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Survival Time of *Ceriodaphnia dubia* in Acid Waters with Metal Contamination

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Many regions are impacted by severe acidification resulting from acid precipitation, acid mine drainage, or natural sources of acidification. Serious ecotoxicological effects, such as loss of fish populations, may be observed in such regions. These can be caused by the direct effects of acidification or by the combined effects of other contaminants that emerge with water acidification (e.g. mobilization and increased solubility of metals) (Kelly 1988, Sengupta 1993). The discrimination between these two factors is not possible with existing assays, as serial dilution methods or the addition of chelating agents seriously alter the chemical characteristics of the water sample (USEPA 1991, Hockett *et al.* 1996). Diluting the acid water increases the pH which promotes solute precipitation and, thus, alters the relative chemical composition. Moreover, pH differences within the dilution range can mask the ecotoxicity due to the high concentration of protons (low pH) and due to other toxicants. Removal of metals by the use of a chelating agent, such as EDTA, also presents other serious disadvantages (Sorvari and Sillanpaa 1996): (i) the chelating agent can be toxic to test organisms thus interfering in final results, and (ii) the chelating agent can remove non-metal ions, like calcium. Furthermore, if other toxicants, besides metals and protons, are present in the water sample, further chemical removals may be necessary. Thus, aiming to distinguish toxic effects due to low pH and other chemicals in acid waters, the sensitivity and discriminatory power of survival time of the cladoceran *Ceriodaphnia dubia* as a toxicity evaluation endpoint was studied at different pHs and at different copper concentrations. A non-linear regression model was then fitted to survival time values as a function of pH and copper concentration.

MATERIALS AND METHODS

The cladoceran *Ceriodaphnia dubia* Richard was cultured in ASTM hardwater medium (ASTM 1988), with the organic additive Marinure '25' (Pann Britannica Industries Ltd. Waltham Abbey, U.K.), an extract from the algae *Ascophyllum nodosum* (Baird *et al.* 1989). Neonates (6 to 24 hr old) from third, fourth or fifth broods were used. Tests were performed in 42 mL glass vessels with 30 mL of medium. An organism was considered dead when it remained immobile during 15 set after gentle prodding. Tests were carried out at 20±1°C, with a 16 hr light and 8 hr dark photoperiod.

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Table 1. Number of test organisms (*Ceriodaphnia dubia*) belonging to validated replicates at each copper concentration and at each pH.

	Copper concentration (µgCu L^{-1})							
pH	control	6	60	600	$6x10^3$	$6x10^4$	$6x10^5$	$6x10^6$	Total
2	30	---	---	15	15	30	15	15	120
2.5	30	---	---	15	15	30	15	15	120
3	30	---	---	15	15	30	15	15	120
3.5	30	15	15	15	15	15	---	---	105
4	20	15	15	15	15	15	---	---	95
5	15	5	15	15	15	15	---	---	80
total	155	35	45	90	90	135	45	45	640

Tests were performed at 6 different pHs: 2, 2.5, 3, 3.5, 4, and 5, obtained by adding sulphuric acid to the synthetic medium. At each pH, a control plus five concentrations of copper sulphate were tested, using a lo-fold scale. From pH 2 to 3, copper concentrations of 600 to $6x10^6$ ugCu L^{-1} were used, while in higher pH solutions, copper concentrations of 6 to $6x10^4$ ugCu L^{-1} were used (Table 1). Sulphuric acid was chosen to adjust pH because it is the commonest in acid mine drainage, due to the oxidation of sulphides, and it is a main acid present in acid rain, due to the oxidation of SO_2 (Kelly 1988, Mason 1989). Copper sulphate was chosen as a reference chemical because data about its toxic effects are available for a considerable number of *taxa* and because both copper and sulphate ions are commonly present in acid mine drainage (Kelly 1988).

Each control and each copper concentration, at each pH, was replicated three times. All controls and some copper concentrations were repeated. In each vessel, five non-fed daphnids were tested. At pH values from 2 to 3, organisms were introduced in each vessel one by one; survival time being recorded for each daphnid before introducing the next organism. At higher pH values, all five organisms were introduced simultaneously. Observations of organisms' immobilisation were made continuously during the first 15 min, every 3 min from 15 to 30 min of test duration, every 5 min from 30 to 120 min, every 15 min from 2 to 6 hr, every hr from 6 to 12 hr, and at 18, 24, 36 and 48 hr. A test ended when all daphnids died; no organism died after 48 hr of exposure. Dissolved oxygen and pH were measured in each vessel at the beginning and at the end of each test. Average pH for each flask was computed by calculating the average concentration of hydrogen ions and then calculating the respective pH. Test results of an experimental run were rejected whenever the pH variation was higher than 10% of initial pH value, or, the average pH differed by more that 5% of nominal pH (e.g. 2.00, 2.50, 3.00,...). Thus, the number of validated replicates at each copper concentration and at each pH was not constant (Table 1). Oxygen was measured with an WTW OX1 92 meter, and pH measured with an WTW 537 meter.

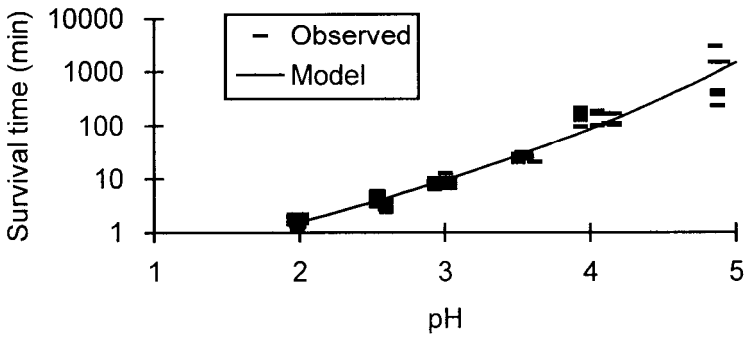

Figure 1. Survival time values (min) of *Ceriodaphnia dubia* in ASTM hardwater medium adjusted with sulphuric acid to pH from 2 to 5. The respective regression line is presented.

Homocedasticity of survival time series at different pHs was achieved by transforming raw data using the Taylor method (Taylor 1961), where each raw value (t, in sec) is transformed to:

$$t[1-(b/2)]$$

where b is the slope of the linear regression between $\log_{10}$ sample variances and $\log_{10}$ sample means.

Overall parameter estimation of the non-linear multiple regression model was accomplished by the Marquardt algorithm (Conway *et al.* 1970).

RESULTS AND DISCUSSION

Survival time of C. *dubia* in ASTM medium at pH 2 was very short, with an average value of about 2 min (Fig. 1). At pH 3, 4 and 5 survival time increased to about 8 min, 2 hr, and 11 hr, respectively (Fig. 1). Survival time means (m) and variances (s^2) were found to be strongly correlated (r = 0.97, n = 30, P < 0.001) (standard errors are presented inside brackets):

$$\log_{10} s^2 = -2.31(\text{s.e.}=0.31) + 2.19(\text{s.e.}=0.10)\ \log_{10} m \quad (r^2 = 94.2\%,\ n = 30)$$

Thus, the ideal exponent to be used in data transformation was found to be -0.1. After transformation, independence between averages and respective variances was achieved. A linear regression model was then fitted to the relationship between transformed survival time (t^{-01}, in sec) and pH, within pH range from 2 to 5 (Fig. 1):

$$t^{-0.1} = 0.8451(\text{s.e.}=0.0046) - 0.1048(\text{s.e.}=0.0014)\ pH \quad (r^2 = 97.3\%,\ n = 155)$$

Copper sulphate additions to ASTM medium adjusted to low pH, from 2 to 5, significantly affected survival time of *C. dubia* (in all five l-way ANOVAs: P<0.00l). By comparing survival time at each copper concentration with the respective control (no copper added), No Observed Effect Concentrations (NOECs, in µgCu L^{-1}) were found to be:

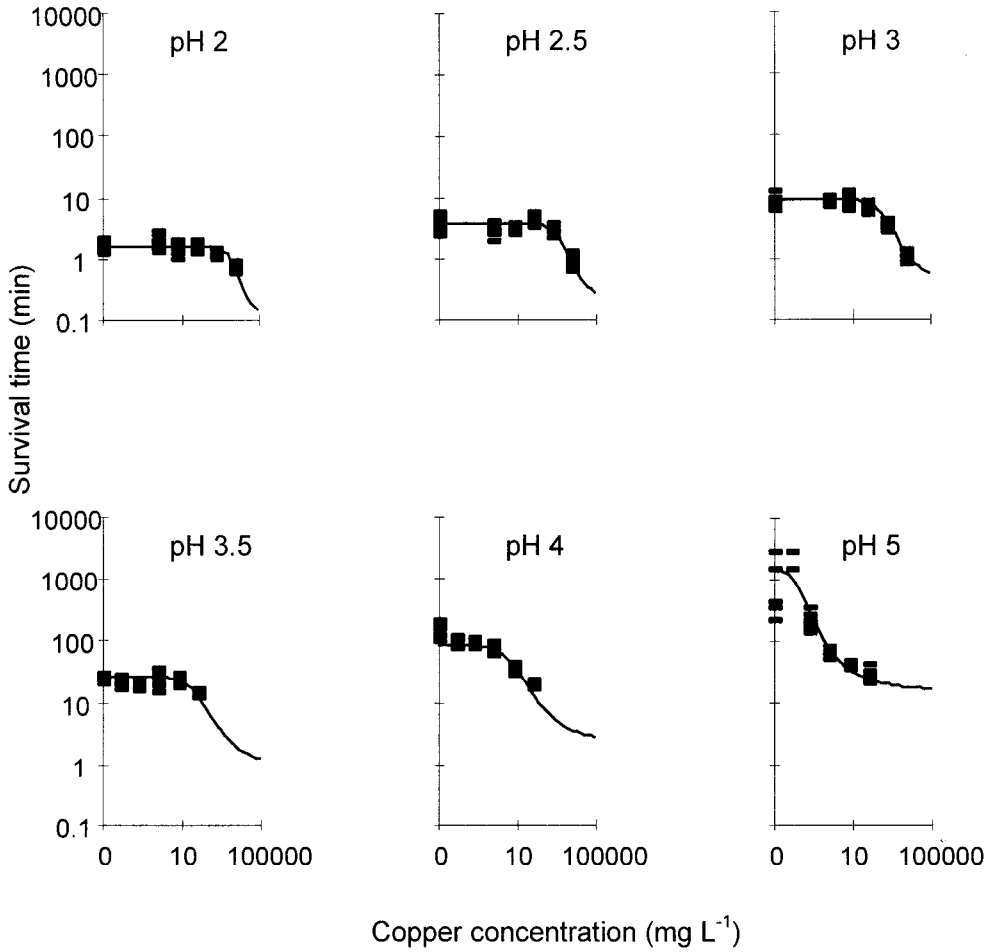

Figure 2. Survival time values (min) of *Ceriodaphnia dubia* in ASTM hardwater medium adjusted with sulphuric acid to pH from 2 to 5, at increasing copper concentrations. The respective regression line is presented.

- $6x10^4$ µgCu L^{-1} at pHs 2 and 2.5,
- $6x10^3$ µgCu L^{-1} at pHs 3 and 3.5,
- 60 µgCu L^{-1} at pH 4, and
- 6 µgCu L^{-1} at pH 5.

The curve shape of the relationship between survival time and copper concentration at pH ranging from 2 to 3.5 was found to be different from the curve shape at higher pHs, mainly pH 5 (Fig. 2). Thus, at pH between 2 and 3.5 the curve shape corresponed to the first half of a sigmoid curve, at pH 4 an inflexion point is present and at pH5 the curve shape corresponded to the second half of a sigmoid curve. The model chosen to integrate all information corresponds to the Type III functional-response equation (Real 1977):

$$Y = aX^n / (b^n + X^n)$$

In the present situation, Y is the transformed survival time after removing pH influence (noted here as v), which, as previously demonstrated, is:

$$Y = v = t^{-0.1} - A - BpH \quad \text{where} \quad A = 0.845 \text{ and } B = -0.105$$

X is the logarithm of copper concentration (noted here as conc = ln (µgCu L^{-1} +l)), a is the maximal v (noted here as V) and b" is the concentration at which v is half-maximal (v = V / 2):

$$v = V\, conc^n / (b^n + conc^n)$$

By fitting this model to v at each pH it was found that n and b were a function of pH:

$$ln\, n = C + D\, pH \quad \text{and} \quad b = E + F\, pH^i$$

Overall parameter estimation of this non-linear model resulted in a good fit (r^2 = 92.3%, n = 640):

V = 0.1846 (s.e.=0.008)

C = 4.303 (s.e.=0.125)

D = -0.6485 (s.e.=0.035)

E = 16.93 (s.e.=0.099)

F = -0.07194 (s.e.=0.013)

i = 3.145 (s.e.=0.095)

Equalling to cone, in order to estimate the copper concentration (Cu, in µgCu L^{-1}) that would immobilise *C. dubia* after an observed time, this expression becomes:

$$conc = ((-b^n v) / (v - V))^{1/n} \quad \text{and} \quad Cu = e^{conc} - 1$$

Survival time of *C. dubia* allows one to predict if, in certain limits, there are other sources of toxicity in an acid water besides pH, and, furthermore, it allows the toxicity comparison of different acid water samples. Such comparison is achieved by computing the relative difference between the survival time in each sample and the survival time in the respective control at the same pH. For instance, a water sample with a relative reduction in the survival time by 50% is more toxic than a sample with a lower relative reduction. However, special caution is needed in the comparison of such toxicity values because other factors, such as hardness, modulate the effects of pH. Therefore, the control water should be consistent with the sample chemistry. Whenever possible, water from nearby reference sites should be adjusted to the sample pH and used to check the appropriateness of the control medium composition. In this situation, the survival time in the control should not be significantly higher than the survival time in the reference water.

The relative reduction in survival time is a measure of toxicity that does not allow any prediction about the contamination level. Actually, detailed chemical analyses are needed to quantify contaminants. However, a rough estimate of the equivalent contamination of a reference toxicant is potentially useful in monitoring acid mine drainage. A mine effluent with fluctuating pH could then be easily surveyed, for both toxicity and relative contamination, with a drastic reduction of detailed metal analyses. The copper concentration dissolved in ASTM medium that would promote a survival time reduction identical to the portion of observed reduction non-explained by the pH is determined with the regression model. Thus, the comparison of relative contamination between

different samples with different pHs is then possible by comparing the "copper equivalent concentrations". Such an approach is only acceptable if the principal toxicant is copper or another chemical whose toxicity varies similarly to pH. Much more work is required before accepting copper as a reference for all acid waters or, even, for all mining effluents.

For the valid application of this survival time test to field situations other specific assumptions must also be met:

1) pH variation must not be higher than 10% of initial pH value, and, the average pH of each replicate must not differ by more than 5% of control pH. Thus, each sample requires a respective control unless several samples present the same pH.

2) survival time of *C. dubia* in each acid water sample replicate must be significantly different from survival time observed in the respective control (ASTM medium adjusted with sulphuric acid to the sample pH).

3) survival time in the control must not be significantly different from survival time predicted by the model. If this assumption is not met, it could mean that sensitivity to low pH of *C. dubia* used in the sample testing was significantly different from the sensitivity of daphnids used here to develop the model, being necessary to re-evaluate model parameters. Or else, it could mean that pH measurements were made with a different accuracy than that obtained in the model development. If differences are strongly suspected to be due to lack of accuracy between pH measurements, then pH values of the control and water samples are to be corrected. This is achieved by calculating, with the model, what is the pH corresponding to the survival time observed in the control. Then the difference between predicted pH and control pH is to be added to both the control pH and the water sample pH. Nevertheless, if the aim is to have a rough evaluation of relative ecotoxicity, then the model could be used with caution.

4) a "copper equivalent concentration" computed by the model is relevant only if it is higher than the NOEC corresponding to the respective pH:

$$\text{NOEC (in } \mu\text{gCu L}^{-1}) = e^{(18.73 - 3.355\ \text{pH})}$$

The use of this survival time test and the respective model in monitoring acid mine drainage and as a screening tool for preliminary evaluations of remediation priority sites has already been field validated at two cupric pyrite mines (Lopes *et al.* in press a,b). At both impacted places, this test revealed the presence of other sources of toxicity besides low pH at the most contaminated sampling stations. Moreover, "copper equivalent concentration" values could be computed for those stations, and were found to be correlated with actual metal concentrations in the water column (Lopes *et al.* in press a,b).

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