wetlands systems have been used to improve the quality of coal mine drainage. Although the impact of coal mining-related toxic substances on fauna community is an important conservation concern, it has not been studied intensively. The objective of this study was to evaluate the possibility of use a microcrustacean (*Artemia* sp.) and pearl cichlid (*Geophagus brasiliensis* Quoy & Gaimard, 1824) to assess the toxicity and the genotoxicity, in a constructed wetland at biopolishing acid mine drainage (AMD) previously treated by conventional physical and chemical processes. Effluent samples were collected at four stations along the treatment system: 1 - pH control and precipitation, 2 - second damping pond output, 3 - wetland input and, 4 - wetland output. Acute toxicity analysis using *Artemia* sp. was performed at AMD concentrations of 0% 25%, 50%, 80%, 90% (diluted with mineral water) and 100% (not diluted). Genotoxicity analysis was performed using the comet assay on peripheral blood and hepatic cells of *G. Brasiliensis*. The *Artemia* sp. test results indicated lethality of 30% at station1 and 0% at station 4 at 100% AMD concentration, indicating that the constructed wetland is effective at reducing the toxicity for this organism. The comet assay indicated that the effluent is genotoxic, with an increase in the DNA damage index from station 1 to 3 and a significant reduction at station 4, in both blood, and hepatic cells. This increase could be due to the presence of oxidated reactive species that are in between station 3 and 4 partially captivated by the living forms present in the wetland, reducing the genotoxic potential of the effluent. The results suggest that the treatment is efficient at removing toxicity and reducing genotoxicity but further improvements are required.

Additional Key Words: Coal, Constructed Wetland, Fish, Passive Treatment, DNA Damage.

Introduction

Known Brazilian reserves of coal total 32 billion tons "in situ", located predominantly in the southern region of the country in the geological dominions of the Paraná Basin. Of this total, the State of Rio Grande do Sul retains 89.25%, Santa Catarina 10.41%, Paraná, 0.32% and São Paulo retains 0.02% (Horbach et al., 1986; DNPM-BRASIL, 2008).

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).

Beginning with a mean crude production of 720,000 tonnes/month of mine runoff (MRO) in the Catarinense coal mining region, with a mean recovery of 36% of the crude coal after improvement, roughly 460,000 tonnes/month of sulfated residues are produced (DNPM-BRASIL, 2007), causing a huge environmental impact (Madeira et al., 2005). Besides the active mines, old mining areas that have not undergone recuperation must also be considered, since these continue to compromise the hydrographic system due to the daily production of untreated AMD.

Normally, the residue obtained after crude coal improvement is discarded into controlled modules as piles of pyritic residues. One of the principal problems related to these deposits, is that they contain significant quantities of potentially polluting compounds, such as pyrites and marcasites, clays, and various trace elements (Madeira et al., 2005); more than 30 such heavy elements had been found worldwide in association with the coal matrix (Goldschimidt, 1930). Contact of these residues with air and water generates AMD, that can contaminate the environment, thus, it should be treated prior to being discarded (Madeira, et al. 2005).

Several AMD 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), giving the appearance that the effluents are nontoxic.

In the last several decades, many systems based on the natural processes of cleansed water have been constructed to improve the water quality. Constructed wetlands are now used to improve the quality of point and nonpoint sources of water pollution, including stormwater runoff, domestic wastewater, agricultural wastewater, and coal AMD. A large number of wetlands have been constructed to treat drainage from active and abandoned coal mines and more than 500 such systems are operating in Appalachia alone (USDA and EPA, 2002). They 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; USDA and EPA, 2002; Ji et al., 2004).

Most wetlands support a dense growth of vascular plants adapted to saturated conditions. This vegetation slows the water, creates microenvironments within the water column, and provides attachment sites for the microbial community (USDA and EPA, 2002). The efficiency of such systems, natural, and or constructed wetlands, for the removal of pollutants and toxic characteristics have been evaluated by physical and chemical monitoring of the quality of the effluent; however, the use of biological indicators has increased in the last few years (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), leading to a demand for bioindicator organisms for monitoring environmental genotoxicity (Klobucar et al., 2003).

Several organisms have been used as bioindicators (Klobucar et al., 2003; Lee and Steinert, 2003; Svensson et al., 2005), all of which respond in very particular ways to a variety of alterations in the environment that they live in, providing physiological, biochemical, genetic and behavioral data (Shugart, 1994). In addition the compounds contained in the effluents can be assimilated by small animals, passing through the food chain, and bioaccumulating over long-term exposure (Sánchez-Chardi et al., 2007). The toxic potential of effluents has been previously demonstrated. Acute lethality tests, using classic bioindicators such *Vibrio fisheri, Daphnia similis* and *Artemia* sp were performed to evaluate the toxicity of crude leachate from an old solid waste landfill in Rio de Janeiro, Brazil, showing an elevated toxic potential (Silva et al., 2004).

Geophagus brasliensis (acará) is Brazilian species widely distributed on costal and Uruguay river basins, inhabiting different physical-chemical conditions (Menezes et al., 2007). Considered omnivorous in preserved environments is widely flexible in relation to feeding

habits, may become detritivores in changed habitats (Oliveira and Bennemann, 2005). Moreover, as a territorial species and non-migratory (Malabarba et al, 2004) theoretically be in continuous contact with possible chemical stressors. Thus, its wide distribution and ecological aspects made it suitable to be used as bioindicator to assess the effects of heavy metal pollution.

Assessment of DNA damage is of primary concern when determining the pollution-related stress in living organisms (Klobucar et al., 2003). Genotoxic substances can cause effects such as carcinogenesis, teratogenesis, embryotoxicity, as well as a suite of health disorders referred to as genotoxic disease syndrome (Kurelec, 1993), which can have adverse effects on stability of ecosystems (Nacci et al., 1996; Mitchelmore and Chipman, 1998). Furthermore, genotoxic agents rarely damage only DNA, because most chemicals exert their effects via genotoxic and metabolically toxic mechanisms operating simultaneously (Depledge, 1998; Henderson et al., 2000). Since the comet assay has been used to assess the ability of potential aquatic contaminants to induce DNA damage (Klobucar et al., 2003), and the advantages of comet assay using aquatic animals are (1) damage to the DNA in individual cells is measured; (2) only small number of cells are needed to carry out the assay (<10,000); (3) the assay can be performed on virtually any eukaryotic cell type; (4) and it is a very sensitive method for detecting DNA damage (Shing et al, 1988), investigations of its applicability in environmental biomonitoring of AMD have come into focus.

The objective of this study was to evaluate the possibility of use *Artemia* sp. and *Geophagus brasiliensis* (Quoy & Gaimard, 1824) to assess the toxicity and the genotoxicity in a constructed wetland at biopolishing AMD previously treated by conventional physical and chemical processes.

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 company, 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.

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.

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 dykes and canals designed to channel the AMD through a primary treatment plant, where the water is neutralized for use in the coal improvement process (1,100m³/h; 38,850ft³/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 (6,000m²), which contain dense vegetation growth of *Eleocharis acutangula* and *Typha domingensis*. The second effluent collection station was installed at the output 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 input and output of this wetland (Fig. 2). After the wetland, the effluent flows into the River Sangão.

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 Mach 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.

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

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 output; 3, constructed wetland input; and 4, constructed wetland output (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 Microcrustacean *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 seawater 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. The acute AMD toxicity of each of the four collection stations was tested in serial concentrations of 25%, 50%, 80%, 90% (diluted with mineral water) and 100% (not diluted) to best determine the LC_{50}. Salinity of the dilutions was corrected at $30g.L^{-1}$. For each concentration tested, four replicas of 10 individual microcrustaceans were performed. A negative control (0% AMD 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).

Genotoxicity in *Geophagus brasiliensis* using the Comet Assay

DNA damage in *Geophagus brasiliensis* exposed for 2 h to effluent (100% AMD concentration since no lethality was observed) collected from each of the four collection stations described above at room temperature of $25^{o}C$ and in a dark room was evaluated by the comet assay, as proposed by Singh et al., (1988). Commercial mineral water (0% AMD concentration) was used for negative controls.

After the exposure period, blood was removed by pulsation and the fish were sacrificed to extract their livers. The blood was diluted in 0.19mL of 0.9% saline solution and the liver was removed, homogenized in a Tamis 200 mesh and placed in an eppendorf plate containing 0.3mL of PBS (phosphate-buffered-saline). Later the blood and liver samples were dissolved in low-melting point agarose (0.75%) and plated on slides precoated in agarose (1.5%). The slides were duplicated for each fish analyzed. Next, the slides were immersed in lyses solution (2.5M NaCl, 100mM EDTA; 10mM de Tris; 1% de Triton X-100 and 10mL DMSO), pH 10, at 4ºC for 2 h, then placed in a horizontal electrophoresis apparatus. To facilitate DNA unraveling, the slides were incubated for 20 min in alkaline buffer (300mM NaOH and 1 mM EDTA, pH 13.00-13.50 at 4°C) prepared at the moment of analysis. An electric current of 300mA and 25V was applied for 15 min at 4°C. The slides were then neutralized with 0.4M Tris, pH 7.5. After drying, the

slides were fixed by in a solution containing 5% Na_2CO_3, 0.1% $NH4NO_3$, 0.1% $AgNO_3$, 0.25% tungstosilicic acid and 0.15% formaldehyde. The reaction was interrupted by adding 1% HOAc. For each fish exposed to the effluent from each sample collection station, 100 nuclei images were randomly analyzed (50 nuclei per slide, in duplicate), with the size of the comet, nuclear region plus tail, were 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). The tail is composed by fragmented DNA that are able to migrate under electrophoresis and its intensity and size are direct indication of the index of fragments generated by the exposure to genotoxic agents.

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 $p \le 0.05$, $p \le 0.01$, and $p \le 0.001$. All the results were expressed as the mean ± standard deviation (SD).

Results

The pH measured at the four sample collection stations showed minimal variation (Table 2), revealing, instead not significant, a slight increase in acidification resulting from the transition from the physical and chemical treatments to the biological treatment adopted by the company. 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.

Table 2. pH, total Fe and soluble Mn^{2+} 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)	6.46±0.23	0.19±0.14	0.37±0.13
Station 2 (Second damping pond output)	6.45±0.25	0.19±0.14	0.37±0.12
Station 3 (Wetland input)	6.18±0.25	1.11±0.81	1.00±0.33
Station 4 (Wetland output)	6.42±0,26	0.20±0.14	0.33±0.08

*Mean values based on monthly lectures in 2008 March.

The effluent collected at station 1 caused the highest level of lethality to *Artemia* sp. at 100% concentration, with 30% of the microcrustaceans exposed presenting immobility. The effluent samples collected at stations 2 and 3 caused 20% and 10% lethality, respectively, while that collected at station 4 did not cause lethality at any of the concentrations tested. In general, 90% concentration marked the onset of toxicity in sample collection stations 1, 2 and 3 (Fig. 3).

Due to the low rates of mortality observed, it was not possible to calculate the LC_{50} for *Artemia* sp. at any of the sample collection stations, leading to the supposition that during the period in which the samples were collected (June 2008), the treatment of effluent adopted by Carbonífera Criciúma S.A, was 100% efficient at eliminating toxicity for aquatic organisms.
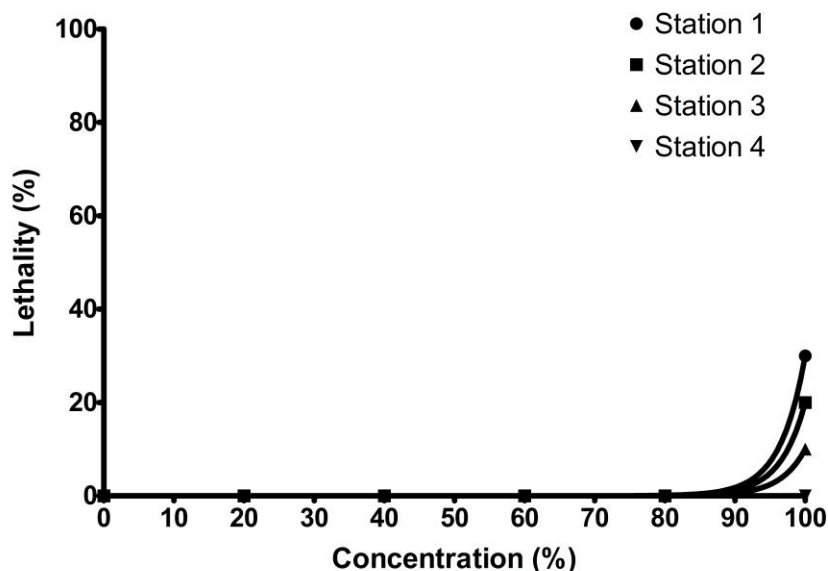

Figure 3. Acute toxicity for *Artemia* sp. at the four sample collection stations 1 (pH control and precipitation), 2 (second damping pond output), 3 (wetland input), and 4 (wetland output).

The comet assay applied to peripheral blood and liver tissue from *Geophagus brasiliensis* showed significantly greater genotoxicity values at all the sample collection stations, when compared to the negative control, in both: blood cells (Fig. 4 A and B) and liver tissue (Fig. 5 A and B). A significant increase in genotoxicity values was observed at sample collection station 3, in comparison with the remaining collection stations and the negative control. This was probably due to the occurrence of untreated acid mine drainage infiltration originating from the

piles of deposited residues, as observed visually on site and later verified by the chemical monitoring realized by Carbonífera Criciúma S.A.
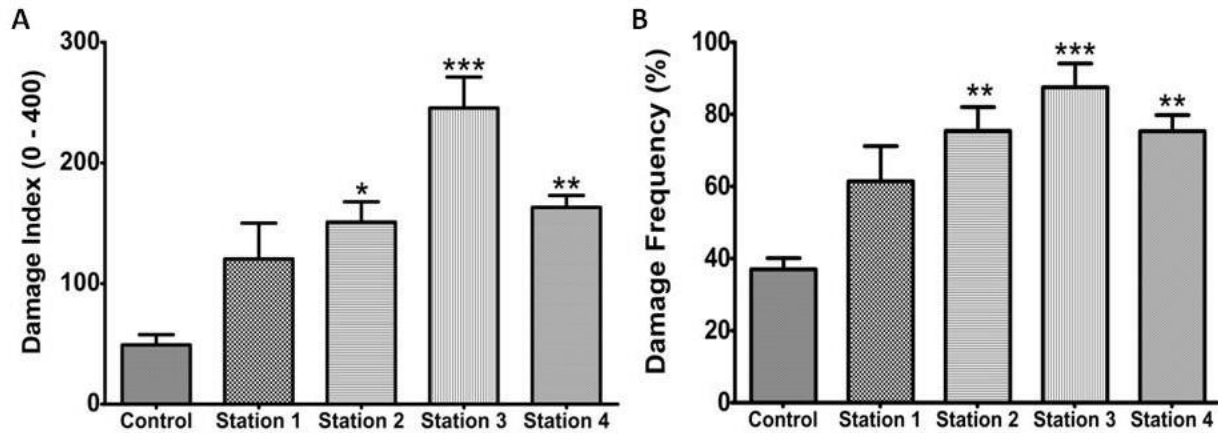

Figure 4. Damage (A) and Frequency (B) Indices of DNA damage to *Geophagus brasiliensis* blood cells exposed to the effluent of coal exploration, collected at four sample collection stations, throughout the treatment system: 1 (pH control and precipitation), 2 (second damping pond output), 3 (wetland input), and 4 (wetland output). * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.
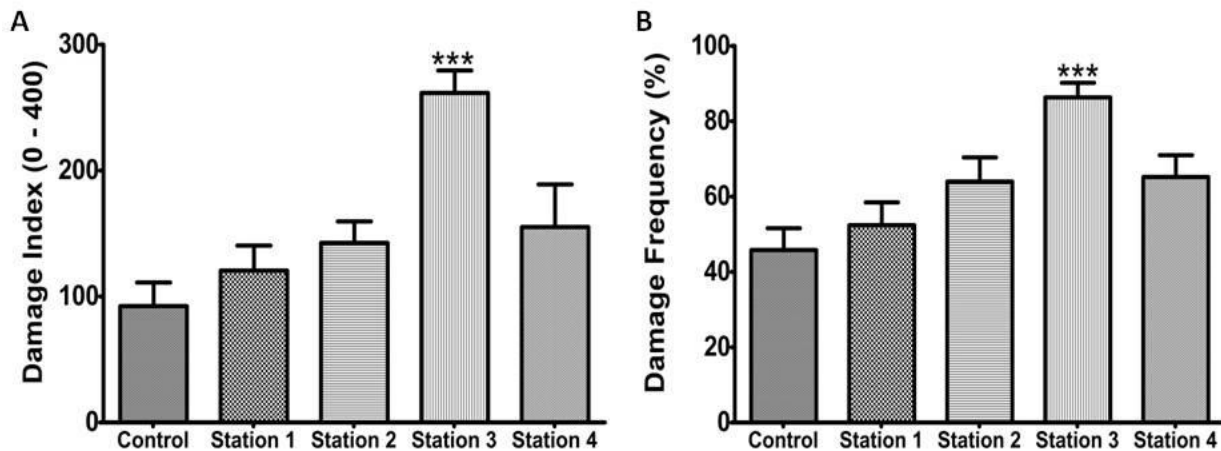

Figure 5. Damage (A) and Frequency (B) Indices of DNA damage to *Geophagus brasiliensis* liver cells exposed to the effluent of coal exploration, collected at four different sample collection stations, throughout the treatment system: 1 (pH control and precipitation), 2 (second damping pond output), 3 (wetland input), and 4 (wetland output). *** $p \leq 0.001$.

Discussion

Evaluation of efficiency of natural and/or constructed wetlands to remove pollutants and toxic characteristics of effluents is of considerable importance for the use of such methods in the treatment of acid mine drainage, especially that produced by coal exploration, which is one of the principal environmental problems generated by this important economic activity.

Evaluation of the efficiency of such systems normally involves 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 immediate or medium-term responses that provide information highly relevant to toxicological or ecological status.

The pH measured at the four sample collection stations, which normally present very low values in untreated acid mine drainage, 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 detected during the realization of this work.

Artemia sp. was used before as a bioindicator in studies which demonstrate the toxicity of pesticide contamination even in low level (Oliveira-Filho and Paumgartten, 2000) and toxicity of fenolic compounds (Guerra, 2001). Although the *Artemia* sp lethality recorded on this work had remained below 50% in all the sample collection stations (indicating that the effluent was not toxic to aquatic organisms), it was observed that this parameter had diminished from 30% in sample collection stations 1 and 2 to 20% and 0% at stations 3 and 4, respectively. The sensibility of the model and the results presented in these work evidence that the chemical treatment and the passage of effluents through the constructed wetland resulted in an improvement in water quality. It has to be considered that *Artemia* sp as a marine organism is less sensitive to ion concentrations in effluents as freshwater organisms are, and due to this the samples can be more toxic than observed.

The fact that a treated effluent does not present toxicity does not mean that it causes no environmental damage, since summarizing the toxicity of heavy metals for aquatic organisms is not easy, as lethal doses vary enormously between biologically close species (Bastos and Freitas, 2000). Allied to the toxicity is the genotoxicity. The DNA damage is of primary concern when

determining the pollution-related stress in living organisms (Klobucar et al., 2003). Many substances can cause several health disorders in organisms, referred to as genotoxic disease syndrome (Kurelec, 1993), promoting adverse effects on the stability of ecosystems (Nacci et al., 1996, Mitchelmore and Chipman, 1998).

In this context, the comet assay is a simple, sensitive and rapid technique for detection of DNA damage (single-, double-strand breaks, alkali-labile sites or DNA-DNA and DNA-protein crosslinks) in individual cells (Fairbairn et al., 1995) and therefore can be very useful in studies of genetic toxicology, especially ecogenotoxicology (Cotelle and Férard, 1999). This test presents certain advantages over biochemical and cytogenetic tests, including the need for a minimal number of cells and that these do not need to present cell division (Fairbairn et al., 1995).

The effluents collected at all the sample collection stations showed greater genotoxicity values in relation to the negative control, as verified both for peripheral blood and liver tissues of *Geophagus brasiliensis*. A further significant increase also occurred at sample collection station 3 in comparison with the remaining stations, brought attention to three facts: first, although the physical and chemical treatment adopted by Carbonífera Criciúma S.A is very good, it is not 100% efficient at eliminating the effluent genotoxicity; second, the system was compromised by AMD infiltration that occurred between the sample collection stations 2 and 3, resulting in worsening of effluent quality; third, the quality of the effluent improved again after passing through the constructed wetland, verifying the effectiveness and applicability of the constructed wetland at biopolishing the effluent of AMD.

This improvement, despite the negative influence of the infiltration, is proof of the applicability of constructed wetlands for biopolishing the effluent of AMD originating from coal exploration; thus, the implantation of wetlands in effluent treatment systems to improve the quality of such effluents prior to being discharged into natural drainage systems is recommended.

Conclusions

The acute toxicity results using the *Artemia* sp. model, indicated that the treatment of effluent adopted by Carbonífera Criciúma, S.A during the winter period was 100% efficient at eliminating toxicity for the aquatic organisms tested, as verified by the low mortality rates observed.

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 increase in genotoxicity observed at station 3 made possible to detect on AMD infiltration process on the system and after passing through the passive treatment in the constructed wetland, the genotoxic potential of the effluent was significantly reduced. This fact proving the efficiency of the passive treatment in improving the quality of the acid mine drainage effluent treated by conventional physical and chemical processes.

The bioindicators used were efficient at detecting effluent genotoxicity, after these had been submitted to conventional treatments. This fact suggests they could be used in programs to monitor the quality of effluent derived from coal exploration, though further research is required to corroborate the conclusions and hypotheses raised in this work.

Acknowledgements

References

Alexandre, N.Z. 1999. Diagnóstico ambiental da região carbonífera de Santa Catarina: degradação dos recursos naturais. Revista Tecnologia e Ambiente. 5, 35-53.

Bastos, A.C.S. and A.C. Freitas. 2000. Avaliação e perícia ambiental. In: Cunha, S.B. and A.J.T. Gerra (eds.), Rio de Janeiro, Bertrand Brasil,

Brenner, F.J. and D.L. Hofius. 1990. Wildlife use of mitigated wetlands on surface mined lands in Western Pennsiylvania. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. (Charleston, WV). https://doi.org/10.21000/JASMR90020445

Calabrese, J.P., D.K. Bhumbla, A.J. Sexstone, G.K. Bissonnette, and J.C. Sencindiver. 1990. Simulated *Typha* wetlands applied to removal of iron and manganese from acid mine drainage. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. (Charleston, WV). Abstract

CETEM – Centro de Tecnologia Mineral. Ministério da Ciência e Tecnologia do Brasil. 2001. Projeto conceitual para recuperação ambiental da Bacia Carbonífera Sul Catarinense. Relatório Técnico 1, 2 e 3.

Collins, A.R. 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular Biotechnology. 26, 249-261. http://dx.doi.org/10.1385/MB:26:3:249.

Cotelle, S., and J.F. Férard. 1999. Comet assay in genetic ecotoxicology: a review. Environ. Mol. Mutagen. 34, 246-255. http://dx.doi.org/10.1002/(SICI)1098-2280(1999)34:4<246::AID-EM4>3.0.CO;2-V.

Depledge, M.H. 1998. The ecotoxical significance of genotoxicity in marine invertebrates. Mutat. Res. 399, 109-122. http://dx.doi.org/10.1016/S0027-5107(97)00270-4.

DNPM-BRASIL. Departamento Nacional de Produção Mineral. 2007. Informativo Anual da Indústria Carbonífera. Brasília. www.dnpm.gov.br.

DNPM-BRASIL. Departamento Nacional de Produção Mineral. 2008. Informe Mineral: Desenvolvimento & Economia mineral. Abril, 2008. Brasília. www.dnpm.gov.br

Fairbairn, D.W., P.L. Olive, and K.L. O'Neil. 1995. The Comet assay: a comprehensive review. Mutat. Res., 339, 37-59. http://dx.doi.org/10.1016/0165-1110(94)00013-3.

Goldschimidt, V. M. 1930. Rare elements in coal ash. Industrial and Engineering Chemistry. 27, 1100-03. http://dx.doi.org/10.1021/ie50309a032.

Guerra, R. 2001. Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents. Chemosphere. 44, 1737-1747. http://dx.doi.org/10.1016/S0045-6535(00)00562-2.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol., 11, 714-719. http://dx.doi.org/10.1021/es60130a004.

Henderson, L., S. Albertini, and M. Aardema, M., 2000. Thresholds in genotoxicity responses. Mutat. Res. 464, 123-128. http://dx.doi.org/10.1016/S1383-5718(99)00173-4.

Horbach, R. et al. 1986. Geologia. In: Levantamento de recursos naturais. 33: Folha SH.22 Porto Alegre e parte das Folhas SH.21 Uruguaiana e SI.22. Rio de Janeiro: SEPLAN e IBGE.

Jamison, E. and H.W. Rauch. 1990. Impacts of volunteer cattail wetlands on drainage quality from reclaimed mined land in Northern West Virginia. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. (Charleston, WV). Abstract

Ji, S.W., J.I. Ko, and S.J. Kim. 2004, the status of the passive treatment systems for acid mine drainage in South Korea, Proceedings America Society of Mining and Reclamation, 2004 pp 971-979 http://dx.doi.org/10.21000/JASMR04010971 9

Klobucar, G.I.V., M. Pavlica, R. Erben, and D. Papes. 2003. Application of the micronucleus and comet assays to mussel Dreissena polymorpha haemocytes for genotoxicity monitoring of freshwater environments. Aquatic Toxicology, 64, 15-23. http://dx.doi.org/10.1016/S0166-445X(03)00009-2

Kurelec, B. 1993. The genotoxic disease syndrome. Mar. Environ. Research. 35, 341-348. http://dx.doi.org/10.1016/0141-1136(93)90101-5.

Lacki, M.J., J.W. Hummer, and H.J. Webster. 1990. Diversity patterns of invertebrate fauna in cattail wetlands receiving acid mine drainage. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. (Charleston, WV). https://doi.org/10.21000/JASMR90010365

Lee, R.F., and S. Steinert. 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutation Research, 544, 43–64 http://dx.doi.org/10.1016/S1383-5742(03)00017-6

Madeira, V.S., Echert, A.B., Schneider, C.H. and Schneider, I.A.H. 2005. Redução de sulfato na drenagem ácida de mina através de tratamento anaeróbio com bactérias redutoras de sulfato. Anais do XXI Encontro Nacional de Tratamento de Minérios e Metalurgia Extrativa (Natal, RN, Novembro, 2005).

Malabarba, L.R., Pereira E.H.L., Silva, J.F.P., Bruschi jr., W. and Flores-Lopes, F. 2004. Avaliação da qualidade da água através da freqüência de anomalias morfológicas em peixes: estudo de caso do lago Guaíba, Rio Grande do Sul, Brasil. Comunicações do Museu de Ciências e Tecnologia. PUCRS, Série Zoologia. 17(2): 97-128.

Menezes, N.A., Weitzman, S.H., Oyakawa, O.T., Lima, F.C.T, Castro, R.M.C. and Weitzman, M. 2007. Peixes de água doce da Mata Atlântica: lista preliminar das espécies e comentários sobre conservação de peixes de água doce neotropicais = Freshwater fishes of Mata Atlântica: list of species and comments on conservation of neotropical freshwater fishes. São Paulo: Museu de Zoologia – Universidade de São Paulo. 408 p. il.

Mitchelmore, C.L., and J.K. Chipman. 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutat. Res. 399, 135-147. http://dx.doi.org/10.1016/S0027-5107(97)00252-2

Mota Marques, D.M.L., M. SUMINSKY, and C.P. BRUM. 2000. Ecossistemas criados (banhados): importância dos macrófitos no controle de pH de drenagem ácida. In: Carvão e

meio ambiente. Centro de Ecologia da Universidade Federal do Rio Grande do Sul (Org.). Porto Alegre, Editora da UFRGS.

Nacci, D.E., S. Cayula, and E. Jackim. 1996. Detection of DNA damage in individual cells from marine organisms using the single cell gel assay. Aquat. Toxicol. 35, 197-210. http://dx.doi.org/10.1016/0166-445X(96)00016-1.

Nawrot, J.R. and W.B. Klimstra. 1990. Biochemical treatment of mine drainage through a reedgrass wetland. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. (Charleston, WV).

Oliveira, D. C. 1. and Bennemann, S. T. 2005. Ictiofauna, recursos alimentares e relações com as interferências antrópicas em um riacho urbano no sul do Brasil. Biota Neotropica, 5 (1): 1-15. http://dx.doi.org/10.1590/S1676-06032005000100011.

Oliveira-Filho, E. C.; Paumgarten, F. J. R. Toxicity of Euphorbia milii Látex and Niclosamide to Snails and Nontarget Aquatic Species. Ecotoxicology and Environmental Safety, v.46, n.3, p.342-350, 2000. http://dx.doi.org/10.1006/eesa.2000.1924.

Sánchez-Chardi, A., Pe-arroja-Matutano, C., Ribeiro, C.A., Nadal, J., 2007. Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The wood mouse, Apodemus sylvaticus. Chemosphere. 70, 101-10. http://dx.doi.org/10.1016/j.chemosphere.2007.06.047.

Shugart, L.R. 1994. Biological monitoring contaminants in the environment. A multidisciplinary assessment of risks to man and others organisms. In A. Renzoni, N. Mattei, L. Lari, and M.C. Fossi (eds). Lewis Publishers, New York.

Silva, A.C., Dezotti, M., Sant'Anna Jr., G.L., 2004. Treatment and detoxication of a sanitary landfill leachate. Chemosphere. 55, 207–214. http://dx.doi.org/10.1016/j.chemosphere.2003.10.0134.

Singh, N.P., M.T. McCoy, R.R. Tice, and E.L. Schneider. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 175, 184-191. http://dx.doi.org/10.1016/0014-4827(88)90265-0.

Svensson, B.M., L. Mathiasson, L. Martensson and S. Bergström. 2005. Artemia salina as test organism for assessment of acute toxicity of leachate water from landfills. Environ. Monit. Assess., 102, 309-321. http://dx.doi.org/10.1007/s10661-005-6029-z.

Taylor, G.J. and A.A. Crowder. 1982. Copper and nickel tolerance in Typha latifolia clones from contaminated and uncontaminated environments. Canadian Journal Botany, 62,1304-1308. http://dx.doi.org/10.1139/b84-176.

Taylor, G.J. and A.A. Crowder. 1983a. Uptake and accumulation of copper, nickel, and iron by Typha latifolia in wetlands of the Sudbury, Ontario region. Canad. Jour. Bot., 61, 63-73. http://dx.doi.org/10.1139/b83-005.

Taylor, G.J. and A.A.Crowder. 1983b. Uptake and accumulation of copper, nickel, and iron by Typha latifolia grown in solution culture. Canadian Journal Botany, 61, 1825-1830. http://dx.doi.org/10.1139/b83-193.

USDA-Natural Resources Conservation Service; EPA - Environmental Protection Agency. 2002. Handbook of constructed wetlands: a guide to creating wetlands for: agricultural wastewater, domestic wastewater, coal mine drainage, stormwater in the Mid-Atlantic Region.-v. 1. Washington, D.C; USDA/NRCS/EPA. 53 p.

Zar, J. 1985. Biostatistical Analysis. New Jersey, Prentice May.

Zocche, J. J. 2005. Metais pesados (Fe, Mn e Zn) no solo construído e na vegetação das antigas bacias de decantação do lavador de Capivari, Capivari de Baixo, SC. Anais do VI Simpósio Nacional e Congresso Latino-Americano de Recuperação de Áreas Degradadas. (Curitiba, PR, 2005, Nov., 2005). Sobrade – Sociedade Brasileira de Recuperação de Áreas Degradadas.

Zocche, J. J. 2008. Efeitos da mineração de carvão sobre os morcegos no sul de Santa Catarina: a presença de metais pesados e a ocorrência de danos celulares. Anais do IV Congresso Brasileiro de Mastozoologia. (São Lourenço, MG, 2008, Julho, 2008).