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# Acute toxicity of Cd in stream invertebrates in relation to pH and test design

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## Abstract

The acute toxicity of Cd to three stream invertebrates (*Baetis rhodani* Pict., *Leptophlebia marginata* (L.) and *Pisidium* sp.) was tested at pH 5 and 7 simultaneously in static (ST) and flow through (FT) systems. In the static design, the animals were kept individually in small boxes containing aerated stream water. In the flow through system, the three species were kept together in circular aquaria simulating stream ecosystems with patches of gravel and recirculating aerated stream water. The nominal Cd concentrations ranged from 0 to 5 mg $l^{-1}$ during an exposure period of 120 h.

The graphically obtained $LC_{50}$ (120 h) values for *B. rhodani* were: pH 7: 2.3 mg $l^{-1}$ (ST), 2.5 mg $l^{-1}$ (FT) and pH 5: 3 mg $l^{-1}$ (ST), 1 mg $l^{-1}$ (FT). For *L. marginata* the following values were found: pH 7: $>5$ mg $l^{-1}$ (ST), 4.4 mg $l^{-1}$ (FT) and pH 5: $>5$ mg $l^{-1}$ (ST), 3.6 mg $l^{-1}$ (FT). *Pisidium* sp. showed a 100% survival in all Cd- and pH-treatments. From thee values it can be concluded that *L. marginata* is more tolerant than *B. rhodani* and that especially both species tolerated Cd better in the ST system than in the FT system, especially at pH 5.

At neutral pH the Cd-concentrations in the animals were higher than at low pH, probably due to surface adsorption of Cd onto the body. *B. rhodani* tended to molt more at low than at neutral pH.

## Introduction

Acidification is one of the most serious pollution problems in Europe. It causes an increase in the hydrogen ion content of lake and stream waters, accompanied by an increase in the concentrations of several metals because of remobilization from sediments or input by watershed materials (Förstner, 1984; Monitor, 1987; Christensen, 1989). The metals, either independently or jointly with hydrogen ions, are suspected to be toxic to aquatic organisms and seem to lower the diversity and production of aquatic communities (Ravera, 1984; Mackie, 1986; Clements *et al.*, 1989).

Knowledge of the relationship between pH and the toxicity of heavy metals in freshwater habitats is limited and contradictory (Campbell & Stokes, 1985). It has been suggested that Crustacea are more sensitive than Ephemeroptera and Plecoptera, while Trichoptera seem to be the most tolerant (Wang, 1987; Gerhardt, 1990).

In this study the toxicity of cadmium (Cd) at pH 5 and pH 7 was determined for three species of stream invertebrates using static water and flow through test systems with recirculating stream water. The following hypotheses were put forward:

1) The animals accumulate more Cd at low than at high pH.

2) Survival of invertebrates is affected by the exposure conditions.

## Methods

### *Animals*

Three common species of invertebrates were selected for the study. *Baetis rhodani* (L.) (Ephemeroptera) occurs fairly abundantly in streams with pH values above 5.5 (Otto & Svensson, 1983), but survives even at pH 4.5 (Harmanen, 1980; Engblom & Lingdell, 1984). *Leptophlebia marginata* Pict. (Ephemeroptera) does not occur below pH 4.5 (Otto & Svensson, 1983; Ökland & Ökland, 1986). *Pisidium* sp. (Bivalvia) is pH tolerant down to pH 4 (Bauer & Fischer-Scherl, 1987). All species were collected in Mullra creek near Perstorp in Southern Sweden, which is characterised by a pH around 7, total hardness of 0.5 mmol $l^{-1}$; concentrations of $Fe_{tot} = 1$ mg $l^{-1}$, $Al_{tot} = 0.1$ mg $l^{-1}$ and $Cd_{tot} < 1.0$ $\mu$g $l^{-1}$. Flow rates vary from 0 in pool sites to 0.4 m $s^{-1}$ in riffle sites, measured at medium water level.

### *Test designs*

In the *static test* (ST), eight PVC boxes each containing 8 l unfiltered stream water were used and aerated by 4 plastic air plates. In these boxes PVC frames with a nylon net bottom (500 $\mu$m mesh size) were submerged providing 65 chambers of 60 $cm^3$ each containing one animal (Fig. 1). 15 animals were used for each treatment and treatments were duplicated.

In the *flow through systems* (FS) stream conditions were simulated by circular glass aquaria (820 $cm^3$), containing stones and aerated unfiltered streamwater, which was recirculated from a 10 l tank at a velocity of ca. 0.3 m $s^{-1}$. In the FS, all three species were kept together, 15 individuals of every species. Each treatment was duplicated (Fig. 2).

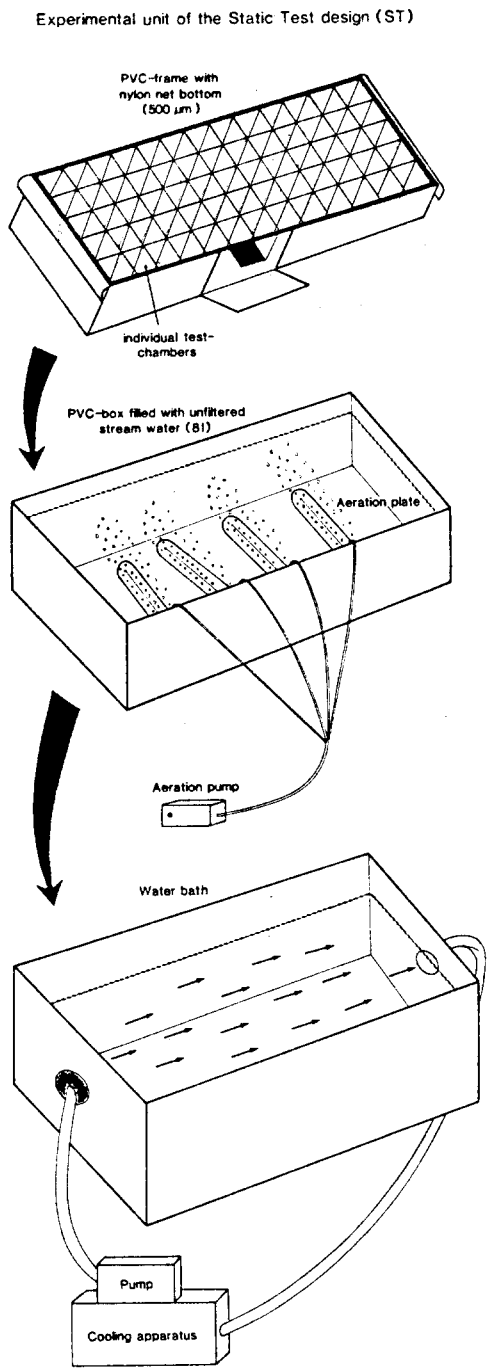


*Fig. 1.* One experimental unit of the static test design (ST). a) PVC frame with net bottom (mesh size 500 $\mu$m), forming 65 test chambers (3.2 x 3.2 x 5.9 $cm^3$). b) PVC box, in which the frame was put. The PVC box contained 8 l unfiltered stream water, which was aerated by 4 plastic air plates on the bottom of the box. c) The whole system was cooled to 10 °C by a water bath.

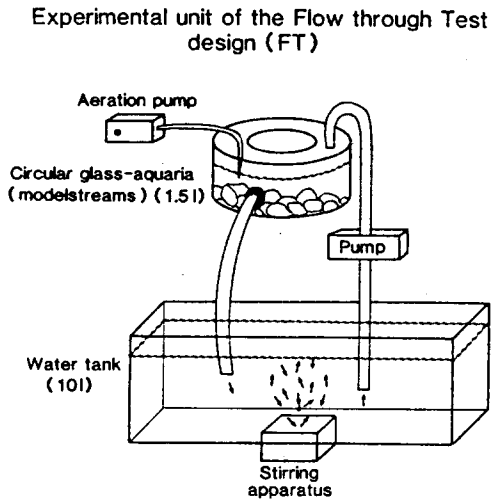


*Fig. 2.* One experimental unit of the flow through test design (FT). a) Circular glass aquarium, simulating a small round model stream ($9 \times 4.5$ cm$^2$) and containing about 1.5 l water, which was aerated by a pump. Stream water was pumped into the aquarium at a flow rate of about 30 cm s$^{-1}$. At a certain water level in the aquarium, the water was led back to the storage tank (b). b) The storage tank was a glass aquarium, containing 10 l stream water, which was magnetically stirred to prevent sedimentation of particles.

The organisms were acclimated to the pH levels for four days and the exposure to Cd lasted 120 h. Survival and moulting was recorded daily. The Cd uptake was measured in living animals prior to and at the end of the experiments. The animals were caught with a pipette and rinsed with distilled water. Pooled samples of three to five animals were dried (48 h, 80 °C), weighed and digested in boiling 100% $HNO_3$. The Cd content was analysed with a Flame Atomic Absorption Spectrophotometer (detection limit: 1 $\mu$g l$^{-1}$).

The pH of the water was initially lowered to pH 5 by steps of 0.5 pH units per day using 96% $H_2SO_4$. Subsequently, pH was measured and adjusted within 0.1 pH unit twice a day with 0.1 M $H_2SO_4$ or 0.1 M NaOH.

Water temperature was kept between 9.5 °C and 11.5 °C and the light regime was a 12 h–12 h light-dark cycle. The stream water was assumed to provide food in the form of fine particulate organic matter and was not changed during the experiments.

The Cd solutions for the different treatments (0, 0.1, 1, 5 mg l$^{-1}$) were prepared from stock solutions containing 10 mg Cd l$^{-1}$ prepared from the salt 3 $CdSO_4$ 8 $H_2O$. The actual cadmium concentrations were adjusted daily for the five day test period.

The following treatments were used:

| Test design | pH | Cd-conc. (mg l$^{-1}$) | pH | Cd conc. (mg l$^{-1}$) |
|---|---|---|---|---|
| Static test (ST) | 7 | 0 | 5 | 0 |
| | 7 | 0 | 5 | 0 |
| | 7 | 0.1 | 5 | 0.1 |
| | 7 | 0.1 | 5 | 0.1 |
| | 7 | 1 | 5 | 1 |
| | 7 | 1 | 5 | 1 |
| | 7 | 5 | 5 | 5 |
| | 7 | 5 | 5 | 5 |

Flow through test (FT) the same treatments as for ST.

*Statistical analysis*

All data (means of the two replicates for each treatment) were analysed by nonparametric statistical methods because of their advantage of being free from specific assumptions. Survival data were analysed by the Friedman test, which represents a nonparametric 2-way ANOVA and by the multiple comparison test (Siegel & Castellan, 1988) to detect significant differences between the treatments. Comparisons between two treatments were made by the Mann-Whitney-U-test (Sokal & Rohlf, 1981). The $LC_{50}$ values were obtained graphically.

## Results and discussion

*Survival*

*Pisidium* sp. was by far the most Cd tolerant species of those tested, since no mortality was observed at any concentration in any of the experiments. It is most likely that mussels can survive short term pollution by retreating into the shell (Mackie, 1986). Therefore, longer test periods of several weeks have to be used for evaluating le-

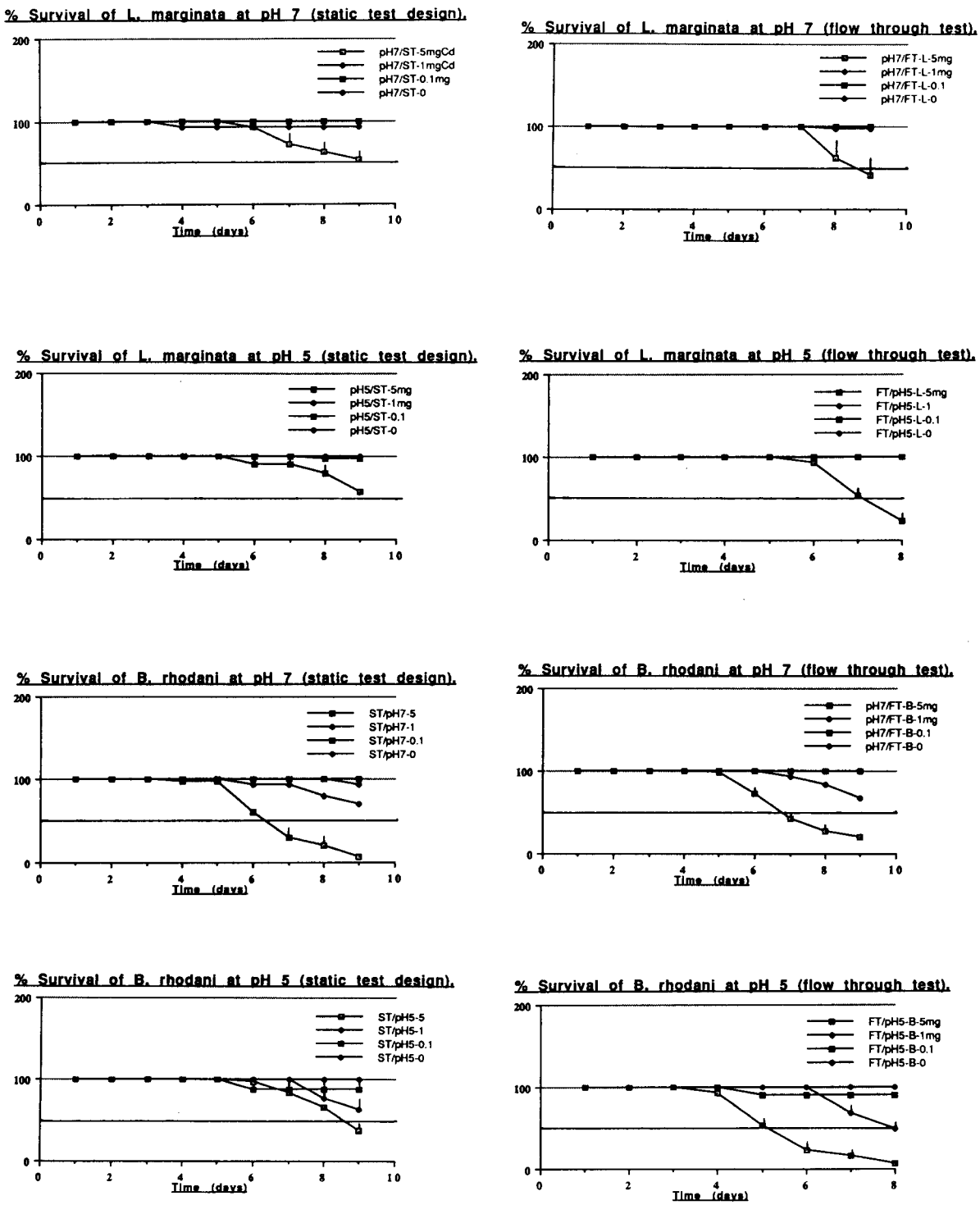


*Fig. 3.* Percentage of invertebrates surviving during the experiment in the different pH- and Cd-treatments. The values represent means of two replicates and the bars are the standard deviation of the mean.

thal effects on mussels. Mackie (1986) reported $LC_{50}$-96 h values for Cd of 0.4 to 2 mg $l^{-1}$ depending on pH within a range of pH 3.5 to 4.5. In that study the low pH itself may have had a lethal effect on the mussels.

Survival of the mayflies was dependent on Cd-exposure (Fig. 3), the differences between the different treatments being significant at the 5% or 1% level (Friedman test), especially between the controls and the 1 mg $l^{-1}$ and 5 mg $l^{-1}$ treatment groups (Table 1). Lethal effects of Cd on *B. rhodani* could be seen at Cd concentrations of 1 mg $l^{-1}$ and 5 mg $l^{-1}$ at both pH values. In the ST, the animals survived better at low than at neutral pH, the $LT_{50}$ was reached on day 6 (pH 7) and on day 8 (pH 5) in the 5 mg $l^{-1}$ treatment. In the FT, the opposite was found with the $LT_{50}$ being reached on day 7 (pH 7) and on day 5 (pH 5). At pH 7 survival was similar in both the FT and ST systems, while at pH 5 the animals survived longer in the ST than the FT system at lethal Cd concentrations (5 mg $l^{-1}$), however the Mann-Whitney-U-test was not significant.

Lethal effects of Cd on *L. marginata* could only be seen at the 5 mg $l^{-1}$ concentration, indicating that this species was more tolerant than *B. rhodani*. The $LT_{50}$ was reached on day 9 in both systems at pH 7, while it was reached on day 7 in the ST system and on day 9 in the FT system at pH 5. Survival of this species was thus slightly better in the ST system than in the FT system but the difference was not significant (Mann-Whitney-U-test). Inter- and intraspecific competition between the animals in the model streams (FT) might be an additional stress factor which was suppressed in the ST by keeping the animals individually.

The $LC_{50}$ (120 h) values were 2.3 mg $l^{-1}$ (ST) and 2.5 mg $l^{-1}$ (FT) at pH 7 and 3 mg $l^{-1}$ (ST) and 1 mg $l^{-1}$ (FT) at pH 5 for *B. rhodani* and > 5 mg $l^{-1}$ (ST) and 4.4 mg $l^{-1}$ (FT) at pH 7 and > 5 mg $l^{-1}$ (ST) and 3.6 mg $l^{-1}$ (FT) at pH 5 for *L. marginata*. There was a difference in test design for *B. rhodani* and *L. marginata* at pH 5 (Fig. 4). *L. marginata* survived better than *B. rhodani* irrespective of treatment conditions. The $LC_{50}$ values of this study are comparable with those for mayflies in earlier studies: *Ephemerella subvaria*: 2.0 mg $l^{-1}$, Warnick & Bell, 1967; *Atalophlebia australis*: 0.84 mg $l^{-1}$, Thorp & Lake, 1974). Canton & Slooff (1982) found $LC_{50}$ values (48 to 96 h) between 0.02 and 11.1 mg $l^{-1}$ depending on the invertebrate species. Great variation thus exists and *Tubifex tubifex* (Diptera) had a $LC_{50}$ value of

*Table 1.* Statistical analysis of the survival data.

| Test | Species | pH | 0 | 0.1 | 1 | 5 | F | D |
|---|---|---|---|---|---|---|---|---|
| ST | *L. marg.* | 7 | x | x | x | x | ** | n.s. |
| | | 5 | x | x | x | x | ** | n.s. |
| | *B. rhodani* | 7 | x | x | x | x | ** | |
| | | | x | | x | | | * |
| | | | x | | | x | | * |
| | | 5 | x | x | x | x | * | n.s. |
| FT | *L. marg.* | 7 | x | x | x | x | n.s. | n.s. |
| | | 5 | x | x | x | x | * | n.s. |
| | *B. rhodani* | 7 | x | x | x | x | ** | n.s. |
| | | 5 | x | x | x | x | ** | |
| | | | x | | | x | | * |

F: Friedman test, D: multiple test; * 5% level, ** 1% significance level. Cd concentrations are given in mg Cd $l^{-1}$.

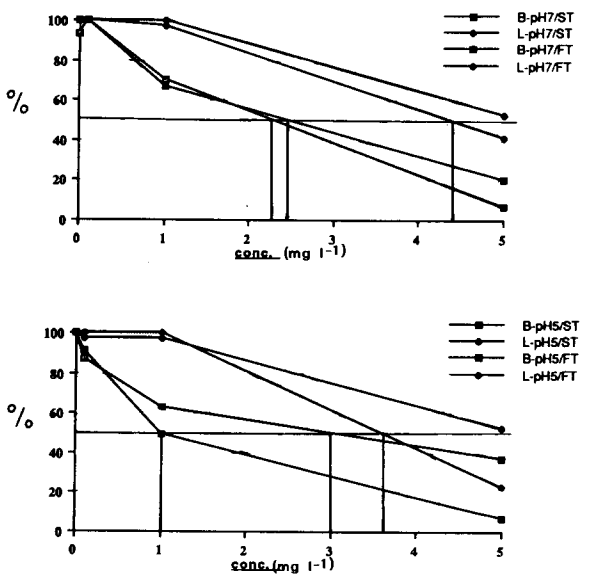


*Fig. 4.* Graphical analysis of the $LC_{50}$ values for the mayflies. B = *Baetis rhodani*, L = *Leptophlebia marginata*; ST = static test design, FT = flow through design.

0.027 mg $l^{-1}$, while Leptoceridae (Trichoptera) seem to tolerate 2000 mg $l^{-1}$ (Thorp & Lake, 1974). The trichopteran *Polycentropus flavomaculatus* showed a $LC_{10}$ of 2500 mg $l^{-1}$ (Gerhardt, unpublished). Crustacea seem to be most sensitive to Cd (Gerhardt, 1990). Mackie (1986) investigated the Cd-$LC_{50}$ for different invertebrates at pH values from pH 3.5 to 6 and found that toxicity increased with decreasing pH for the crustacean *Hyallela azteca*, while it decreased for the bivalve *Pisidium compressum* and the gastropod *Amnicola limosa*. In this experiment, Cd toxicity increased at low pH for both mayflies.

*Uptake of Cd*

In general, all three species contained more Cd (mg $g^{-1}$) when exposed to a higher Cd concentration in the water (Fig. 5). At pH 7, higher Cd body burdens were reached than at pH 5, especially in the 5 mg $l^{-1}$ treatment. As the individual variation in Cd body burden was considerable, these observations are only tendencies. In the FT, mayflies seemed to take up more Cd than in the ST, perhaps because of the availability of fine detritus with adhering Cd as food. The uptake of Cd after the whole exposure period was measurable at all concentrations, supporting the results of Canton & Slooff (1982) and Dressing *et al.* (1982), which show that Cd uptake is fast and linear during the first 2–4 days of exposure. Cd was more toxic at pH 5 than at neutral pH, however the total Cd body burdens were higher at neutral than at low pH; thus adsorption of Cd onto the body without leading to toxic effects could be an important factor at neutral pH. Surface adsorption of Cd on chironomids increased with increasing pH (Krantzberg & Stokes, 1985). Perhaps the washing procedure in distilled water was unappropriate to remove all of the adsorbed Cd and should be replaced by washing the ani-

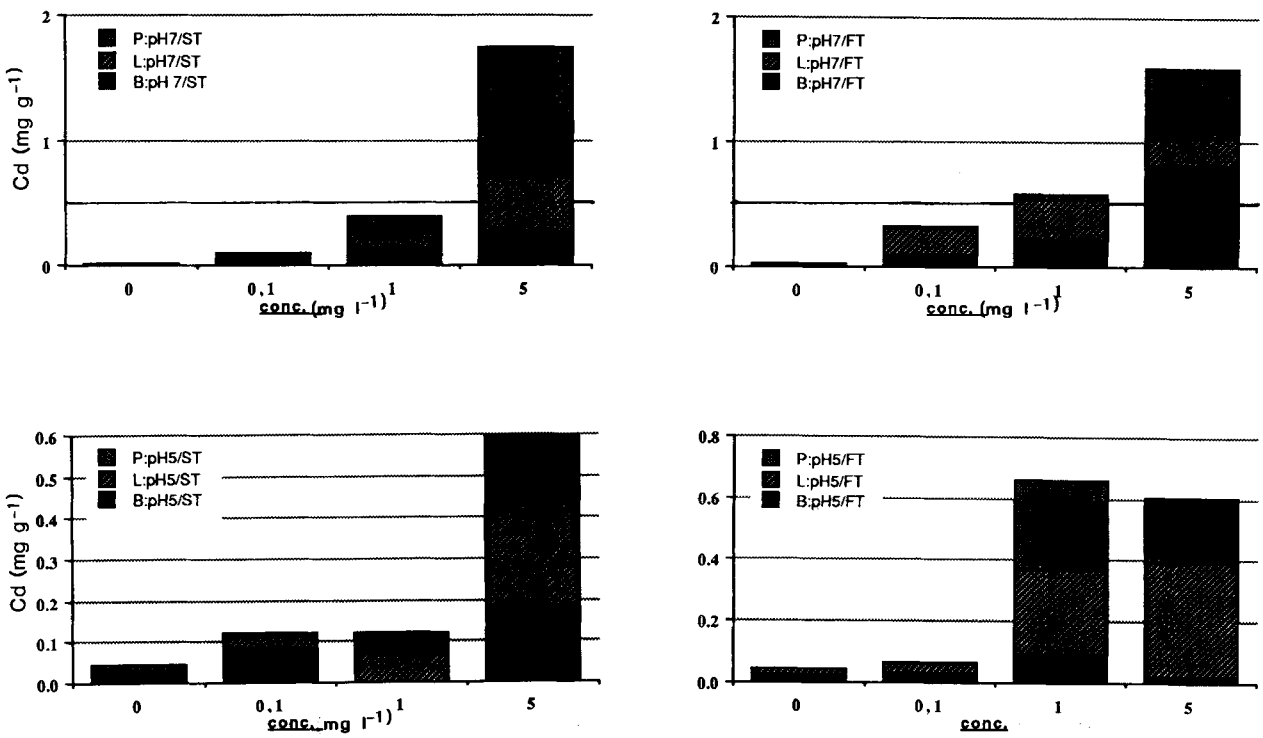


*Fig. 5.* Cd concentrations (means) (mg $g^{-1}$ DW) in the animals after 120 h exposure to different Cd levels in the water (mg $l^{-1}$). P = *Pisidium* sp., B = *Baetis rhodani*, L = *Leptophlebia marginata*; ST = static test design, FT = flow through design.

mals in acidic water. The lower Cd-body burdens at pH 5 can also be caused by a lower uptake due to competition between $H^+$ and $Cd^{2+}$ at the uptake sites.

*Moulting*

In both test systems, moulting of *B. rhodani* and *L. marginata* was independent of Cd concentration in the water (Table 2). More *B. rhodani* tended to moult at pH 5 than at pH 7 regardless of test design, however the difference was not statistically significant. In the ST, more specimens of mayflies seemed to moult than in the FT. Increased moulting may also have been a reaction to sublethal chemical stress (pH, test design).

## Conclusions

All tested species accumulated more Cd at neutral than at low pH, even if Cd was more toxic (survival) at pH 5 than at pH 7. At lethal Cd concentrations, Cd was more toxic in the modelstreams, probably because of additional stress factors like inter- and intraspecific competition, especially at low pH. This indicates that even acute toxicity tests should also be performed under field relevant conditions (model streams with several species) and that such a test design can lead to lower $LC_{50}$ values than traditional designs.

*Table 2.* Percentage of moulting in the mayflies during the experiments.

| Test design | Species | pH | 0 | 0.1 | 1 | 5 |
|---|---|---|---|---|---|---|
| ST | *B. rhodani* | 5 | 80 (0) | 53 (0) | 56 (4) | 60 (13) |
| | | 7 | 0 (0) | 53 (0) | 20 (9) | 10 (3) |
| FT | *B. rhodani* | 5 | 44 (9) | 20 (7) | 40 (14) | 26 (0) |
| | | 7 | 27 (6) | 17 (10) | 30 (2) | 10 (10) |
| ST | *L. marginata* | 5 | 17 (4) | 13 (0) | 24 (4) | 60 (0) |
| | | 7 | 27 (0) | 13 (0) | 10 (10) | 23 (10) |
| FT | *L. marginata* | 5 | 0 (0) | 23 (10) | 3 (3) | 0 (0) |
| | | 7 | 0 (0) | 17 (3) | 0 (0) | 3 (3) |

The values given are means of two replicates and the standard deviation is shown in brackets. Concentrations are given in mg Cd $l^{-1}$.

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## Zusammenfassung

Die akute Toxizität von Cadmium wurde in drei Wirbellosenarten untersucht. Die Teste erfolgten bei pH 5 und pH 7 und wurden gleichzeitig in einem statischen System (ST) und einem Durchflussystem (FT) durchgefuehrt. Während in dem statischen System die Tiere einzeln in belueftetem Bachwasser gehalten wurden, wurden in dem Durchflussystem all drei Arten zusammen in Rundaquarien mit Sediment und fliessendem, belueftetem Bachwasser gehalten. In beiden Systemen wurden die Tiere fuer 120 h verschiedenen Konzentrationen (0, 0.1, 1 und 5 mg Cd $l^{-1}$) ausgesetzt.

*Pisidium* sp. war die am meisten tolerante Art (100% Ueberleben in allen Konzentrationen). Die $LC_{50}$ (120 h) Werte fuer *Baetis rhodani* lagen bei 2.3 mg Cd $l^{-1}$ (ST), 2.5 mg Cd $l^{-1}$ (FT) bei pH 7 und 3 mg Cd $l^{-1}$ (ST), 1 mg Cd $l^{-1}$ (FT) bei pH 5; fuer *Leptophlebia marginata* bei $>5$ mg Cd $l^{-1}$ (ST), 4.4 mg Cd $l^{-1}$ bei pH 7 und $>5$ mg Cd $l^{-1}$ (ST), 3.6 mg Cd $l^{-1}$ bei pH 5.

Bei lethaler Cd Exposition wirkte das Durchflusstestsystem als zusätzlicher Stressfaktor, wahrscheinlich aufgrund von Konkurrenz zwischen den Individuen und Arten.

*B. rhodani* häutete sich mehr bei pH 5 und im statischen System, wahrscheinlich ein Ausdruck fuer Stress.

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