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Occurrence of Aluminium in Chloride Cells of *Perla marginata* (Plecoptera) after Exposure to Low pH and Elevated Aluminum Concentration

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As a consequence of acid depositions on poorly buffered catchments underlain by hard rocks, aluminum is mobilized and transported from terrestrial systems to the aquatic environment. Loss of fishes has been related to low pH and elevated aluminum concentrations in surface waters which present a low ionic content, especially during acid stress such as snowmelt and heavy rainfalls (Leivestad and Muniz 1976). Among the causes of fish population decline in acid waters, aluminum is considered a toxic cofactor. Different studies have clearly shown that aluminum is accumulated in different organs such as kidneys, liver and gills (Karlsson-Norrgren 1986; Galle et al. 1990). Research on fish has demonstrated that aluminum may be toxic, but the toxicity is markedly influenced by the pH, organic compounds and calcium content of the water (Potts and McWilliams 1989).

Field surveys have shown clearly that macroinvertebrates are also affected by surface-water acidification (Sutcliffe and Carrick 1973). However, little is known about the possible effects of aluminum on aquatic invertebrates and, particularly, on aquatic insects exposed to acidic conditions. Hall et al. (1988) have shown that the whole-body concentration of aluminum decreases in blackflies and mayflies transplanted from neutral water to acid water. Similar results have been reported for *Daphnia* (Havas 1985) and chironomid (Young and Harvey 1988). On the contrary, Ormerod et al. (1988) demonstrated the absence of relationship between water pH and insect aluminum concentrations. When aluminum occurs in aquatic insects, it has been shown that it is primarly adsorbed on the external surface (e.g., cuticle) and/or accumulates in gut contents (Frick and Hermann 1989; Hall et al. 1988).

To our knowledge, the subcellular location as well as the toxicity of aluminum to acid-sensitive aquatic insects remains unclear and existing hypotheses are often based on research on fish. In this context the purpose of this study was to investigate the presence of aluminum at a subcellular level in the acid-sensitive species of stonefly, *Perla marginata*, after exposure to low pH and elevated aluminum concentrations.

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MATERIALS AND METHODS

Full-grown nymphs of the stonefly *P. marginata* (Plecoptera Setipalpia), a widespread species in neutral headwater streams from the Vosges mountains (North-Eastern France), was selected for this study as it is an acid-sensitive species and easy to collect and to maintain in well-oxygenated water. Nymphs were collected in a neutral stream of the Vosges Mountains.

Twenty nymphs were kept in synthetic, low conductivity (18.0 ± 2 µS cm^{-1}; $Ca^{2+} = 20 \pm 1.5$ µ*M* L^{-1}) acidic water (pH = 5.2 ± 0.2) with elevated aluminum concentrations (600 ± 11 µg L^{-1}). To provide a control test, twenty nymphs were also kept in synthetic non-acidic water (pH: 6.8 ± 0.2; conductivity = 60 ± 3 µS cm^{-1}; $Ca^{2+} = 80 \pm 2$ µ*M* L^{-1}) with low aluminium concentrations (30 µg ± 5 µg L^{-1}). The aluminum and calcium concentrations used in these exposure media were similar to those found, respectively, in acidified streams and in neutral streams from the Vosges mountains during snowmelt. During the experiment, the nymphs were fed daily with midges. In order to reduce the possibility of uncontrolled variations, the synthetic water was changed every day.

After twenty-one days of exposure, five nymphs of each exposure medium were randomly sampled, fixed in liquid propane cooled to -196°C, freeze-dried at a temperature below -80°C and embedded in low viscosity Spurr resin. The following organs or tissues were investigated: foregut, midgut, hindgut, Malpighian tubules, fat body, cuticle, chloride cells of the intersegmental membrane of the thoracic segment and gills. Two complementary methods were used to locate aluminum in semi-thin histological sections: Laser Microprobe Mass Spectrometry (LAMMS) and histochemical staining (HS). LAMMS has recently established a new concept in microprobe analysis with a high degree of sensitivity (in the ppm or to the sub-ppm range). We used a modified version of the LAMMA 500^{r} instrument (Leybold Herraus, Köln, FRG) equipped with a UV-microscope in order to visualize unstained biological samples. Principles, limitations and merits of this analytical method are reviewed by Kaufmann (1982) and Verbueken et al. (1985)

The aluminon method was used for histochemical demonstration of aluminum. This method provides a high degree of specificity for aluminum. Reaction with aurine-tricarboxylic acid gives a bright red color with aluminum salts. .

RESULTS AND DISCUSSION

Aluminum was detected only in nymphs exposed to high aluminum concentrations. As shown in Table 1, aluminum was not detected in digestive and excretory system epithelia, although these organs are known to accumulate metals (Sumi et al. 1991). The presence of aluminum in gut content and aluminum deposited on the external cuticle layer have been reported by different authors.

Table 1. Results of HS and LAMMS samplings of aluminium-exposed organs and tissues of *P. marginata* (presence of aluminum = +; absence = -)

Organs and tissues	H S	LAMMS
foregut	-	-
midgut	-	-
hindgut	-	-
Malpighian tubules	-	-
fat body	-	-
gut content	+	+
cuticle	-	+
chloride cells (gills and thorax)	+	+

Frick and Herrmann (1989) found that adsorbed aluminum on the cuticle represented the major part of whole-body metal burdens in *Heptagnia sulfurea* (Ephemeroptera). Consequently, most of the aluminum is lost at molting. As a result of molting and gut content excretion, the aluminum fraction should not be toxic to the organism itself but it may represent a significant source of metal to the next trophic level.

HS and LAMMS revealed aluminum in the chloride cells of the thoracic segment and gills. Spectra results of microanalysis of chloride cells are given in Figure 1a. Aluminum is associated with sulphur, phosphorus and chloride. Analysis of adjacent epithelial cells showed that they were free of aluminum (Figure 1b). It is important to note that with LAMMS aluminum was detected in all the chloride cells whereas HS had a positive reaction only with the apical portion (e.g., the cuticular disc), which is in contact with the external medium. As suggested by Martoja and Martoja (1973), the aluminon method does not permit the visualization of masked aluminum. This may be a consequence of a modification of aluminum form between the outer and the inner part of the cell.

Our results confirm that in aquatic insects exposed to high aqueous aluminum concentrations, significant amounts of aluminum are present in the gut content and/or are deposited externally. It is interesting to note the presence of aluminum in the chloride cells. The specificity of these well-defined and highly specialized cells, which are in direct and permanent contact with the external medium, has been previously established (Kapoor and Zachariah 1973; Wichard and Komnick 1973). Experiments with radioactive Cl have clearly demonstrated the essential role of chloride cells in ionic-regulation. Disturbance of ionic-regulation is a characteristic of fish and crayfish exposed to low mineralized water and elevated aluminum and proton concentrations (Potts and McWilliams 1989). Similarly, sensitive aquatic insects seem to display disturbance of ionic-regulation (Lechleitner et al. 1985; Herrmann 1987), but results are limited and based only on whole-body ion contents.

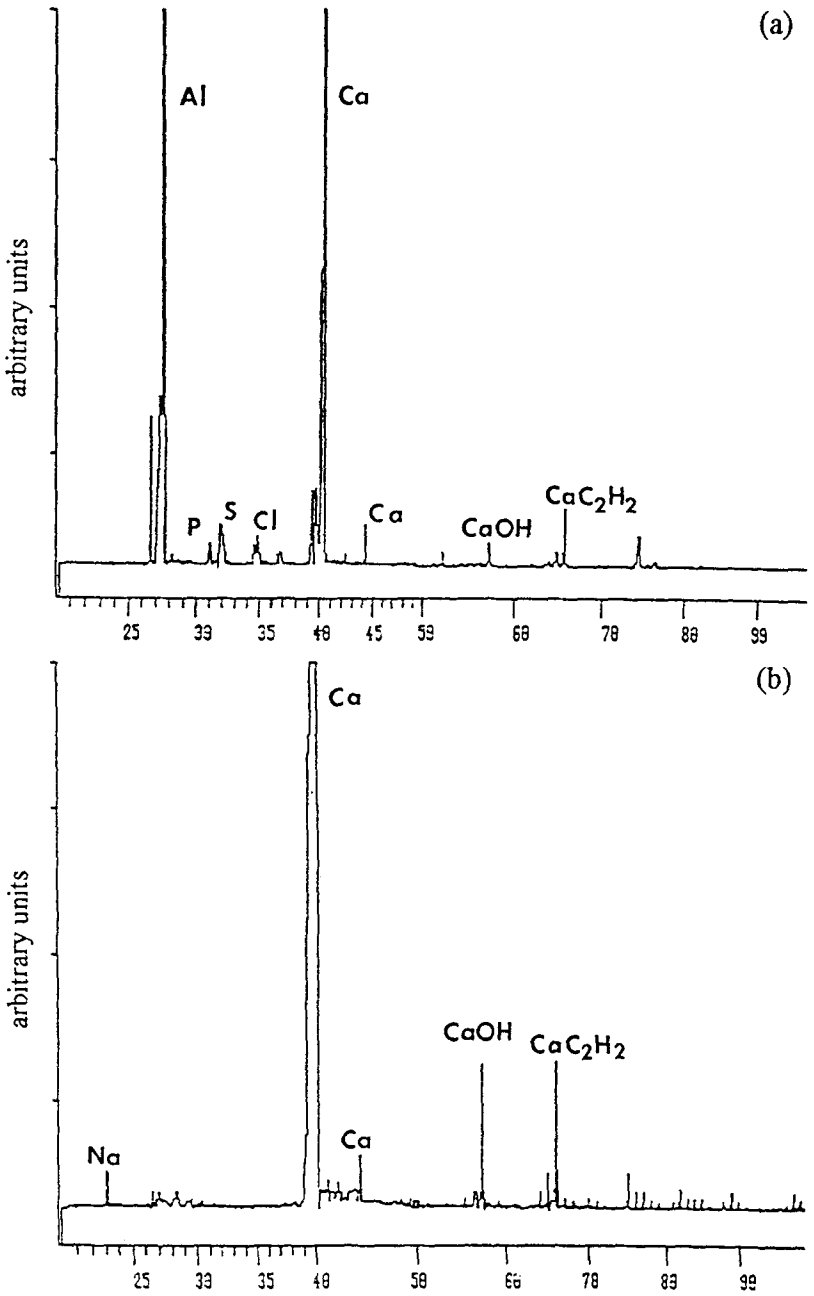

Figure 1. Ion mass spectra of semi-thin sections of gill of *P. marginata*: (a) chloride cells and (b) adjacent epithelial cells.

In our study the identification of subcellular loci for aluminum deposits (e.g., chloride cells) provides a better understanding of aluminum toxicity. As it has been

demonstrated for fishes, the main target structures for aluminum effects are those involved in ion-exchange with the external medium.

We found aluminum on the cuticular disc of the chloride cell which is the site of ion uptake, and despite the certainty that very low amounts of aluminum accumulated, it may be significant enough to induce or to enhance osmoregulatory deficiency.

As the toxicity of aluminum depends on the speciation of aluminum within the aquatic system, which in turn is modified by different factors such as pH, Ca and organic compounds, we hypothesize that certain forms of aluminum may act synergistically with protons and interfere with ionic-regulation of acid-sensitive insects which possess numerous chloride cells.

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