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Short term toxicity of iron (Fe) and lead (Pb) to the mayfly *Leptophlebia marginata* (L.) (Insecta) in relation to freshwater acidification

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Abstract

The mayfly *Leptophlebia marginata* was exposed to different concentrations of Fe^{2+} or Pb^{2+} at pH 4.5 and pH 7.0. The effects of the metals on escape behavior and survival of the mayflies were investigated during an exposure of 120 hours.

(1) Whole-body metal loads (Fe; Pb) of the mayflies increased in a dose-dependent way at both pH levels. A significant effect of pH on metal concentration in the mayflies was only found for Pb ($p < 0.001$).

(2) In terms of mortality, both metals were more toxic at pH 4.5 than at pH 7. The 96 h-LC_{50} values for Fe were 106.3 mg Fe l^{-1} at pH 7 and 89.5 mg Fe l^{-1} at pH 4.5. Those for Pb were > 5 mg Pb l^{-1} at pH 7 and 1.09 mg Pb l^{-1} at pH 4.5.

(3) The mayflies lost their escape behavior, when exposed to the metals, the effects being more pronounced at low than at circumneutral pH for both metals ($p < 0.05$). The 96 h-EC_{50} values for Fe were 70.0 mg Fe l^{-1} at pH 7 and 63.9 mg Fe l^{-1} at pH 4.5.

Introduction

Lead (Pb) is a widely distributed contaminant. Lead concentrations in freshwater can reach up to 9 $\mu g\, l^{-1}$ (Jørgensen *et al.*, 1991). Acidification of surface waters down to pH 5 causes an increase of aqueous Pb if the content of organic ligands in the water is low (Nelson & Campbell, 1991). The presence of organic matter or amorphous Fe-hydroxides, however, reduces the availability of Pb (Tessier *et al.*, 1984). Investigations of the role of Pb in aquatic ecosystems have concentrated on its bioaccumulation and biomagnification in the aquatic food web. Toxicity studies have mostly been performed with fish, crustaceans, molluscs or insects as test organisms, however, the effect of pH on Pb toxicity is not uniform (Wren & Stephenson, 1991; Gerhardt, 1993). Some of the reasons for the contradictory results of the effects of pH on Pb toxicity may be differences in water chemistry parameters and in the tolerance of the various test species.

Iron (Fe) is an essential trace metal for all organisms because it is a mediator in oxygen and energy transport (Huebers, 1991). The concentration of iron in freshwater varies from 0.01 to 1.4 mg Fe l^{-1} (Jørgensen *et al.*, 1991). Only a few studies of Fe-toxicity have been performed, mostly without considering pH and Fe-speciation (Jørgensen *et al.*,1991; Gerhardt, 1993). There is, however, an indication that Fe may be more toxic at low pH to *Asellus aquaticus* (Maltby *et al.*, 1987) and to *Leptophlebia marginata* (Gerhardt, 1992a).

Mayflies are important links in the aquatic food web. However, they are under-represented in studies on metal toxicity (Jørgensen *et al.*, 1991; Gerhardt, 1993). *Leptophlebia marginata* and the closely related species *Leptophlebia vespertina* occur at pH levels down to 4.0 and may be increasing in abundance in acidified streams in Sweden (Økland & Økland, 1986). Since metal solubility and bioavailability increase at low pH, these mayflies may be exposed to increased metal stress. As *L. marginata* is a particle feeder, it will be exposed to metals with a tendency to bind to organic matter, such as Pb and Fe.

Mortality is one of the most frequently used endpoint in toxicity studies. However, physiological and behavioral responses to a toxicant are more sensitive (Warner, 1967) and in terms of response time, they are among the first reactions against toxicant stress at sublethal doses (Beitinger, 1990).

To compare the lethal (mortality) and sublethal (behavior) effects of Fe and Pb the mayfly *Leptophlebia marginata* was exposed to the metals for 120 hours under circum-neutral and acidic conditions.

Material and methods

Mayfly nymphs were collected from a small, softwater stream, rich in humic matter (Table 1). The nymphs were kept in aquaria containing unfiltered streamwater and stones serving as shelter. For a period of two days, the animals were acclimated to the system (10 °C ± 1°, 12 h/12 h light/dark cycle) and to the two pH conditions of a) circum-neutral and b) pH 4.5 by stepwise adjustments twice a day with 0.1 M H_2SO_4 and 0.1 M NaOH.

Two groups with 20 mayflies each were subsequently exposed to one of the following nominal metal concentrations (Pb^{2+}: 0, 0.1, 0.5, 1.0 and 5.0 mg l^{-1}; Fe^{2+}: 0, 10, 50, 100, 250 and 500 mg l^{-1}) at both pH conditions. As the background levels for Pb and Fe are ≤0.01 mg Pb l^{-1} and ≤1 mg Fe l^{-1} respectively, the concentrations used in the experiments were up to 500 times higher than in natural surface waters. The stream water in the aquaria was renewed daily and the metal concentrations (Fe_{tot}, Fe^{2+}, Pb_{tot}) were determined before a fresh addition of the metal solutions ($PbCl_2$; $FeSO_4 \times 7H_2O$) occurred. Fe_{tot} and Pb_{tot} were determined with flame AAS, whereas Fe^{2+} was measured spectrophotometrically at 522 nm using 2,2′ Bipyridine. The mayflies were not fed during the 120 h exposure period.

Survival of the mayflies and their reaction to a mechanical stimulus with a forceps (escape reaction) were recorded daily. A positive escape reaction was noted if the mayflies moved a distance of more than one body length away from the stimulus, irrespective of the kind of movement (swimming, creeping).

At the end of the experiments, alive animals from all treatment groups were prepared for metal analysis (Fe_{tot}, Pb_{tot}) with flame AAS. After rinsing with destilled water, the mayflies were dried to constant weight (48 h, 80 °C) and then digested in HNO_3 suprapur at 80 °C (Gerhardt, 1990, 1992b).

Statistical analysis

All data were analyzed by non-parametric statistical methods because of the lacking knowledge about the underlaying frequency distribution due to poor replication ($r = 2$). According to Manly (1991) an estimation of the distribution is only useful at $r \geq 10$. Differences in the pH values between the various metal-concentration levels were

Table 1. Chemical characterization of the water used for the experiments.

pH	$Fe_{tot.}$ mgl^{-1}	$Pb_{tot.}$ μgl^{-1}	Ca^{2+} mgl^{-1}	Cond. μScm^{-1}	DOC $mgCl^{-1}$	NO_3^- mgl^{-1}	PO_4^{3-} mgl^{-1}	$Al_{tot.}$ mgl^{-1}	FPOM mgl^{-1}
6.5	1.3	2	7.2	7.0	21.6	0.1	0.015	0.034	12.5

Table 2. pH and metal concentrations during the experiments.

Group	pH		Fe_{tot} (mgl^{-1})		Fe^{2+} (%)
	mean	sd	mean	sd	
Fe					
control	4.64	0.17	1.3	0.2	n.d.
10 mgl^{-1}	4.65	0.08	9.6	0.3	98
50 mgl^{-1}	4.56	0.10	47.0	2.8	97
100 mgl^{-1}	4.53	0.11	97.0	2.4	96
250 mgl^{-1}	4.42	0.21	241.3	8.5	95
500 mgl^{-1}	4.46	0.18	450	30	95
control	6.74	0.45	1.3	0.2	n.d.
10 mgl^{-1}	6.51	0.32	9.4	0.3	93
50 mgl^{-1}	6.42	0.34	45.8	2.0	94
100 mgl^{-1}	6.31	0.40	95.0	2.2	91
250 mgl^{-1}	5.95	0.29	230.0	5.2	90
500 mgl^{-1}	6.06	0.48	423.0	18	92
Pb					
control	4.60	0.2	0.002	0.0003	
0.1 mgl^{-1}	4.61	0.15	0.11	0.02	
0.5 mgl^{-1}	4.90	0.21	0.49	0.10	
1.0 mgl^{-1}	4.7	0.17	0.80	0.11	
5.0 mgl^{-1}	4.6	0.19	4.10	0.35	
control	6.7	0.20	0.002	0.0003	
0.1 mgl^{-1}	6.5	0.454	0.09	0.02	
0.5 mgl^{-1}	6.6	0.51	0.42	0.13	
1.0 mgl^{-1}	6.7	0.49	0.70	0.20	
5.0 mgl^{-1}	6.6	0.43	3.91	0.30	

The values are means of 2 experimental units, with 10 measurements in each.

analysed with the Kruskal Wallis multiple comparison test. The data for metal concentrations in the mayfly nymphs from different metal treatments were compared with Likelihood ratio tests. The Friedman test served as a non-parametric two-factor repeated measurements ANOVA to find differences in survival or display of escape behavior between the treatments, due to pH or metal concentration. After a significant Friedman test, post-hoc pairwise comparisons were made to indicate significant differences (Siegel & Castellan, 1988).

LC_{50}/EC_{50} values were obtained by using the Maximum Likelihood Estimate based on probit percent survival concentration-response relationships. The Maximum Likelihood Estimate was preferred to simple least square regression because it seems to be a more accurate method for scattered data (Weber, 1986).

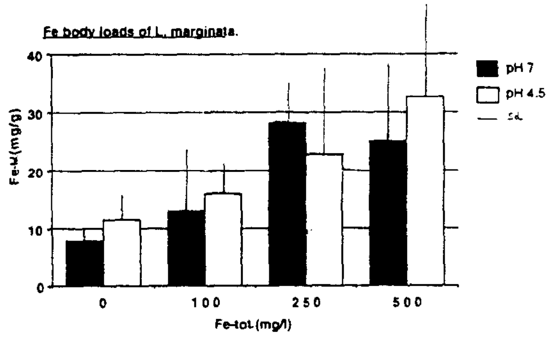

Fig. 1. Uptake of Fe (mg g^{-1} DW) by *Leptophlebia marginata* in relation to pH levels in the water.

Results

Chemical parameters

Between the daily renewals of the water and metal solutions in the aquaria, the Fe_{tot} concentrations were reduced by up to 10% at pH 4.5 and 20% at circumneutral pH and $\geq 90\%$ of the Fe_{tot} was present as Fe^{2+} at both pH levels (Table 2). The Pb concentrations decreased by 20% within 24 hours at both pH levels. At circumneutral pH, exposure to 500 mg Fe l^{-1} caused a decrease in pH down to pH 6.06, whereas in the corresponding acidified Fe-treatment, the mean pH values were only slightly lower than in the controls (Table 2). However, neither in the Fe-experiments nor in the Pb experiments, the differences in pH between the various metal exposures were significant ($p > 0.05$, Kruskal Wallis).

Whole body concentrations of the metals

The uptake of Fe by the mayflies increased in a dose dependent way (Fig. 1). No significant differences in Fe-uptake due to pH were found ($p > 0.05$, Likelihood ratio test).

At both pH levels, the uptake of Pb by the mayflies was dose dependent ($p < 0.001$; Likelihood ratio test) (Fig. 2). Uptake of Pb was significantly lower at acid compared to circumneutral conditions ($p < 0.001$, Likelihood ratio test).

Table 3. Post-hoc pairwise comparisons of the response-time curves (*:$p > 0.05$).

Pb

Differences between the concentration levels.

	Survival		Escape behavior	
	pH 7	pH 4.5	pH 7	pH 4.5
control vs. 0.1 mg Pb l^{-1}	n.s.	*	n.s.	n.s.
control vs. 0.5 mg Pb l^{-1}	n.s.	*	n.s.	*
control vs. 1.0 mg Pb l^{-1}	*	*	*	*
control vs. 5.0 mg Pb l^{-1}	*	*	*	*
0.1 vs. 0.5 mg Pb l^{-1}	n.s.	*	n.s.	*
0.1 vs 1.0 mg Pb l^{-1}	*	*	*	*
0.1 vs. 5.0 mg Pg l^{-1}	*	*	*	*
0.5 vs. 1.0 mg Pb l^{-1}	*	*	*	n.s.
0.5 vs. 5.0 mg Pb l^{-1}	*	*	*	*
1.0 vs. 5.0 mg Pb l^{-1}	*	n.s.	n.s.	*

Differences between pH 7 and 4.5 at various concentration levels.

	Survival	Escape behavior
controls	n.s.	*
0.1 mg Pb l^{-1}	n.s.	n.s.
0.5 mg Pb l^{-1}	*	*
1.0 mg Pb l^{-1}	*	n.s.
5.0 mg Pb l^{-1}	*	*

Fe

Differences between the concentration levels.

	Survival		Escape behavior	
	pH 7	pH 4.5	pH 7	pH 4.5
control vs. 10 mg Fe l^{-1}	n.s.	n.s.	n.s.	n.s.
control vs. 50 mg Fe l^{-1}	n.s.	n.s.	*	n.s.
control vs. 100 mg Fe l^{-1}	*	*	*	*
control vs. 250 mg Fe l^{-1}	*	*	*	*
control vs. 500 mg Fe l^{-1}	*	*	*	*
10 vs. 50 mg Fe l^{-1}	n.s.	n.s.	*	n.s.
10 vs. 100 mg Fe l^{-1}	*	*	*	*
10 vs. 250 mg Fe l^{-1}	*	*	*	*
10 vs. 500 mg Fe l^{-1}	*	*	*	*
50 vs. 100 mg Fe l^{-1}	*	*	n.s.	*
50 vs. 250 mg Fe l^{-1}	*	*	*	*
50 vs. 500 mg Fe l^{-1}	*	*	*	*
100 vs. 250 mg Fe l^{-1}	*	*	*	*
100 vs. 500 mg Fe l^{-1}	*	*	*	*
250 vs. 500 mg Fe l^{-1}	*	*	n.s.	n.s.

Table 3. Continued.

Differences between pH 7 and 4.5 at various concentration levels.

	Survival	Escape behavior
controls	*	n.s.
10 mg Fe l^{-1}	n.s.	n.s.
50 mg Fe l^{-1}	*	*
100 mg Fe l^{-1}	*	n.s.
250 mg Fe l^{-1}	n.s.	n.s.
500 mg Fe l^{-1}	n.s.	n.s.

Survival

Fe

At both pH levels, survival of Fe-exposed mayflies decreased significantly in a dose-dependent way at ≥ 50 mg Fe l^{-1} (Fig. 3) (pH 7: $p = 0.003$; pH 4.5: 0.002 Friedman). Post-hoc pairwise comparisons between pH 4.5 and pH 7 revealed a significant better survival at pH 7 compared to pH 4.5 for Fe-concentrations of 50 and 100 mg l^{-1} (Table 3). The 96 h-LC$_{50}$ values for *L. marginata* nymphs were 106.3 (95% C.I.: 111.3; 101.5) for pH 7 and 89.5 (95% C.I.: 101.1; 78.5) for pH 4.5. After 120 h exposure, the pH-dependent difference in survival of Fe-exposed mayflies became more clear as the LC$_{50}$ value at pH 4.5 decreased to 65.3 (95% C.I. 65.7; 64.9), whilst that at pH 7 was the same as after 96 h. The differences between the LC$_{50}$ values at pH 4.5 and 7 were, however, not significant ($p > 0.05$, Likelihood ratio test).

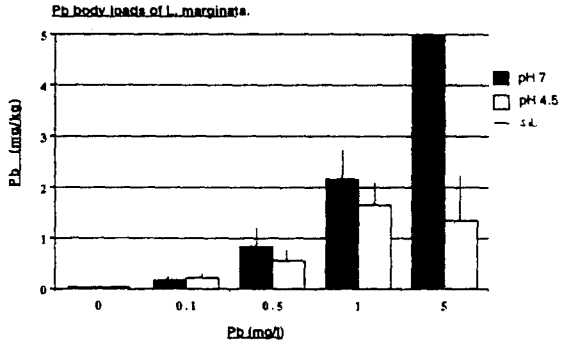

Fig. 2. Uptake of Pb (mg kg^{-1} DW) by *Leptophlebia marginata* in relation to pH levels of the water.

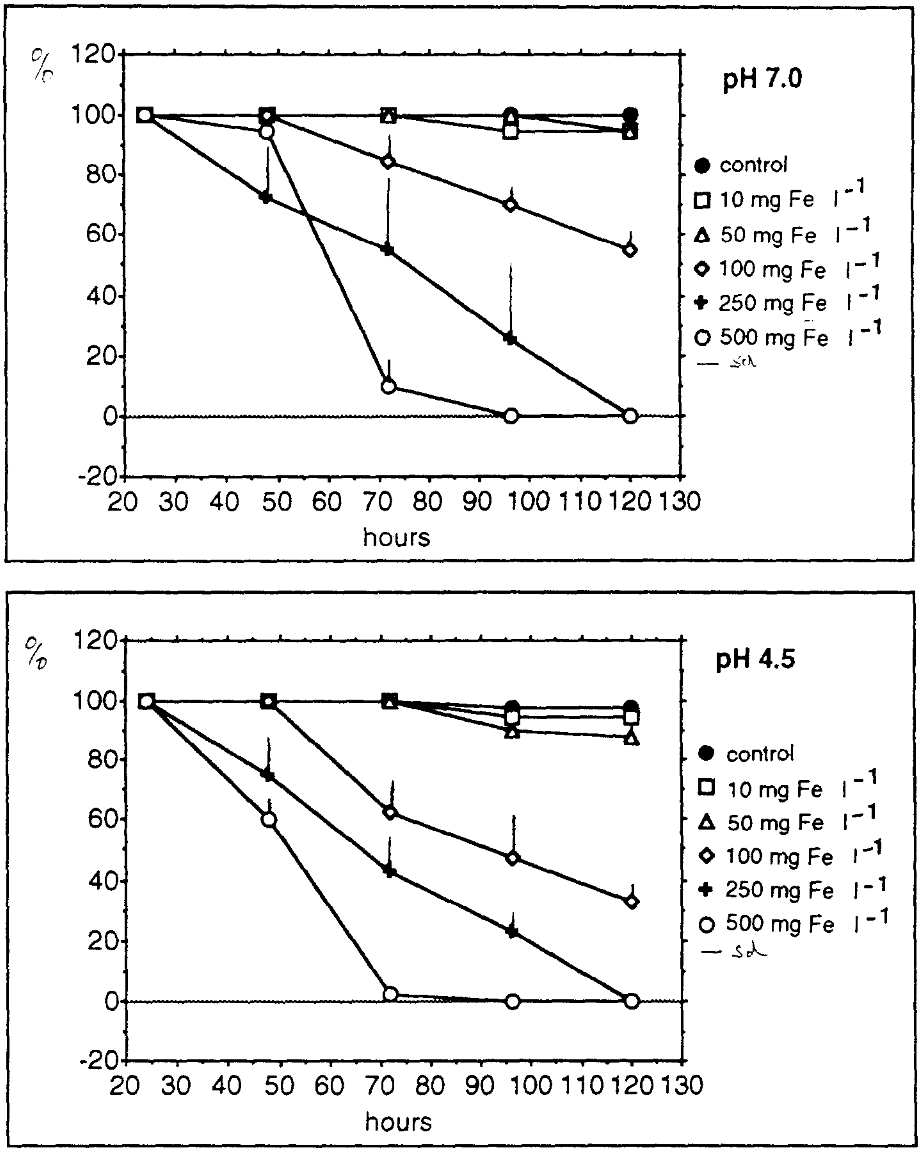

Fig. 3. Survival of *L. marginata* exposed to different Fe concentrations and pH in the water.

Pb

At both pH levels, survival of *L. marginata* decreased with increasing Pb-concentration in the water (Fig. 4) as significant differences between the Pb-concentrations were found (pH 4.5: $p = 0.004$; pH 7: $p = 0.03$; Friedman). A detailed pairwise analysis showed significant differences in survival due to pH in the treatments ≥ 0.5 mg l^{-1} (Table 3).

The 96 h-LC_{50} values for Pb were > 5 mg Pb l^{-1} at pH 7 and 1.09 mg Pb l^{-1} (95% C.I: 133.2; 0.4) at pH 4.5.

Escape behavior

Fe

The decrease in mayflies that displayed escape behavior after prodding was correlated with increasing exposure time and Fe concentration

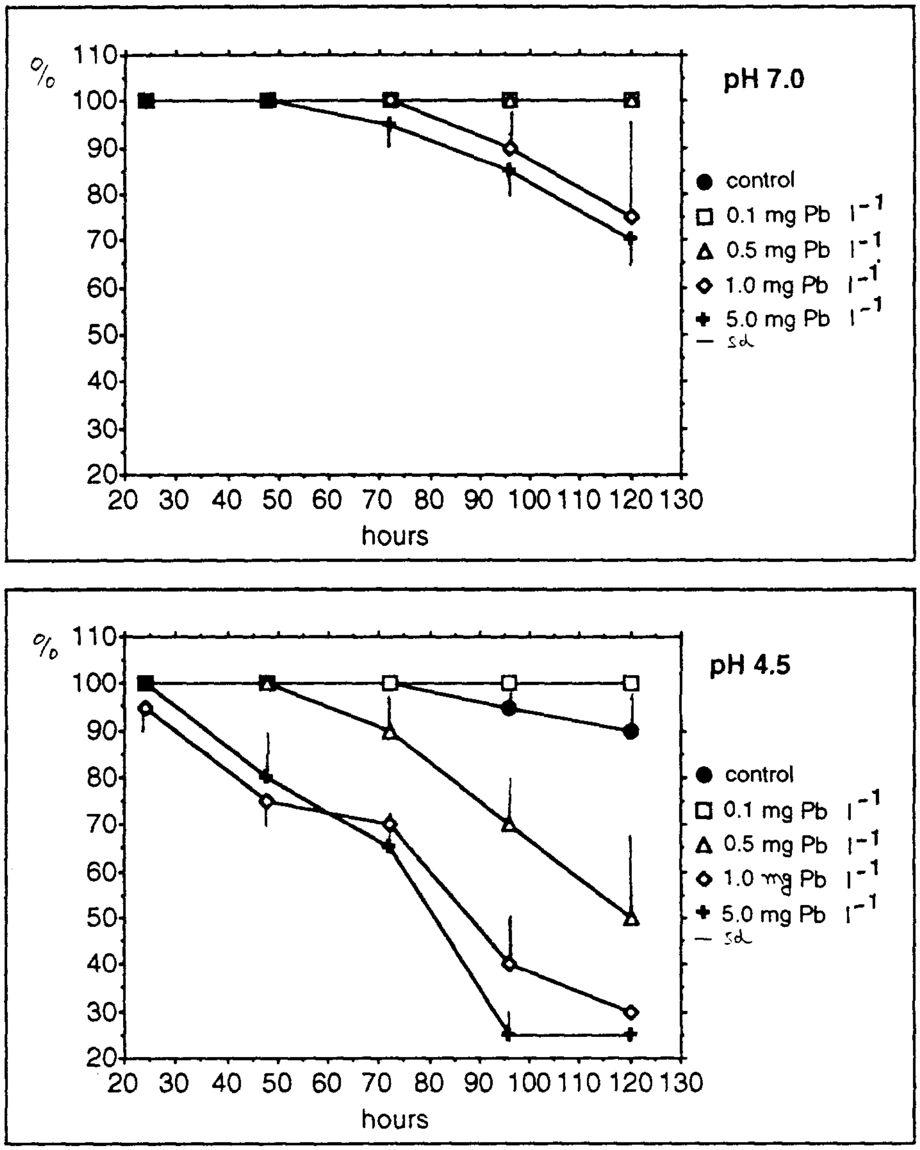

Fig. 4. Survival of *L. marginata* exposed to different Pb concentrations and pH in the water.

(pH 7: $p = 0.0004$; pH 4.5: $p = 0.0004$; Friedman) (Fig. 7). For example, significantly more mayflies in the controls displayed escape behavior than those exposed to concentrations ≥ 50 mg Fe l^{-1} at pH 7 or ≥ 100 mg Fe l^{-1} at pH 4.5 ($p < 0.05$, post-hoc pairwise comparisons) (Table 3). No significant differences due to pH were found, except at 50 mg Fe l^{-1}, where more mayflies at pH 7 expressed escape reactions than at pH 4.5 ($p < 0.05$). The EC_{50} values for *L. marginata* were 70.0 mg Fe l^{-1} at circumneutral pH (95% C.I.: 103.9; 47.1) after 96 and 120 h of exposure. At pH 4.5, the corresponding EC_{50} values were 63.9 (95% C.I.: 80.2; 51.0) after 96 h and 40.2 (95% C.I.: 66.7; 24.2) after 120 h of exposure. The EC_{50} value for pH 4.5 did not differ significantly from that at pH 7 ($p > 0.05$, Likelihood ratio test).

Pb

At pH 7, $\geq 65\%$ of the mayfly nymphs exposed to 0.1 to 5 mg Pb l^{-1} showed normal escape behavior until the end of the experiments (Fig. 8).

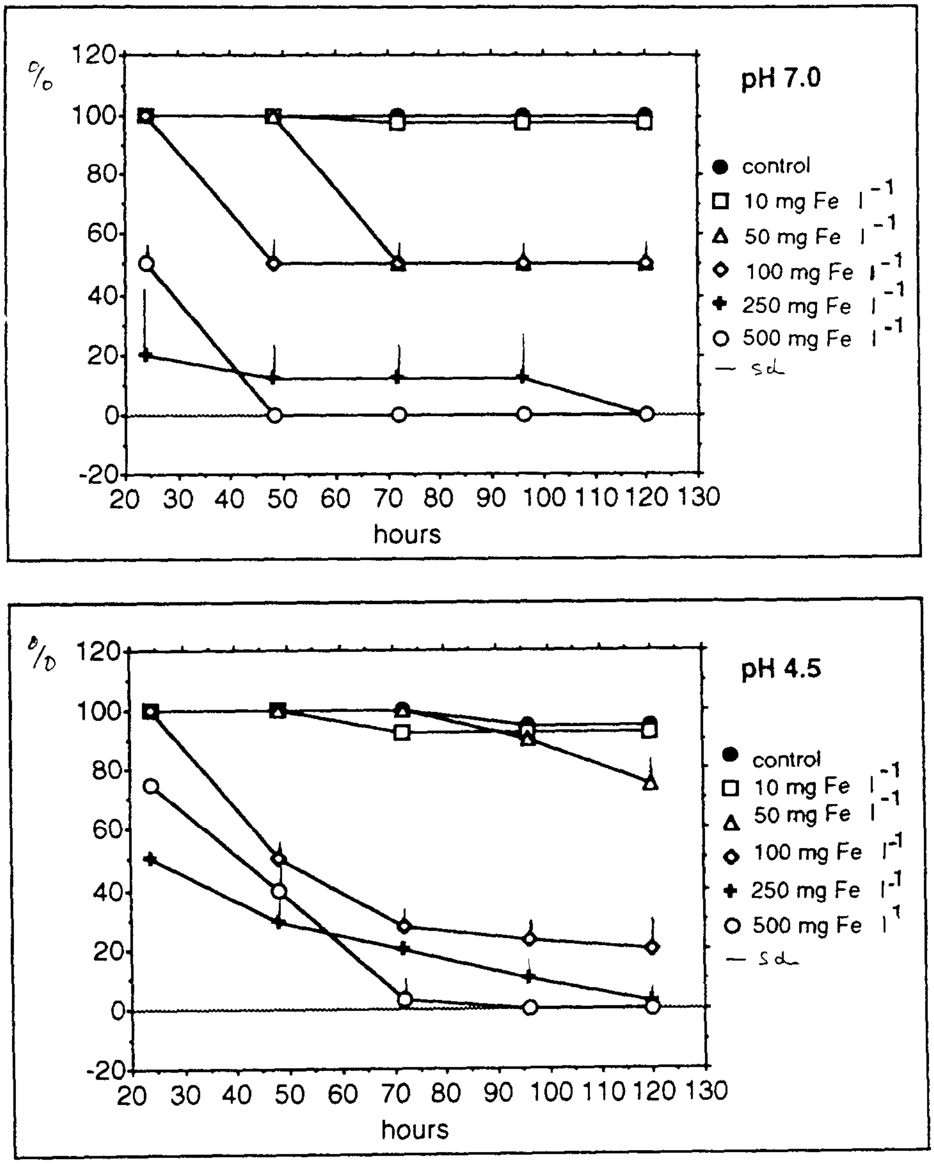

Fig. 5. Escape behavior of *L. marginata* exposed to different Fe concentrations and pH in the water.

At pH 4.5, however, lead concentrations of ≥ 0.5 mg l^{-1} affected drastically the escape behavior of the mayflies. Significant differences in behavior between the concentration levels tested were found at pH 4.5 ($p = 0.001$) and at pH 7 ($p = 0.016$; Friedman). Pairwise comparison tests revealed significant differences caused by pH at 0.1, 0.5 and 5.0 mg Pb l^{-1}. EC_{50} calculations for the Pb-experiments were not appropriate due to non-linearity of the probit response function.

Discussion

Chemical parameters

In the Fe-experiments, changes in speciation due to oxidation of Fe^{2+} to Fe^{3+} occurred at both pH levels. Oxidation processes resulted in a decrease of the pH, which was most obvious at circumneutral pH. Kirk *et al.* (1990) found that the oxidation of Fe^{2+} to Fe^{3+} caused a decrease in pH of up to 2 units in soils. At circumneutral pH and Fe concentrations ≥ 250 mg Fe l^{-1}, Fe-hydroxides coprecipitated with organic matter on the bottom of the aquaria, followed by clearence of the water. Due to daily renewal of the water, these processes could be minimized. Fe-speciation in artificially acidified freshwaters was found to be mainly regulated by pH, organic matter and oxygen with chemical, photochemical and microbial processes being involved (McKnight & Bencala, 1990).

The concentrations of Pb in the water decreased by 20% within a few hours after addition. Vighi (1981) found that the actual Pb values were lower than the calculated ones in a flow through system with artificial water and $Pb(NO_3)_2$. This may be due to fast coprecipitation of Pb with humic matter. Wren and Stephenson (1991) reported that the bioavailability of Pb may be reduced by organic material and Fe/Mn-hydroxides.

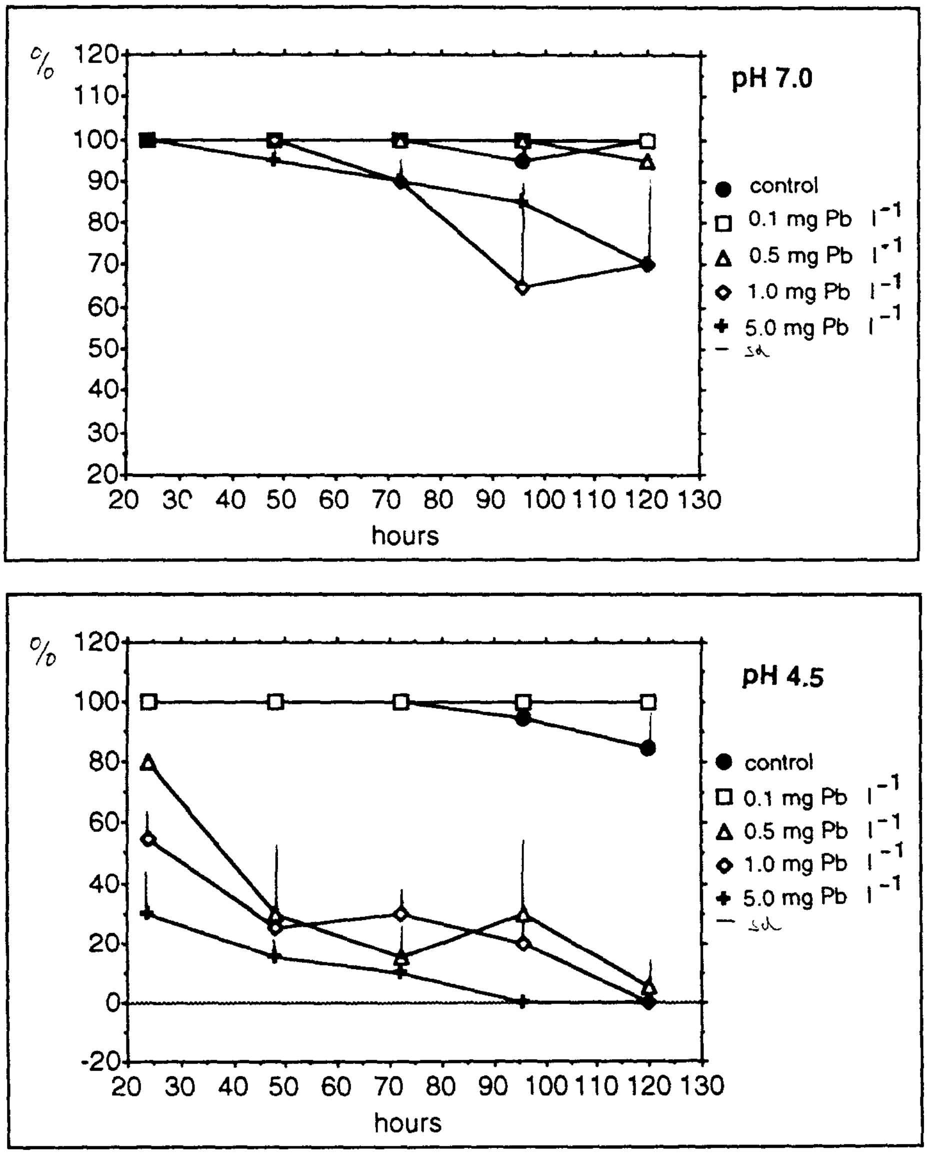

Fig. 6. Escape behavior of *L. marginata* exposed to different Pb concentrations and pH in the water.

Whole body metal loads

Fe

At both pH levels, Fe-concentrations in the insects increased with the exposure concentrations. Also the variations in Fe-concentrations between various individuals increased in that direction. This may be due to Fe-hydroxide precipitations on the mayflies' body surface, which may not have been removed by rinsing the animals in water. No pH-dependent differences in the Fe-body-loads were found, most likely due to the short exposure time. This agrees with a long-term exposure of *L. marginata* to Fe-concentrations up to 50 mg l^{-1}, in water from the same stream, where the insects did not take up significant amounts of iron during the first two weeks of exposure (Gerhardt, 1992a). Low accumulation factors for Fe in aquatic insects have also been reported by Hare (1992). This may be due to the fact that Fe-concentrations can be regulated in insects (Locke & Nichol, 1992).

Pb

At pH 7 and concentrations ≥ 1 mg l^{-1}, the mayflies contained significantly more Pb than at pH 4.5. Pb-body-loads increased with exposure concentrations in the water, probably due to precipitation of Pb together with organic matter on the body surface of the mayflies. Adsorption processes may be responsible for the 'uptake' of Pb within 5 days of exposure as they occur faster than metal uptake in the organism and they are elevated at neutral pH compared to low pH. Surface adsorption can account for up to 75% of the Pb-body burden of chironomids (Krantzberg & Stokes, 1988) and the mayfly *Hexagenia rigida* (Hare *et al.*, 1991).

Survival

Fe

A comparison of the LC_{50} values for Fe reported for different invertebrate species reveales great variation. For example, 50% of *Asellus aquaticus* tolerated 200–400 mg Fe^{2+} l^{-1} for 50 h (Maltby *et al.*, 1987) and *Daphnia magna* tolerated 152 mg Fe^{2+} l^{-1} for 16 h (Anderson, 1944), whilst other invertebrates were less tolerant. *Gammarus roeseli* had a LC_{50} (108 h) of 20 mg Fe^{2+} l^{-1}, *Asellus aquaticus* had a LC_{50} (62 h) of 3 mg Fe^{2+} l^{-1} (Walter, 1966). *Ephemera vulgata* was most sensitive to Fe^{2+} (LC50 (100 h) 3 mg Fe^{2+} l^{-1}) (Walter, 1966). In conclusion, *L. marginata* with a LC_{50} (120 h) of 65–106 mg Fe l^{-1} has to be considered as tolerant to Fe-exposure.

At intermediate Fe-concentrations (50 and 100 mg Fe l^{-1}) survival of *L. marginata* was lower at pH 4.5 than at circumneutral pH. This may be due to a synergistic effect of Fe and low pH. Dave (1985) reported that Fe-toxicity to embryolarvae of fish at pH 4 was 32 times higher than at pH 7–9 within a range of 0 to 10 mg Fe l^{-1}. At low pH, mechanisms such as photoreduction of Fe^{3+} to Fe^{2+} or the destabilisation of metastable Fe-complexes should lead to increased Fe-toxicity at low pH (Shaw *et al.*, 1992). Maltby *et al.* (1987) found that mortality of *A. aquaticus* caused by Fe^{2+} decreased if organic buffers were added to the water (pH 6.5) than at pH 4.5 without buffers. Complexation of Fe^{2+} with buffers may reduce the toxicity of iron.

Pb

A survey of the LC_{50} values for Pb reported in the literature reveals great variation between different test organisms. Molluscs (LC_{50} (96 h): *Pisidium casertanum*: 45.8–66.2 mg Pb l^{-1}; *Amnicola limosa*: 7–12 mg Pb l^{-1}) seem to tolerate more Pb than crustaceans (LC_{50} (96 h): *Hyalella azteca*: 0.01 mg Pb l^{-1}, *Daphnia magna*: 0.45 mg Pb l^{-1}) or insects (LC_{50} (96 h): *Chironomus tentans*: 1.89 mg Pb l^{-1}) (Mackie, 1986; Oladimeji & Offem, 1989; Jørgensen *et al.*, 1991). The Pb-LC_{50} values for *L. marginata* were higher than those previously reported for chironomids (1.09– >5 mg l^{-1}).

The toxicity of Pb was also enhanced at pH 4.5 compared to that at pH 7, probably due to pH-induced changes in Pb-speciation, combined with a release of Pb from organic ligands. In general, Pb is more toxic to algae and invertebrates at low than at neutral pH (Campbell & Stokes, 1985;

Wren & Stephenson, 1991). Mackie (1986) found for some freshwater invertebrates, that Pb toxicity increased with decreasing pH (pH 6.0–3.5) except for *Enallagma* sp. and *Hyalella azteca*, where mortality was independent of pH.

In comparison to Fe, Pb was about 80 times more toxic to *L. marginata* at pH 4.5. This may be due to the fact that Fe is an essential metal, its uptake through the gut mucosa cells being regulated by transferrin and its transport through the gut cells being regulated by ferritin (Locke & Nichol, 1992), whereas Pb is not essential and may therefore cause toxic effects at smaller doses.

Escape behavior

Fe

Usually the mayflies escaped from prodding by swimming to the nearest available shelter. Metal-stressed animals (≥ 50 mg Fe l^{-1}) lost this ability and remained inactive irrespective of the pH conditions in the water. Chronic Fe-stress also caused decreased activity and food consumption in *L. marginata* at pH 4.5, whereas no effects were found at pH 7 (Gerhardt, 1992a). There is limited knowledge concerning the influence of Fe on the behavior of aquatic organisms. However, also increased activity due to Fe-exposure was observed. For example, Fe-hydroxide suspensions at concentrations between 4.25 and 6.5 mg Fe l^{-1}, the salmonid *Oncorhynchus kisutch* showed avoidance behavior (Updegraff & Sykora, 1976; Beitinger, 1990).

Pb

The escape response of the mayflies was reduced by Pb-exposure especially at pH 4.5. Just before death, the nymphs no longer hid under the stone. This may indicate a disturbance of the negative phototactic behavior of the mayflies, which is a typical behavior and was also reported for the closely related species *L. vespertina* (Kjellberg, 1972).

Exposure to Pb has been shown to cause behavioral changes, such as spiral movements, in fish (Oladimeij & Offem, 1989), increased variability in the spontaneous locomotory activity of frog tadpoles (Steele *et al.*, 1989), and decreased reaction distance, combined with increased handling time of prey by the zebrafish (Nyman, 1981). Moreover, Pb has been found to reduce locomotion of earthworms (Beeby, 1991).

Both, Fe and Pb caused a decrease in escape behavior of the mayfly *L. marginata*. However, different mechanisms may be responsible. Fe seems to affect the animals by forming crusts on the body surface and aquarium bottom, which may prevent the animals' free movements and oxygen/ion uptake. As Fe was not significantly taken up by the organisms, cytotoxic effects by Fe^{2+} ions cannot be responsible for the observed effects on survival and behavior. Pb, however, was taken up by the mayflies and the observed effects seemed to be caused by Pb-toxicity within the organisms. Moreover, pH-dependent changes in Pb-speciation caused differences in Pb-toxicity. Changes in behavior occurred at somewhat lower metal concentrations than mortality. A lost in escape behavior and negative phototaxis can have severe ecological consequences for the individual, e.g. increased vulnerability to predation. This may result in decreased abundance of the species in metal polluted freshwaters.

Zusammenfassung

Die akute Toxizität von Fe und Pb wurde für die Eintagsfliegenlarve *Leptophlebia marginata* in versauertem (pH 4.5) und circumneutralem (pH 6–7) Bachwasser bestimmt.

In allen Experimenten war Fe^{2+} die dominante Ionenspecies. Bei neutralem pH und Konzentrationen von ≥ 250 mg Fe l^{-1} fiel jedoch vermehrt Fe-hydroxid aus. Auch die Konzentration von Pb sank im Wasser bei beiden pH Werten innerhalb von 24 Stunden.

Die Metall-Konzentrationen in den Tieren stiegen proportional zu den Konzentrationen im Wasser. Unter versauerten Bedingungen enthielten die Insektenlarven signifikant weniger Pb als unter neutralen Verhältnissen.

Beide Metalle reduzierten das Überleben der

Eintagsfliegen signifikant mehr unter versauerten als unter neutralen Bedingungen. Pb war 80 mal mehr toxisch als Fe. Subakute Effekte der Metalle waren der Verlust der Fluchtreaktion und der negativen Phototaxis, besonders in den Pb-Experimenten bei pH 4.5.

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