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Discriminating the Ecotoxicity Due to Metals and to Low pH in Acid Mine Drainage

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Several techniques have been developed to assess the ecotoxicity of contaminated watercourses. Most of these techniques involve chemical alterations of water samples, by diluting it or by adding chelating agents. These changes become particularly severe when assessing the toxicity of samples with very low pH and with high quantities of contaminants. Trying to overcome this problem, a novel toxicity test, specific for acid waters, was previously developed and field validated. The toxicity of acid samples is assessed using the survival time of *Ceriodaphnia dubia*. During this study, the novel test was applied to a field situation, where an aquatic system is seriously impacted with acid mine drainage. Its efficiency was tested and compared with two classical toxicity tests: the Microtox and the median lethal dilution with *C. dubia*. The survival time test was performed without adjusting pH and after adjusting pH to a fixed value (pH 2, 3, and 4). At pH 2 and 4 no acceptable results were obtained; at pH 3 it was possible to distinguish the toxicity due to pH from the toxicity due to other toxicants. The test conducted at local pH was able to discriminate toxicity sources only for highly contaminated samples. The toxicity evaluation of acid mine drainage samples was possible neither with the median lethal dilution test nor with Microtox.

***Key Words*: test development; toxicity discrimination; *Ceriodaphnia dubia;* acid waters; acid mine drainage.**

INTRODUCTION

Acidification is one of the most severe causes of contamination in aquatic systems, not only as a toxicant itself, but also through its effects on the speciation, mobility, and bioavailability of other toxicants. Assessing the toxicity in acidified systems, also contaminated with toxicants, is difficult using the existing toxicity tests. This difficulty is especially noticeable when dealing with acid mine drainage (AMD), because it combines high heavy metal concentrations and low pH. It is important to develop new methods to assess the toxicity of such extremely acidified waters (pH < 4). Existing tests imply the chemical manipulation of the sample by adding alkalis to raise pH to circumneutrality, or by diluting it with an artificial water or, yet, by removing some contaminants with chelating agents. A specific toxicity test should not change the chemical characteristics of the effluent to permit a more realistic assessment of the water toxicity and to allow the discrimination of the toxicity due to low pH from the toxicity inherent to other contaminants (Gerhardt, 1995). Such a test was proposed by Ribeiro *et al.* (in press). Using the survival time of *Ceriodaphnia dubia* neonates as the endpoint, toxicity tests were conducted exposing cladocerans to different concentrations of copper with pH adjusted to different values. A nonlinear multiple regression model was then fitted to the survival time as a function of pH and of varying copper concentrations. The toxicity of each water sample is computed by comparing the survival time values of cladocerans exposed to the sample and in the respective control. The relative contamination of the sample is screened by calculating the copper equivalent concentration (CEC), i.e., the copper concentration dissolved in an artificial medium adjusted to the sample pH that will promote a survival time reduction identical to the portion of the observed reduction non-explained by the pH.

The aim of this study was to field validate and develop this test by assaying its efficiency in an AMD-contaminated aquatic system. An acid mine drainage impacted site was chosen for this study. The main contaminants in AMD are high quantities of H^+ and heavy metals (Kelly, 1988; Sengupta, 1993; Evangelou and Zhang, 1995). This test should discriminate between H^+ and heavy metal toxicities and be sensitive to different levels of contamination. Running the survival time test without adjusting the pH of the sample, i.e., at local pH, implies the preparation of a control specific to each sample with exactly the same pH. Although the test at local pH has the advantage of assessing the toxicity of the unchanged sample, it requires more test organisms and more effort to be performed. Conducting the survival time

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test after adjusting sample pH to a fixed value would save time and a lower number of organisms would be required, because only one control, at the same pH, would be needed. Thus, the survival time test was performed at fixed pH of 2, 3, and 4 and compared to the results at local pH. To compare survival time test results with classical assays in the toxicity evaluation of very acid waters, the Microtox and the median lethal dilution test with *C. dubia* were performed.

MATERIALS AND METHODS

An abandoned cupric pyrite mine, located at the south of Portugal, was chosen to develop this study (Fig. 1). The oxidation of this pyrite environment produces an effluent with high concentrations of heavy metals and with very low pH. The treatment given to the mine effluent, before being discharged to the Chança river, was evaporation and settlement in a series of ponds. Thirty years after the closure of the mine, the effluent still runs due to the continuous oxidation of mine tailings (Pereira *et al.*, 1995).

Surface water samples were made at the open pit, which was inundated just after the mine closure, and at some settlement ponds along the effluent. Two reference sites, free of acidification and heavy metal contamination, were also sampled: stations 0 and 00 (Fig. 1). At each sampling site water samples were collected with a glass jar, and pH (WTW 537 pH meter), conductivity (WTW LF92 conductivity meter), dissolved oxygen, and temperature (WTW OX192 oxygen meter) were measured. These parameters were also measured in all test vessels at the beginning and at the end of each test. Average pH for each flask, during the test, was computed by calculating the average concentration of hydrogen ions and then backcalculating the respective pH. Test results of an experimental run were rejected whenever the pH variation was higher than 10% of the initial value, or the average pH differed by more than 5% from the control pH (Ribeiro *et al.*, in preparation).

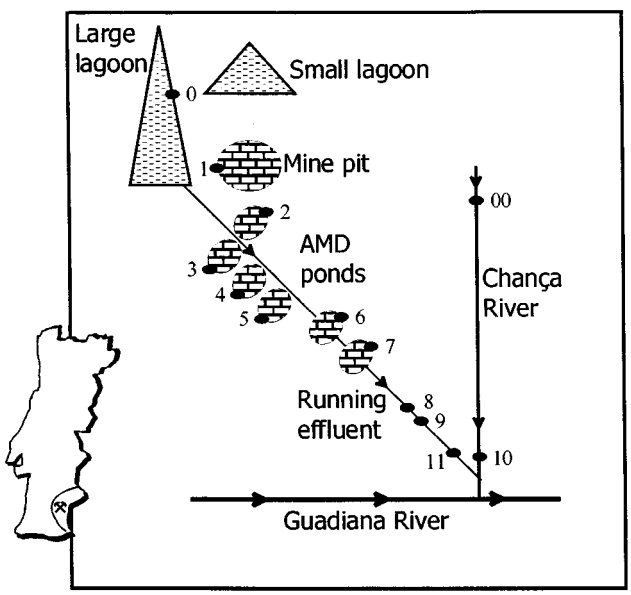

FIG. 1. Scheme of the studied aquatic system and location of sampling stations.

Six toxicity tests were performed with these water samples: the Microtox, the median lethal dilution test at pH 8, and survival time tests at fixed pH (pH 2, 3, and 4) and at local pH (no pH adjustment).

Studies were conducted in a stepwise manner with three experimental phases. In the first phase (A samples), the Microtox test was assayed as an ecotoxicological tool for AMD, using water samples from sites 1 through 4, 6, and 7 (water samples A1 through A4, A6, and A7). In the second phase (B samples), five types of tests were performed with *C. dubia* Richard: the median lethal dilution test (at pH 8) and survival time tests at fixed pH (pH 2, 3, and 4) and at local pH. In the third phase (C samples), after having rejected other tests as adequate for AMD samples, only two tests were compared: survival time tests at pH adjusted to 3 and at local pH.

Tests with the Microtox were done, following the Microbics Corp. detailed protocol for the Basic Test, with observations at 2, 5, and 10 min and using a Microtox Model 500 Analyzer (Microbics Corp., 1992). Microtox median effective dilutions (EC_{50}s) were expressed in U.S. EPA toxic units (toxic units $= 100/EC_{50}$) (Microbics Corp., 1992).

Only *C. dubia* neonates, 6 to 24 h old, from third or fourth broods, were used in toxicity testing (U.S. EPA, 1991). *C. dubia* was cultured at $20 \pm 1°C$ with a photoperiod of 16:8 h light:dark, in American Society for Testing and Materials (ASTM) hardwater medium (ASTM, 1988), with the organic additive "Marinure 25" (Soares, 1989), and were fed daily with the green algae *Selenastrum capricornutum* Printz (3×10^5 cells/ml/day).

Toxicity tests with cladocerans were carried out at $20 \pm 1°C$, with a photoperiod of 16:8 h light:dark, and were performed in 42-ml glass vessels with 30 ml of water. Five *C. dubia* neonates were introduced in each test vessel. Three and four replicates were used in each control and in each sample, for the survival time and for the median lethal dilution tests, respectively. No food was added during the tests. An organism was considered dead when it remained immobile for 15 s after gentle prodding.

Five dilutions and an ASTM control were used in the median lethal dilution test: 25, 12.5, 6.25, 3.125, and 1.625%. After the dilution of the water sample with ASTM medium, the pH was adjusted to 8 by adding $Ca(OH)_2$, with continuous stirring. Survival time was checked after 24 and 48 h of exposure.

Survival time tests at fixed pH (2, 3, or 4) were performed after adjusting sample pH with $Ca(OH)_2$ or H_2SO_4. In survival time tests without pH adjustment, an ASTM medium control was prepared, for each sample, by equaling its pH to the sample pH with H_2SO_4. Since pH is different from sample to sample, survival values cannot be compared directly. Therefore, for each organism, the survival time relative to the respective control was computed by equaling to 100% the average survival time in the control. The differences between samples could then be analyzed by comparing the "relative survival time" values.

When pH was lower than 3.5, organisms were introduced into each vessel one by one, survival time being recorded for each daphnid before the next one was introduced. At higher pH values, all five organisms were introduced at the same time. Mortality was checked continuously for the first 15 min, every 3 min from 15 to 30 min, every 5 min from 30 to 120 min, every 15 min from 2 to 6 h, every hour from 6 to 12 h, and at 18, 24, 36, and 48 h. A test ended when all daphnids died or after 96 h of exposure if at least one organism survived that long.

The evaluation of the relative contamination level was achieved by calculating the CEC for each water sample. The CEC of an AMD sample is the copper concentration dissolved in ASTM medium adjusted to the sample pH that would promote a survival time reduction identical to the portion of the observed reduction non-explained by the pH. CEC was computed using the respective nonlinear regression model developed by Ribeiro *et al.* (in preparation). Such calculations were carried out only if certain assumptions were met, as discussed in Ribeiro *et al.* (in preparation). (a) cladoceran survival time in each water sample must be significantly different from the survival time observed in the respective control; (b) survival time in the control must not be significantly different from the survival time predicted by the regression model; (c) copper equivalent concentration must lie above the NOEC values corresponding to the respective pH, given by Ribeiro *et al.* (in preparation).

Concentrations of dissolved metals (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Co, Zn, and Hg) were determined in water samples from the third phase step by ICP, except for Hg, which was determined by cold vapor AAS, and As, which was determined by AAS with hydrate generator.

RESULTS

First Experimental Phase

The pH values measured in the field were similar among the different stations, (ranging from 2.31 to 2.46). The highest conductivity was measured at the mine pit (sample A1) (Table 1). Samples A2, A4, and A7 presented similar conductivity values, which were higher than at stations 3 and 6 (Table 1).

TABLE 1
First Experimental Phase: Physical and Chemical Parameters Measured in the Field

	pH	T (°C)	Cond. (mS/cm)	DO[a] (mg/L)
A1	2.46	26.0	7.63	6.1
A2	2.31	25.9	6.22	8.4
A3	2.37	25.8	4.12	7.7
A4	2.34	26.2	6.28	7.1
A6	2.44	26.3	4.39	8.6
A7	2.41	25.5	5.98	8.0

[a]Dissolved oxygen.

Sample A2 was the most toxic, with the highest values of EPA toxic units (TU) for all three Microtox exposure periods (Fig. 2). Sample A3 was the least toxic to Microtox. A toxicity pattern related to the exposure time was not noticed. Only samples A2 and A3 presented increasing TU values with increasing exposure times (Fig. 2).

Second Experimental Phase

The pH values measured at stations 1 through 8 were very similar (Table 2). At stations 10, 0, and 00, pH was close to neutrality. Conductivity values registered at stations 1 and 5 were very high (Table 2). Samples B9, B10 and at the reference sites (samples B0 and B00) presented the lowest conductivities (Table 2).

In the median lethal dilution test, the water dilution induced the formation of precipitates. Furthermore, the subsequent addition of $Ca(OH)_2$, to raise the pH until 8, caused additional precipitation. No mortality was observed in the control. At 24 h of exposure, the highest values of mortality were observed at the highest dilutions. Cumulative mortality, in sample B2, increased with the increasing dilution factor, with 80% of mortality at dilution 1.625%.

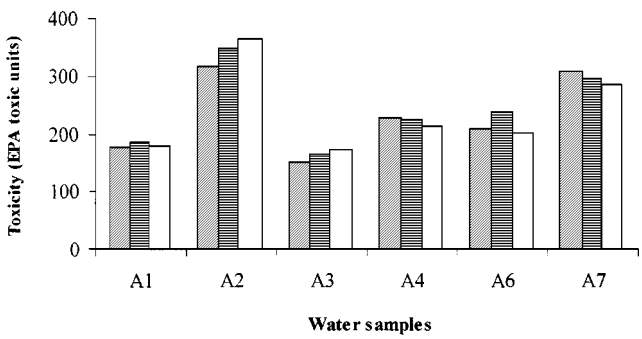

FIG. 2. First experimental phase: Microtox results of water samples after 2 (dark bars), 5 (light bars) and 10 min of exposure (open bars).

TABLE 2
Second Experimental Phase: Physical and Chemical Parameters Measured at the Field

	pH	T (°C)	Cond. (mS/cm)	DO[a] (mg/L)
B1	2.46	18.6	7.39	6.6
B2	2.66	11.3	4.22	11.8
B3	2.39	18	3.32	8.8
B4	2.58	18.8	4.55	7.9
B5	2.10	18.2	7.30	8.8
B6	2.39	13.6	3.71	10.7
B7	2.45	14.2	3.65	10.7
B8	2.72	14.1	2.45	10.2
B9	5.76	—[b]	0.374	—[b]
B10	6.42	—[b]	0.250	—[b]
B0	7.06	15.8	0.268	13.4
B00	7.85	—[b]	0.370	—[b]

[a]Dissolved oxygen.
[b]Not recorded due to technical constrains.

At sample B1, B5, and B7 mortality only occurred at the three highest dilutions; the same being registered at B3, where mortality also occurred at the lowest dilution (25%) (Fig. 3). These results did not allow the computation of an LC_{50} for any of the tested samples. At 48 h of exposure, cumulative mortality values increased and, once again, no LC_{50} values could be computed (Fig. 3). No correlation was found (in all cases Spearman correlation coefficients: $P > 0.01$; $n = 5$) between the dilution gradient and the cladoceran cumulative mortality neither at 24 nor at 48 h of exposure (Fig. 3).

Results obtained in the survival time test at pH 4 indicated significant differences only between the control and the water samples (ANOVA: $P < 10^{-4}$, $df = 2,55$, $F = 99.508$, followed by the Tukey multiple range test) (Fig. 4).

A survival time test was also performed with samples B1 through B8, at pH 2. No significant differences were found between the control and the samples (ANOVA: $P = 0.353$, $df = 8,111$, $F = 1.123$). The survival time of *C. dubia* neonates, considering all the samples, was 136 ± 30 s (average $\pm$ standard deviation) (Fig. 5). The survival time test was then conducted at pH 3, where significant differences were found (ANOVA: $P < 10^{-4}$, $df = 12,182$, $F = 18.489$, followed by the Tukey multiple range test). The control was significantly different from the most contaminated sites: B1 through B9.

At local pH, six samples were tested (B1 through B4, B6, and B7). Sample B5 results were discarded because the sample pH differed in more than 5% from the control pH. The relative survival time could not be calculated because the survival time in the control was never significantly higher than those of the samples.

Third Experimental Phase

At this experimental phase, pH values were similar at all stations except for sample C11 (Table 3). Samples C1 and C2 presented the highest values of conductivity and C11 the lowest value (Table 3).

At the survival time test conducted at pH 3, samples C11 and C1 were significantly different from all other samples (ANOVA: $P < 10^{-4}$, $df = 7,152$, $F = 44.641$, followed by the Tukey multiple range test). The sample presenting more toxicity to *C. dubia* was C2 closely followed by C6, C7, and C4 (Fig. 6).

At local pH, only C1 and C2 were significantly different from the respective control. Thus, the relative survival time

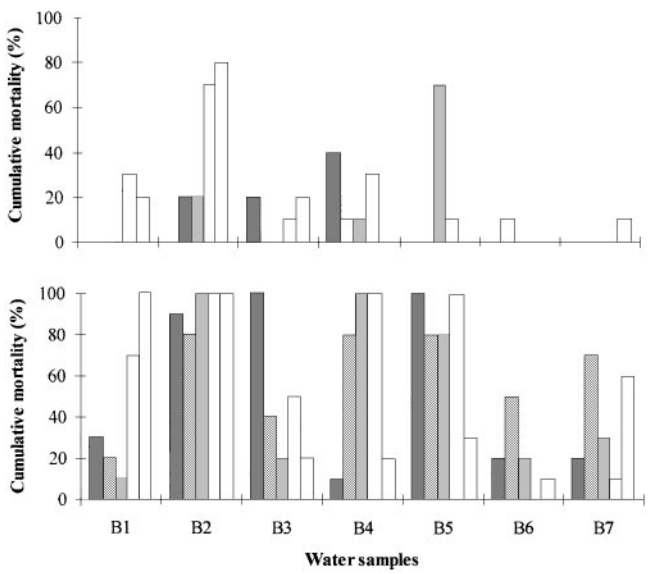

FIG. 3. Second experimental phase: cumulative mortality of *Ceriodaphnia dubia* exposed for 24 (above) and 48 h (below) to five dilutions of water samples: 25, 12.5, 6.25, 3.125, and 1.625% (from dotted to open bars).

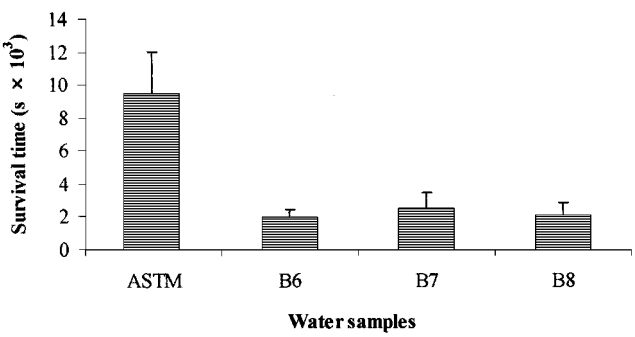

FIG. 4. Second experimental phase: survival time of *Ceriodaphnia dubia* (average and standard deviation) in water samples with pH adjusted to 4.

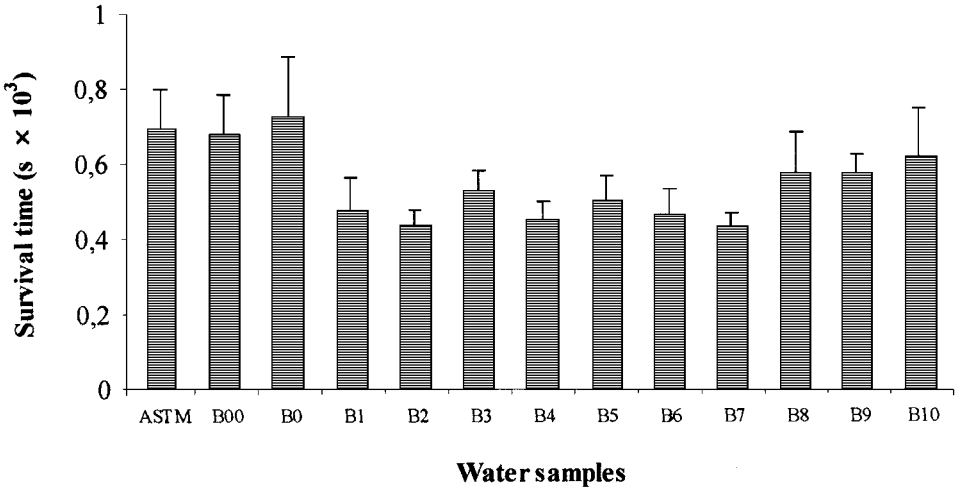

FIG. 5. Second experimental phase: survival time of *Ceriodaphnia dubia* (average and standard deviation) in water samples with pH adjusted to 3.

was only calculated for these samples. The comparison between relative survival times at local pH and at pH 3 indicated that C2 was the most toxic sample on both tests (Fig. 7).

The computation of CEC was only possible, at local pH, for samples C1 and C2. These were also the two samples presenting the highest values of all the metals, except for As and Pb, which presented the highest concentrations at C7 and C6, respectively (Table 4). Worth noting is the quantity of As present in sample C7, which is 443 times higher than the highest value registered for other samples. Sample C1, at pH 3, despite the big amounts of heavy metals, presented the lowest CEC value.

DISCUSSION

Physical and Chemical Parameters

Physical and chemical parameters, measured along the sampling sites, remained quite similar during the study period. However, at the third experimental phase, pH and conductivity presented a slight decrease and increase, respectively, which was probably due to an extremely hot summer, and, thus, to high evaporation rates at AMD ponds. All ponds presented similar conductivities, except for stations 1 and 2, the most contaminated ones.

TABLE 3
Third Experimental Phase: Physical and Chemical Parameters Measured in the Field

	pH	*T* (°C)	Cond. (mS/cm)	Cond.3[a] (mS/cm)	DO[b] (mg/L)
C1	2.23	14.5	7.99	3.65	6.9
C2	2.00	15.0	8.65	6.40	9.7
C3	2.00	15.0	4.34	3.14	9.0
C4	2.26	15.5	5.13	4.37	8.2
C6	2.19	14.5	3.90	4.35	9.0
C7	2.18	15.5	4.98	4.60	9.3
C11	3.24	14.5	0.536	0.753	7.9

[a]Conductivity values after pH adjustment to 3.
[b]Dissolved oxygen.

Microtox

In the first experimental phase, the pH of all water samples was very similar, and, therefore, Microtox results and conductivity were expected to be correlated. However, Microtox revealed sample A2 as the most toxic one, although it presented an intermediate conductivity, and A1, with the highest conductivity, was one of the least toxic samples. Such unexpected results can be due (a) to the very low pH, which was between 2.31 and 2.46, thus being outside the range of the optimum sensitivity of the bacteria (pH between 6 and 7) (Kross and Cherryholmes, 1993), (b) to the interaction between metals and Microtox osmotic regulator

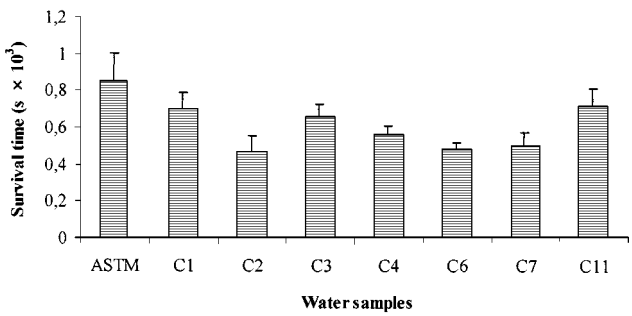

FIG. 6. Third experimental phase: survival time of *Ceriodaphnia dubia* (average and standard deviation) in water samples with pH adjusted to 3.

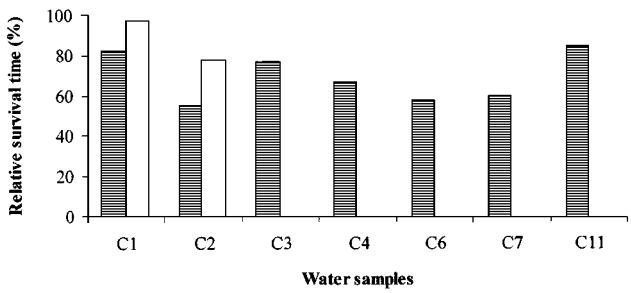

FIG. 7. Third experimental phase: relative survival time of *Ceriodaphnia dubia* (average) in water samples with pH adjusted to pH3 (gray bars) and without pH adjustment (open bars).

solution, or (c) to the interactions between metals and the pH. The protocol for the basic test is that pH produce a minimum effect between values 6 to 8; when pH is above 8 or below 6, and the sample buffer capacity is high, those effects can be dramatic (Microbics Corp., 1992). When diluting the sample, the pH was altered along the dilution range. Thus, at the EC_{50}, the pH was higher than the initial sample pH, and an apparent toxicity change can be induced since luminescence can be altered with pH changes (Kross and Cherryholmes, 1993). To adjust the pH to higher values before running the Microtox test would cause metal precipitation, and, thus, it would interfere with the sample toxicity.

Chloride ions, from the osmotic regulator, most probably reacted with dissolved metals, some of which precipitated, thus reducing the concentration of free metal ions. Furthermore, chloride ions can change the speciation of metals, thus altering the true toxicity of the effluent (Kross and Cherryholmes, 1993; Carlson-Ekvall and Morrison, 1995).

Many interactions, such as antogonistic, additive, and synergistic effects, can occur among metals. These interactions were not constant among sample dilutions because different precipitation levels occurred. Giesy *et al.* (1988) found that Microtox is particularly sensitive to toxic effects of metals such as copper, which is present in high concentrations in these samples and is known to form strong complexes with organic ligands (Giesy *et al.*, 1988).

In conclusion, the Microtox was not adequate to assess the toxicity of AMD contamination because several interactions can interfere with toxicity results. To prevent some of these interactions, pH adjustment would be required, thus altering the chemical characteristics of samples and disallowing a relevant assessment.

TABLE 4
Third Experimental Phase: Dissolved Metal and Copper Equivalent Concentrations (CEC) Determined for Each Water Sample (mg/L)

	C1	C2	C3	C4	C6	C7
Al	144.8	440.0[a]	164.8	362.5	164.3	255.0
As	0.01044	0.00084	0.00626	0.01074	0.03724	16.5
Cd	0.840	0.483	0.0487	0.0987	0.280	0.740
Cr	0.529	0.156	0.0564	0.0867	0.0802	0.142
Cu	61.25	10.25	17.88	38.00	20.15	30.75
Fe	702.5	655.0	90.75	350.0	265.0	109.3
Mn	116.8	89.00	11.03	22.78	13.15	19.13
Ni	1.800	1.980	0.397	0.780	0.383	0.613
Pb	0.100	<0.100	<0.100	<0.100	0.254	<0.100
Co	3.660	4.120	1.070	2.410	1.110	1.560
Zn	145.75	257.5	13.98	24.43	47.75	85.50
Hg	<0.0071	<0.00071	<0.00071	<0.00071	<0.00071	<0.00071
CEC pH3	8.5	133	47	62	113	57
CEC local pH	190	591				

[a]The highest values are underlined.

Median Lethal Dilution Test

Results at 24 and 48 h of exposure indicated the median lethal dilution test to be inappropriate for evaluating AMD toxicity. In fact, no correlation was found between the dilution gradient and the cumulative mortality. Unexpectedly some samples (B1, B2, B5, and B7) were found to be more toxic to *C. dubia* at the highest dilutions. These artifacts may be explained by the severe alteration induced in the water chemistry of the samples when they were diluted and when the base $Ca(OH)_2$ was added.

Different dilutions raised the pH to different values, and, therefore, the changes on the heavy metals speciation were also unequal. The low pH favors the existence of free ions, usually the species presenting more toxicity to aquatic life, increasing the competition with the essential ions at binding and uptake sites of the organism (Mance, 1987). With increasing pH, free ions form complexes with other ions, organic acids and others. An example is aluminium, which at pH 4 occurs in the free form (Al^{3+}), but raising the pH one unit, to 5, results in $AlOH^{2+}/Al(OH)_2^+$, and to pH 7.5 results in $Al(OH)_4^-$; each of these species presents different degrees of ecotoxicity (Wren and Stephenson, 1991; Gerhardt, 1995). Furthermore, the dissimilar amounts of $Ca(OH)_2$ needed to raise the pH along the dilution range led to both quantitative and qualitative differences in precipitates. This base reacts with metals, forming metal hydroxides, which precipitates (Kuyucak *et al.*, 1995). As at the highest dilutions a small amount of $Ca(OH)_2$ was necessary to raise the pH to 8, some metals, like Ni^+, Cd^{2+}, and Zn^{2+}, which are particularly difficult to precipitate, could remain in solution (Riveros, 1995). Therefore, a proportional precipitation along the dilution range, most probably, did not occur. In conclusion, the severe alterations made in the effluent's physical and chemical characteristics, resulting

from the dilution and the addition of $Ca(OH)_2$, disallowed the computation of LC_{50}s and, therefore, the ecotoxicity comparison among sites.

Survival Time Tests

The survival time test at pH 4 was able to discriminate heavy metals as a toxicity source, since control was significantly different from samples B6, B7, and B8, contaminated with heavy metals. Nevertheless, this test was not sensitive to different quantities of heavy metals, because it was not able to differentiate the toxicity among the three AMD samples. This incapability of distinguishing levels of toxicity among samples is probably linked with similar contents of heavy metals in the water columns adjusted to pH 4. Increasing pH to 4 may be sufficient to cause heavy metal saturation, which begin to precipitate. Different quantities of heavy metals, present among the samples, before the base is added, will not remain proportional, since different rates of heavy metals precipitation will take place within the samples. Nevertheless, the amount of each heavy metal remaining in solution will probably be similar, near to saturation limit, and, thus, similar toxicity results, to *C. dubia*, were achieved for each water sample.

The survival time test at pH 2 did not allow the discrimination of the toxicity due to low pH from the toxicity due to heavy metals. The extremely rapid death of *C. dubia* neonates did not allow the differentiation of the influence of any other toxicity source besides pH. Heavy metal toxicity might result from interactions with mediated transport systems at biological membranes. Nevertheless, at such extremely high H^+ concentrations, the water samples cause not only a generalized disruption of the vital functions, but also the destruction of organism structures.

The local pH survival time test could only distinguish the severely heavy metal contaminated samples from other tested samples. Only when quantities of heavy metals were extremely high, their effects were noticed over the pH effects. This is also related to the low pH of the samples (between 2 and 2.5), which causes a rapid death of *C. dubia* not allowing toxicity discrimination.

The survival time test at pH 3 achieved both sensitivity and discrimination. It was sensitive because it was able to detect the presence of heavy metals as a toxicant besides the low pH. It was discriminative because it was able to differentiate levels of toxicity among samples. At the second experimental phase, survival time test at pH 3 was able to differentiate the samples presenting higher conductivity (B1 through B7) from all others. At the third experimental phase, despite conductivity alterations, due to the pH adjustment, the test maintained samples C11 and C2 as the least and the most toxic ones, respectively. This fact indicated that the adjustment of pH to 3 did not seem to alter too much the water toxicity.

Comparing CEC with actual heavy metals concentrations, at pH 3, C2 presents the highest value of CEC corresponding to the highest values of heavy metals. This sample, C2, presented the highest concentrations of Al, Ni, Co, and Zn, and the second highest values of Cr, Fe, and Mn. Sample C1 exhibited the lowest value of CEC, despite presenting one of the highest levels of heavy metals concentrations. The pH adjustment of sample C1 led to a notorious decrease of its conductivity (which became one of the lowest values) and is probably the main cause of such low toxicity and CEC. The smallest CEC value corresponds to sample C3, the one with fewer quantities of heavy metals. This sample, C3, demonstrated the lowest values of Cd, Cr, Fe, Mn, Co, and Zn, and the second lowest values of Al (*ex aequo* with C6), Cu, and Ni.

CONCLUSIONS

The survival time test at pH 3 was confirmed as appropriate to assess the toxicity of AMD effluents. It presents two main advantages: (a) it uses a sensitive toxicity endpoint (survival time of *C. dubia*) that allows the separation of the toxicity due to heavy metals from the toxicity due to low pH, and (b) it is performed in conditions close to the field; chemical alterations induced at pH 3 seemed not to be too relevant. This test can become a very useful tool in AMD studies, not only for assessing the toxicity but also for detecting toxicity sources other than pH, which will aid in the prospect of finding other remediation actions. CEC could be used to establish a toxicity limit for the discharged effluent, in an effort to avoid ecological disasters at receiving waters.

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