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EFFECT OF SUB-LETHAL CONCENTRATIONS OF ALUMINIUM ON THE FILTRATION ACTIVITY OF THE FRESHWATER MUSSEL *ANODONTA CYGNEA* L. AT NEUTRAL pH*

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Significant amounts of aluminium (Al) are commonly present in rivers and lakes, largely in particulate form in neutral waters. Freshwater bivalves, as filter feeders are therefore exposed to both particulate and dissolved metal and are potentially vulnerable to Al.

The effect of Al on filtering behaviour of the freshwater mussel *Anodonta cygnea* L. was investigated during short (1 hour) and long-term (15 days) exposure to environmentally relevant concentrations (250 and 500 μg l^{-1}) at neutral pH. Water flow through the outflow siphon was monitored as an indicator of pumping capacity.

Short-term (1 hour) exposure to 500 μg l^{-1} added Al produced an irreversible decrease in the duration of filtering periods, presumably as an avoidance response to the toxicant. One-hour exposure 250 μg l^{-1} Al had no detectable effect. When mussels were exposed to 250 or 500 μg l^{-1} added Al for 15 days, siphon activity measured in days 11–15 of exposure was inhibited by 50% and 65%, respectively, compared to pre-exposure levels. Recovery occurred following transfer of mussels to uncontaminated water. Interaction between Al and freshwater bivalves at neutral pH may affect both the performance of the mussels and the chemical speciation of the metal in the natural environment.

Keywords: Aluminium – siphon activity – filtration – bivalve – *Anodonta cygnea* L.

INTRODUCTION

The toxicity of aluminium (Al) is now well recognised, and occurs even at low concentrations in plants [13] and fish [9]. Despite its insolubility at neutral pH [7] it has been shown that grazing organisms such as the fresh water snail *Lymnaea stagnalis* exposed to environmentally relevant concentrations of Al may accumulate significant quantities in their tissues under neutral pH conditions [8], probably by ingesting the metal as a colloidal hydroxy-polymer associated with the mucus biofilm [14]. Furthermore, Al exposure at neutral pH significantly affected the behaviour of this snail [4, 25].

* Dedicated to Professor György Ádám on the occasion of his 80th birthday.
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Fresh water bivalves are potentially exposed to high levels of Al in nature. As filter feeders they process large volumes of water and, in doing so, come into contact with both particulate and dissolved forms of the metal. Therefore, these molluscs might be expected to be more vulnerable to the effects of Al than many other invertebrates, and may also provide a route of entry of the metal into the food chain.

Behaviour provides a good indicator of sub-lethal metal toxicity, which may be more sensitive than physiological or biochemical measurements [5]. The extent of exposure of a bivalve to metals in water will depend on the duration of filter feeding activity. The present study examines filtering behaviour, specifically duration of siphon activity coupled with water flow intensity measurements, to assess the response of the ubiquitous freshwater bivalve *Anodonta cygnea* L. to Al at neutral pH. Short-term effects were investigated in order to assess the immediate impact of Al following contact with the chemoreceptive epithelial cells surrounding the siphon, which are known be sensitive to a variety of metals [18]. Experiments were also carried out to examine the long-term (15 days) effect of Al on siphon activity. The concentration dependency of the response to Al exposure was addressed by using two concentrations, both environmentally relevant; 250 µg l^{-1} Al is commonly found in unpolluted surface waters in UK whilst 500 µg l^{-1} has been measured in areas subjected to mine pollution [10].

MATERIALS AND METHODS

Animal maintenance and experimental conditions

Adult *A. cygnea* (shell length 9–11 cm) were collected from a fishpond close to Lake Balaton, Hungary (46 °90.22′ N, 18 °04.91′ E) and acclimated to laboratory conditions for two weeks prior to the experiment. For long-term exposure to Al, animals were immersed in 3 l aquaria placed in a 100 l exposure tank containing sand-filtered Lake Balaton water, which was continuously replenished via a pump from a mixing tank at a rate of 4 l h^{-1}, to provide a natural food supply. The calculated residence time of Al in the tanks was about 24 hours. Aluminium was added to the mixing tank as $Al(NO)_3\,9H_2O$ from a stock solution of 500 mg l^{-1} at acidic (1.7) pH, yielding a final tank concentration of 250 mg l^{-1} and 500 mg l^{-1} added Al, respectively. Near neutral pH (7–7.5) was maintained by adding HNO_3 (analytical grade). Water temperature was between 19 and 22 °C as the experiments were carried out at ambient temperature in the laboratory from October 1998 (250 µg l^{-1} Al exposure) and February 1999 (500 µg l^{-1} Al exposure). Mussels were subject to a natural dark: light regime (typically 8–10 hours dark: 14–16 hours light). Water Al concentrations were measured daily using inductively coupled plasma optical emission spectroscopy (ICPOES) [11]. Measurements were taken first from the water supplied in the laboratory from Lake Balaton (after passing through settling system and sand filter), and then following addition of Al and pH adjustment. Mean Al concentrations in the water column were 525.3 ± 96.7 ($n = 15$) and 241.3 ± 24.8 µg l^{-1} ($n = 15$), respectively, during the exposure periods of the two long-term experiments. Mean conductivities of the water were 640 µS ± 19 and 720 µS, respectively.

Short-term (1 hour) exposure to Al

Two groups of 5 animals were used in short-term experiments to investigate the effect of the two Al concentrations (250 and 500 µg l^{-1}) on siphon activity. These experiments involved a 1 hour control recording of siphon activity prior to addition of Al, followed by a 1 hour period of exposure to the metal and 1 hour wash-out (post-exposure) in untreated lake water. The monitoring device consisted of a small plastic "umbrella" placed at the opening of the analis siphon and linked to a transducer, amplifier and recording unit [26]. This device records both the total duration of siphon activity and the intensity of the water flow from the exhalant siphon, which is proportional to the degree of movement of the umbrella. The product of the active period length and the amplitude of the umbrella movement is proportional to the volume of water pumped [16] and is referred to as water flow index. One shell was fixed to a glass holder with glue in order to prevent movement disrupting the recording. The experiment was repeated in duplicate runs on the same 5 animals, allowing 10 days recovery in lake water between the two runs. Each 1 hour period was divided into three 20 min time frames for analysis.

Long-term exposure to Al

The siphon activity of another two groups of 5 animals was recorded using the same device over 45 days to assess the exposure-time-dependent effect of Al on filtration activity, again using added Al concentrations of 250 and 500 µg l^{-1}. The animals were kept in 3 l aquaria immersed in the 100 l experimental tank containing lake water and added Al in order to minimise the stress of handling when removed for the 1 hour recordings. Recording of siphon activity was only possible on mussels with open shells and with the siphon extended normally. Therefore three 5-day "time frames" were defined within each successive 15 days treatment, i.e. control, exposure and recovery. Within each time frame two 1-hour measurements were taken for each animal resulting in ten sets of recordings in each time frame. This experimental design made it possible to compare the 9 time periods as well as the 3 treatments using analysis of variance.

Statistical analysis

Statistical significance was tested by factorial analysis of variance. Specific differences between treatments were examined *a posteriori* using the S-N-K multiple range test [24]. Calculations were made using the statistical package SPSS.

RESULTS

Exposure to 250 and 500 µg l^{-1} added Al was confirmed to be sub-lethal since there were no deaths recorded during the 1 hour exposure experiment or during or following the 15 days exposure. During the pre-exposure period of the short-term experiment, duration of siphon activity and, in particular, water flow index were greater in animals that were subsequently exposed to 250 µg l^{-1} added Al compared to those exposed to 500 µg l^{-1} Al (Figs 1, 2). This was probably due to seasonal differences, with animals more active during October than February.

Exposure to 250 µg l^{-1} added Al for one hour had no effect on duration of siphon activity (Fig. 1A) or water flow index (Fig. 2A), either during or immediately fol-

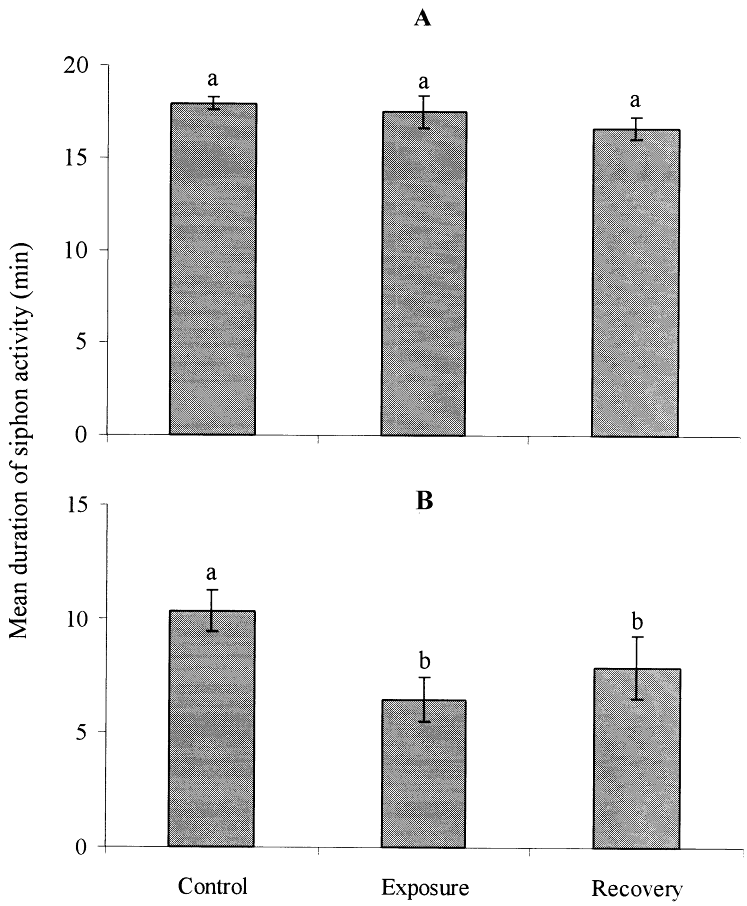

Fig. 1. Effect of short-term (1 hour) exposure to Al on mean duration of siphon activity of *A. cygnea* exposed to A) 250 and B) 500 µg l^{-1} added Al. Columns with the same letter are not significantly different according to S-N-K multiple range test ($p < 0.05$). Vertical bars represent standard error of the mean ($n = 30$)

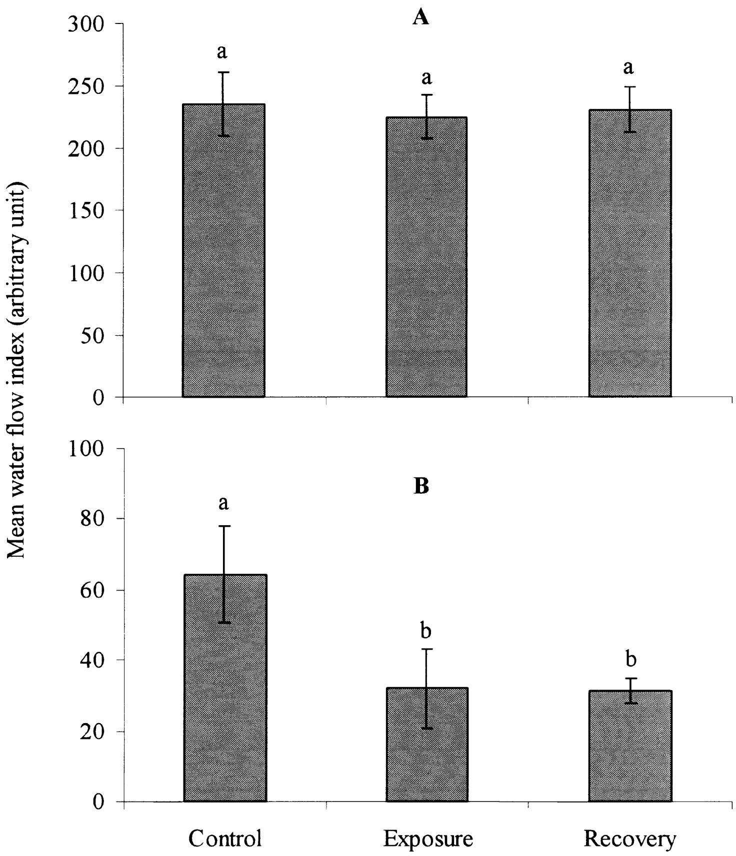

Fig. 2. Effect of short-term (1 hour) exposure to Al on mean water flow index of *A. cygnea* exposed to A) 250 and B) 500 μg l^{-1} added Al. Columns with the same letter are not significantly different according to S-N-K multiple range test ($p < 0.05$). Vertical bars represent standard error of the mean ($n = 30$)

lowing the exposure period. However, 500 μg l^{-1} added Al significantly depressed filtering activity. The mean duration of siphon activity was reduced by 37% ($p < 0.05$) and water flow index by 50% ($p < 0.05$), compared to the pre-exposure period (Figs 1B, 2B). During the 1 hour recovery period, neither parameter showed any significant recovery ($p > 0.05$).

In the long-term (15 days) exposure experiment, animals subsequently exposed to 250 μg l^{-1} added Al again showed greater siphon activity during the initial control period than those to be exposed to 500 μg l^{-1} added Al. Exposure to 250 μg l^{-1} added Al resulted in a significant ($p < 0.05$) increase in mean duration of siphon activity above pre-exposure levels for the first 5 days, followed by depression of activity to 50% of pre-exposure levels between days 6 and 15 (Fig. 3A). In 500 μg l^{-1} added Al, there was no change in siphon activity over the first 5 days of exposure. However, activity was significantly ($p < 0.05$) depressed from days 6 to 15; this effect was

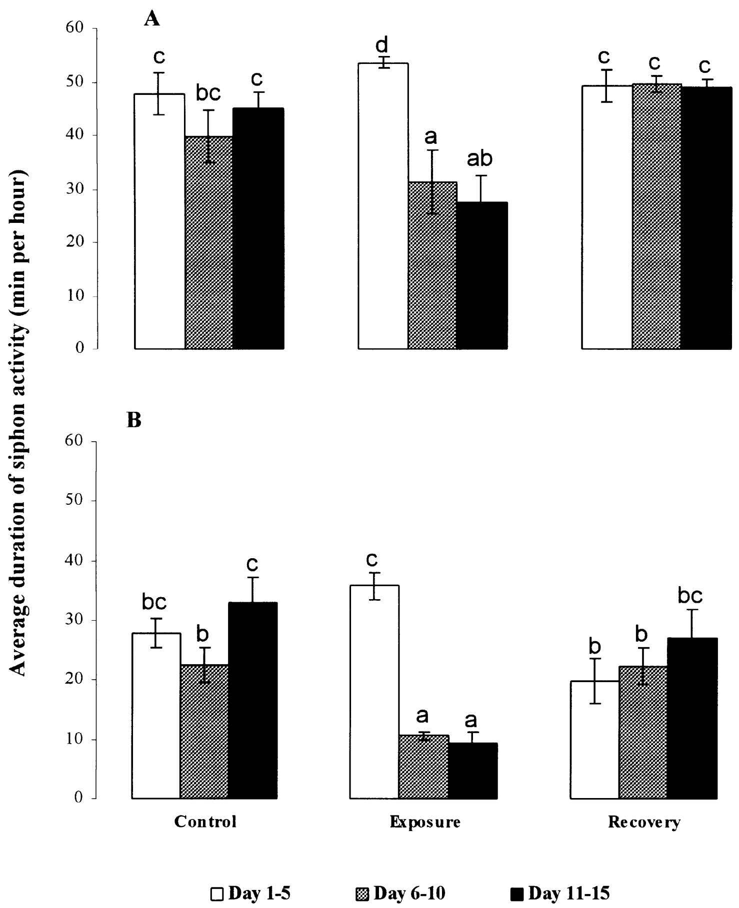

Fig. 3. Mean duration (±SEM, n = 24) of siphon activity of *A. cygnea* prior to (control), during (exposure) and following (recovery) long-term (15 days) exposure to A) 250 µg l^{-1} and B) 500 µg l^{-1} added Al, plotted for 5 day time frames. Columns with the same letter are not significantly different according to the S-N-K multiple range test ($p < 0.05$). Vertical bars represent standard error of the mean (n = 10)

greater than with the lower concentration of Al, with activity reduced to 35% of pre-exposure levels. Recovery was observed in both groups of animals during the initial 5 days following transfer to clean lake water (Fig. 3).

DISCUSSION

This investigation reveals sub-lethal toxicity of Al to the mussel *A. cygnea* at concentrations found in natural running and standing waters at neutral pH [10] when most of the Al would be predicted to be in its insoluble form [7]. Considerable amount of Al was maintained in the water column throughout the 15 day exposure probably due to the presence of high levels of organic chelators, common in shallow lakes such as Balaton. Levels of dissolved organic carbon (DOC) in Balaton water ranges between 20 and 40 mg l^{-1} [1] and high levels of Al in waters of high organic content have been reported previously [3, 6]. At neutral pH these Al-DOC interactions are likely to involve Al hydroxide, resulting in the formation of colloids kept in solution. This assumption is in accordance with studies showing that soluble polynuclear species (e.g. $[Al_{13}(OH)_{32}]^{7+}$) are dominant in solutions containing high Al concentrations (above 200 mM) between pH 5 and 8 [11]. In bivalves there is evidence for direct uptake of metals bound to colloids [22] and also for enhanced metal uptake by oysters with increasing DOC in the water column [10]. Uptake of Al by *A. cygnea* at neutral pH therefore, could involve either direct uptake of organically complexed metal, through ingestion, as observed in clams exposed to Cd, Cr and Zn [17] or by pinocytosis in the gills [27].

One-hour exposure to Al (500 μg l^{-1}) at neutral pH inhibited filtration activity of *A. cygnea*, as indicated by a reduction in duration of siphon activity and water flow. The siphon is known to be under central and peripheral neuronal control [12] and it has been suggested that the chemoreceptive epithelial cells activate local neural circuits leading to changes in the system responsible for water flow. Since the chemoreceptors on the surface of the siphon are in direct contact with Al in the water, the mussels are able to detect the metal and reduce exposure by reducing siphon activity. In the long-term experiment, the first 5 days of exposure to 250 g l^{-1} Al was accompanied by an increase in siphon activity. This may represent a period during which the mussel enhances filtration in order to flush out toxic material. Exposure to both concentrations then resulted in marked inhibition of filtration from day 6 of exposure. This suggests that continued exposure to the toxic metal leads to prolonged shell closure as an avoidance response. Alternatively, depression of filtering activity may be a secondary effect of stress-induced physiological changes such as decrease in respiration and feeding of mussels during shell closure [23]. Kontreczky and coworkers [16] observed similar changes in filtration activity of *A. cygnea* in response to deltamethrin exposure, suggesting that these changes are more likely to be general stress responses rather than specific to Al.

According to the toxicity sequence proposed by Nieborer and Richardson [21] and based on the classification of metal ions in relation to their binding preferences to biomolecules within an organism, Al with its high ionic index should be highly toxic. However, the toxicity of Al would be expected to be reduced by its strong tendency to form hydroxy complexes at physiological pH [19]. In contrast, the data presented here show that Al in its hydroxy form is toxic. Furthermore, our previous studies have demonstrated significant accumulation of Al by the tissues under similar exposure conditions [15].

The interaction between bivalves and Al in the water column could have environmental significance. Alterations in filtration activity in response to Al may affect the performance of the mussels, for example by reducing food uptake. Since freshwater bivalves can reach population densities capable of turning over the whole water body within days [20], changes in the filtration process are likely to affect the chemical speciation of Al in the environment.

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