ALUMINIUM ACCUMULATION AND DISTRIBUTION IN THE FRESHWATER CLAMS (UNIONIDAE)

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Abstract—1. Freshwater unionids (*Anodonta anatina* L. and *Unio pictorum* L.) were exposed to aluminium (300 and 900 μg/l, nominal) in continuous (3 weeks) and fluctuating (24 days) acid exposures.

2. In addition, accumulation was monitored for 2 weeks under semi-static acid (pH 4–5) and circumneutral (pH 6.6–8.3) conditions in hard (35 mg Ca/l) and soft water (3.5 mg Ca/l).

3. In addition, a fluctuating exposure of 24 days, consisting of three intermittent pulses which combined low pH (4–5) and high Al (900 μg/l) concentration, was performed.

4. The various organs of *A. cygnea*, collected from a watershed with relatively high heavy metal concentrations, were analyzed for their Al and Cd concentrations.

5. The ultimate order of the Al and Cd concentration in these clams was identical: kidney $\geq$ midgut gland $\geq$ rest $\geq$ gill $\geq$ mantle.

6. During the 3 weeks of exposure, the Al concentration in the gills and kidney increased linearly, and saturation level was not reached.

7. The Al concentration in the calcium concretion material isolated from the gills was lower than that of the whole organ.

8. An elimination period of 12 weeks was needed to reach the background level of Al in the gills, whereas in the kidney the initial Al concentration was reached after 4 weeks of elimination.

9. In both species, the ambient pH had a significant effect on the Al accumulation in the gills, whereas the effect of the water hardness was only of minor importance.

10. Rapid elimination of the Al accumulated in the gills of *U. pictorum* during the episodic 3-day exposure was recorded.

INTRODUCTION

During the last few decades, the freshwater chemistry of lakes and rivers of poorly buffered watersheds has been frequently affected by sulphur dioxide emissions. The general water pH decreases or severe, episodic pH depressions occur. The decreasing water pH often leads to a rapid increase in the total metal concentration and in the amount of bioavailable forms of metal (Borg, 1986). Metals generally found in elevated concentrations are Cd, Cu, Pb, Zn and Al (Wright and Gjessing, 1976; Schindler *et al.*, 1980). The Al concentration in an acidified lake can reach a level of 500 μg/l (Hall and Likens, 1984), and concentration as high as 1500 μg/l has even been reported (Sharpe *et al.*, 1984).

The accumulation kinetics of Al, however, are still poorly understood. Aluminium is known to lead to Al accumulation in the gills, kidney and gastrointestinal tissues of *Salmo gairdneri* (Lee and Harvey, 1986; Buergel and Soltero, 1983). High Al concentrations have been measured in the gills of fishes killed by low pH in nature (Lee and Harvey, 1986). The sites of Al accumulation in the fish gills are respiratory and chloride cells (Youson and Neville, 1987). The pH at which Al is most toxic to fish is reported to be between 4.5

and 5.1 (Ramamoorthy, 1988). In *Daphnia magna*, by contrast, the optimum pH for Al accumulation was 6.5 (Havas, 1985), Al even decreased the H^+ toxicity at pH 4.5.

Both freshwater and marine molluscs can accumulate bivalent metals to high levels in their tissue (Salanki *et al.*, 1982; Elliot *et al.*, 1986; Hemelraad *et al.*, 1986a). In the acidified field situation, however, no significant bioaccumulation of Cd, Zn or Al was found in *Elliptio complanata* (Servos *et al.*, 1987). In earlier studies, a water pH of between 4.0 and 4.5 was found to cause ionic disturbances in unionid clams in the laboratory (Chang *et al.*, 1988; Pynnönen, 1990) as well as in the field (Malley *et al.*, 1988), but in no cases was the ionic imbalance severe enough to kill adult clams.

There is a lack of information concerning the Al accumulation kinetics in aquatic animals. This study was performed in order to extend our knowledge about the Al kinetics and toxicity in freshwater clams. Differences in species sensitivity to pH and Al have been found earlier (Palmer *et al.*, 1988; Hollet *et al.*, 1986), therefore the accumulation of Al was followed in two different unionid species. Since freshwater unionids inhabit waters with a wide range of hardness (Mackie and Flippance, 1983) and this factor is one of the most important ones in regulating the accumulation and toxicity of heavy metals in aquatic animals (Rand and Petrocelli, 1985), experiments were performed in both hard and soft water. Because a considerable amount of data is available about the

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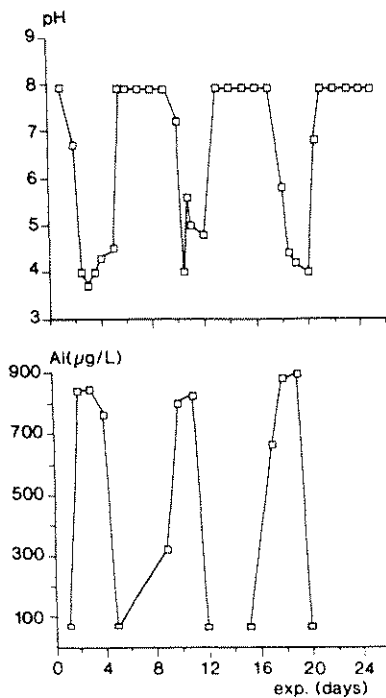
Fig. 2. Water pH and Al concentrations during the intermittent Al-rich pulses (fluctuating exposure). The detection limit of the flame-AAS for Al was 70 $\mu g/l$. The chemical composition of the exposure water is given in Table 1.

Fluctuating exposure

In order to simulate the spring snowmelt with acid run-offs, a group of 24 *U. pictorum* were exposed to a fluctuating pH (from 8 to 4) and to Al concentrations (from ≤ 70 to 900 $\mu g/l$) (Fig. 2). Cu-free tapwater (compositions as given in Table 1) was pumped, at a continuous rate of 7.2 l/hr, into the control (10 clams) and experimental aquaria (24 animals), both having a volume of 100 l. During a period of 3 days an acidified Al solution was pumped three times into the experimental aquarium to achieve gradually a pH between 4 and 5 and a nominal Al concentration of 900 $\mu g/l$ (see Fig. 2). After each exposure period the pump dosing the acid Al solution was turned off and the pH was left to normalize gradually. Before the next exposure period, clams were allowed to recover for 5 days in Al-free (≤ 70 $\mu g/l$), circum-neutral pH 7–8) water. It took about 12 hr to reach the circumneutral pH and the background level of Al.

Sampling

After 1, 2 and 3 weeks from the onset of the exposure, as well as after 4 and 12 weeks of elimination in the flowing system, 6 animals were dissected for the analysis. After 2 weeks of exposure, all the animals from the semi-static (4 *A. anatina* and 4 *U. pictorum* from each experimental arrangement) system were sampled. Animals acclimated for 3 weeks in the aquarium were sampled as controls. In the fluctuating exposure controls ($N = 6$) were sampled before the onset of the exposure and simultaneously (C-II, $N = 6$) with the last experimental group. After each acid, Al-rich period of 3 days, as well as after each recovery period of 5 days, 6 animals were sampled for the analysis.

Gills, labial palps, kidney, mantle, midgut gland (hepatopancreas), adductors and foot muscle, and the remainder (rest) from the specimens of *U. pictorum* exposed for 3 weeks to 300 μg Al/l were dissected and lyophilized. From the animals exposed for 1 and 2 weeks to 300 μg Al/l, and from the animals exposed in the semi-static conditions, only gills

and kidney were sampled. The gill demibranches from one side were used for the Al analysis, and demibranches from the opposite side were used for the isolation of the calcium concretions. The calcium concretions were isolated from lyophilized and weighed gills with 1 N NaOH according to the method described earlier in Pynnönen *et al.* (1987). If glochidia or eggs were found in the outer demibranches of the gills, only the inner demibranches were used for the analysis.

Chemical analysis

Animals ($N = 4$) collected from the Nieuwersluis area were analyzed for their element composition using an induced coupled plasma atomic emission spectrophotometry (ICP–AES). Aluminium from the decomposed shells, tissues and calcium concretions of the exposed animals was measured by AAS using a GTA-96 graphite tube atomizer (Varian). A nitrous oxide–acetylene flame was used for the water samples.

The analyses were based on lyophilized tissues and calcium concretions decomposed by concentrated HNO_3 according to the method described in detail earlier in Hemelraad *et al.* (1986a). The shell valve was decomposed, without heating, in 80–100 ml concentrated HNO_3. The periostracum of the shell did not dissolve in HNO_3 and it was removed before the analyses. A digestion procedure with HNO_3 was chosen, since according to Cedergren and French (1987) this method minimizes the possible losses of the organic-bound Al and allows larger samples to be employed, which levels out errors resulting from an uneven distribution of Al.

Statistical analysis

Data from the semi-static exposures were statistically evaluated for the significances of differences in Al accumulation by one-way analysis of variance (ANOVA). A probability limit of $P \leq 0.05$ was considered as significant.

RESULTS

In the *A. cygnea* collected from the Nieuwersluis area, the order of Al concentrations in the different organs was identical to the order of Cd. Kidney had the highest concentration (Al 266 $\mu g/g$, Cd 35 $\mu g/g$), and mantle the lowest (Al 24 $\mu g/g$, Cd 4 $\mu g/g$) (Table 3). Differences were found between the rate of Al accumulation and the rate of Cd accumulation measured earlier in *U. pictorum* (Pynnönen, submitted, see Table 4). In both cases gills were the first target organs for accumulation and had the highest metal concentrations. Kidney and midgut gland accumulated Cd more effectively than Al. After 3 weeks of Al exposure and after 4 weeks of Cd exposure, the Al concentration in the total soft parts was 76.3 ($\pm$ 19.3) $\mu g/g$ and the Cd concentration 44.3 (± 4.9) $\mu g/g$, which yields the concentration factor of 250 for Al and 900 for Cd. The partitioning of these two metals in the soft part of *U. pictorum* is shown in Table 4.

Table 3. Cadmium and aluminium concentrations in the organs of *A. cygnea* collected from the Nieuwersluis area as measured by ICP–AES

Organ	Concentration ($\mu g/g$) $\pm$ SD	
	Al	Cd
Gill	34.46 $\pm$ 2.49	4.61 $\pm$ 0.99
Mantle	23.75 $\pm$ 7.27	3.97 $\pm$ 1.48
Midgut gland	109.46 $\pm$ 11.94	12.67 $\pm$ 1.64
Kidney	266.46 $\pm$ 36.39	34.60 $\pm$ 6.03
Rest	37.80 $\pm$ 6.74	5.63 $\pm$ 1.05

Mean of 4 clams $\pm$ SEM is given. Rest = the remaining soft parts.

Table 5. Probability values derived from two-way ANOVA for the Al accumulation in the gills and kidney of *U. pictorum* and *A. anatina*

| | A. anatina | | U. pictorum | |
	Gill	Kidney	Gill	Kidney
pH	**0.002**	0.102	**0.000**	0.288
Hardness	0.259	**0.000**	0.086	0.109
Interaction	**0.005**	0.583	0.096	0.155

P values are given for water pH (4–5 vs 7–8), hardness (35 vs 3.5 mg Ca/l) and the interaction of the two factors. Values for $P \leq 0.05$ are printed bold.
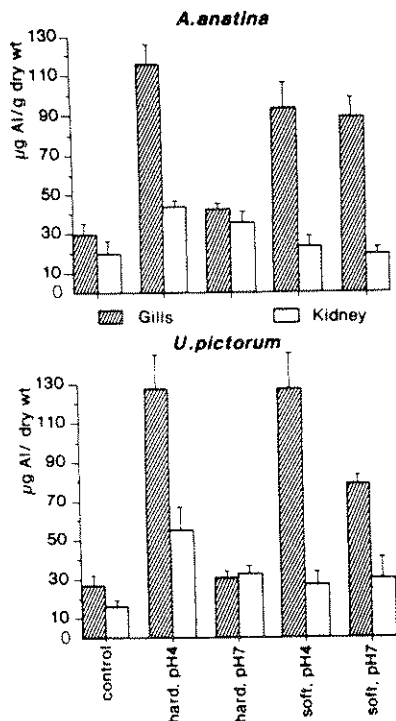

Fig. 6. Aluminium accumulation in the gills and kidney of *A. anatina* and *U. pictorum* exposed for 2 weeks to a nominal Al concentration of 300 μg/l in circumneutral (pH 6.6–8.3) and acid (pH 4–5) hard or soft water. The true Al concentrations are given in Table 2. The metal concentrations in the controls are measured from clams acclimated for 3 weeks in the aquarium. Mean of 4 individuals ± SEM. The statistical significances of the differences in the accumulation are given in Table 4.

Fig. 7. Al accumulation in the gills (A) and kidney (B) of *U. pictorum* during acid, intermittent pulses containing 800–900 μg Al/l. The true concentrations are given in Fig. 2. For the gills, mean of 6 clams ± SEM is given. For kidney, a pooled sample of 6 clams was measured. Acid 1, 2, 3 = concentrations measured directly after a 3-day pulse. Rec. 1, 2, 3 = concentrations measured after a recovery period of 5 days. Aluminium concentrations from the control animals were measured from the animals kept continuously in the flowing circumneutral water before (control I) and after (C-II) the exposure of 24 days.

the Al level in the kidney had reached the control level, but in the gills the Al level was still clearly above that of the controls. Not until 12 weeks of elimination was the Al concentration in the gills of the Al-exposed clams equal to the Al concentration in the gills of the unexposed clams.

In the unionid clams, the Al accumulation rate in the soft part was more affected by the pH of the exposure water than by its hardness (Table 5, Fig. 6). *A. anatina* was more sensitive than *U. pictorum* to the effect of water hardness on the Al accumulation. In circumneutral hard water, no significant Al accumulation was recorded in the gills of either of these clam species.

In fluctuating low pH and Al exposure, the water pH and the Al accumulation rate were highest during the second 3-day acid pulse (Figs 2 and 6). During the exposure to repetitive pulses of acid, Al-rich water,
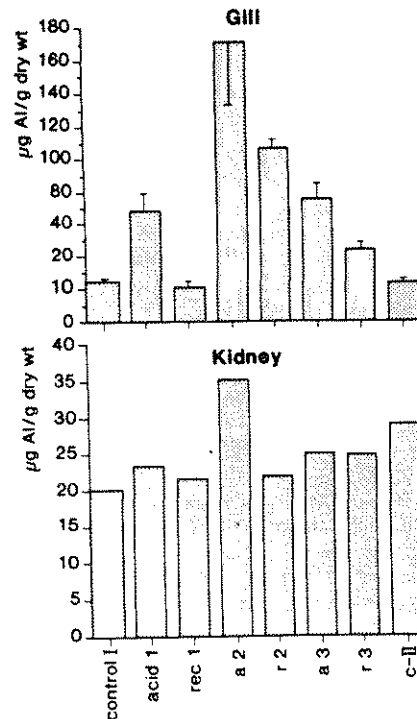
only the gill Al levels were significantly elevated. In the kidneys, no significant Al accumulation was found (Fig. 7), although elevated kidney concentrations were found at the same time as the high Al concentration in the gills. During the 5-day recovery period following the first 3-day acid pulse, all the accumulated Al was eliminated and the Al concentration in the gills returned to the control level. After the second and third pulse, the 5-day recovery period was not sufficient to clear the gills of aluminium.

Although the calcium concretions made up 24% of the total gill dry weight in *U. pictorum*, no more than 3.8% of the total amount of aluminium in the gills was found in the concretions at the end of the exposure (Fig. 5). No change in the amount of calcium concretion in the gills was seen during the continuous or the fluctuating exposures. The amounts of concretions in the gills are given in Pynnönen (1991). Aluminium concentration in the concretions increased linearly, the accumulation pattern following the one seen in the whole gill tissue.

DISCUSSION

In the clams collected from the Nieuwersluis area, Al partition in the soft parts was almost identical to the partition of Cd. This points to the similar uptake

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