# A Study of Aluminium-Exposed Fish using a Scanning Proton Microprobe

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## ABSTRACT

A major problem has arisen in Europe with the depopulation of fresh water fish in lakes and streams collecting acid rain. The sensitivity to acidification is species specific and appears to be associated with metal levels.

The Scanning Proton Microprobe (SPMP) at the Micro Analytical Research Centre of the University of Melbourne was used to study the subcellular distribution of aluminium and other elements in the gills of fish exposed to acidified water with elevated Al-levels. Experiments were performed on thin sections taken from fish exposed to media with different pH and aluminium concentration.

Aluminium was found on the surface of the gill lamellae, but also inside the tissue. Bulk analysis of the gills showed much higher concentrations in the aluminium-exposed fish, compared to the control ones, but no information regarding the actual accumulation sites can be inferred.

Extensive study of damage done to the sample by intense proton beams during elemental analysis was perform ed with scanning transmission ion microscopy (STIM).

## INTRODUCTION

One of the consequences of the acidification of our environment is a higher mobility of metals in soils. They are washed out into the ground water and transported to rivers and lakes. Especially aluminium has been put forward as being toxic for fish. By using bulk analysis one obtains an idea about the amount of aluminium accumulated in or on the gills, but not about the accumulation sites. Information about the actual localization of insoluble aluminium deposits on or in the gill tissue is becoming accessible due to the availability of micro-analytical techniques. Laser microprobe mass analysis (LAMMA) and energy dispersive X-ray analysis in a scanning transmission electron microscope (STEM-EDX) and in a scanning proton microprobe (SPMP-EDX), often known as micro-PIXE, were used to study the (sub-) cellular distribution of aluminium at the gill level of different aluminium-exposed fish species. The morphological information obtained by LAMMA and micro-PIXE is inferior to that from electron microscopy, but these techniques offer a greater sensitivity as compared to X-ray micro-analysis in an electron microscope. In this paper, we report the findings obtained with a scanning particle microprobe.

## MATERIALS and METHODS

The fish used in this study are Bullhead (Ictalurus nebulosus), Pumpkinseed (Lepomis gibbosus) and Rainbow trout (Salmo gairdneri). The first two species are known to be "acid-resistant", the latter is an "acid-sensitive" type of fish. Bullhead and Pumpkinseed were exposed to acidified water (pH 4.2) containing 1.4 mg Al/l for 10 and 21

days. Rainbow trout, being much more sensitive, were exposed to lower levels of acidity and aluminium (pH 5.00, 200 ug Al/l, for 16 and 24 hours). Control fish were only exposed to acidified water.

At the end of the exposure periods, the fish were killed with a blow on the head. The gills were cut into small pieces of approximately 1 $mm^3$, fixed in a buffered 2.5 ethanol series, passed through propylene oxide and embedded in epoxy resin (LX-112). Sections of approximately 2 um were cut and mounted on formvar- covered sample holders. No staining was applied for the micro-analytical measurements.

## EXPERIMENTS and RESULTS

Individual control and Al-exposed fish gill samples, were scanned with a microprobe beam of 3 MeV protons at the Micro Analytical Research Centre in Melbourne, Australia. A continuously moving 2 micron diameter beam spot was scanned over the sample for approximately 2 hours and all PIXE data were collected by TQSA [1]. The spectra and all the elemental maps could be observed during data collection, so that errors in identifying and centring the specimen or any movements could be detected. Fig. 1 shows the energy spectrum from a sample and Fig. 2 shows the distribution of sodium (Na), aluminium (Al), phosphorus (P) and chlorine (Cl).

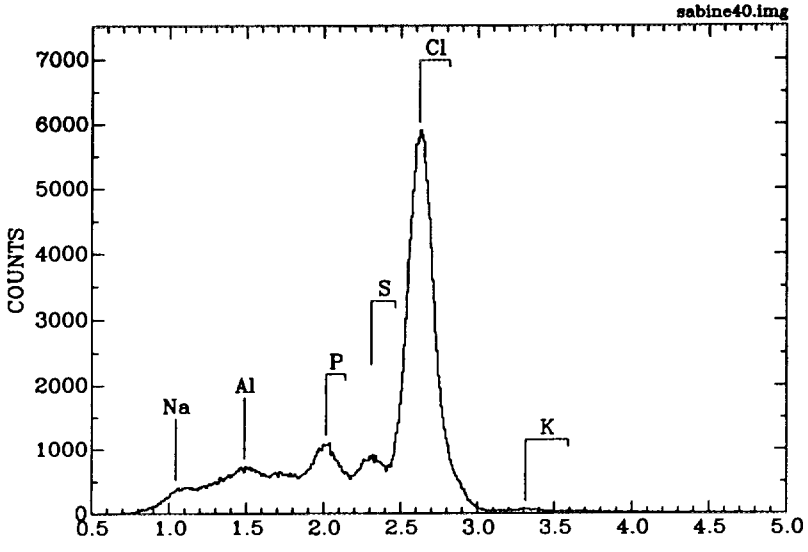
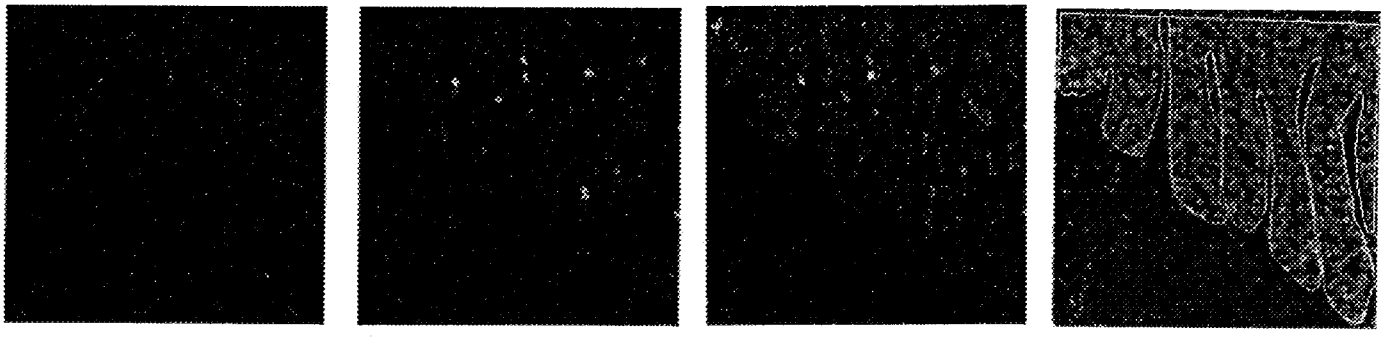



**Fig.1** X-ray energy spectrum collected from the sample.



(Na) (Al) (P) (Cl)

**Fig.2** X-ray intensity maps from a scanned area for sodium (Na), aluminium (Al), phosphorus (P) and chlorine (Cl).

Knowing that these samples were sensitive to irradiation, we examined them with scanning transmission ion microscopy (STIM) [2,3]. A 2 MeV beam of alpha particles was used in order to maximise the density sensitivity.

This beam was focussed to less than 500 nm and scanned over a sample region. The bright field median image in Fig. 3a shows the specimen before PIXE data were collected. Fig. 3b shows a second STIM image of this sample,

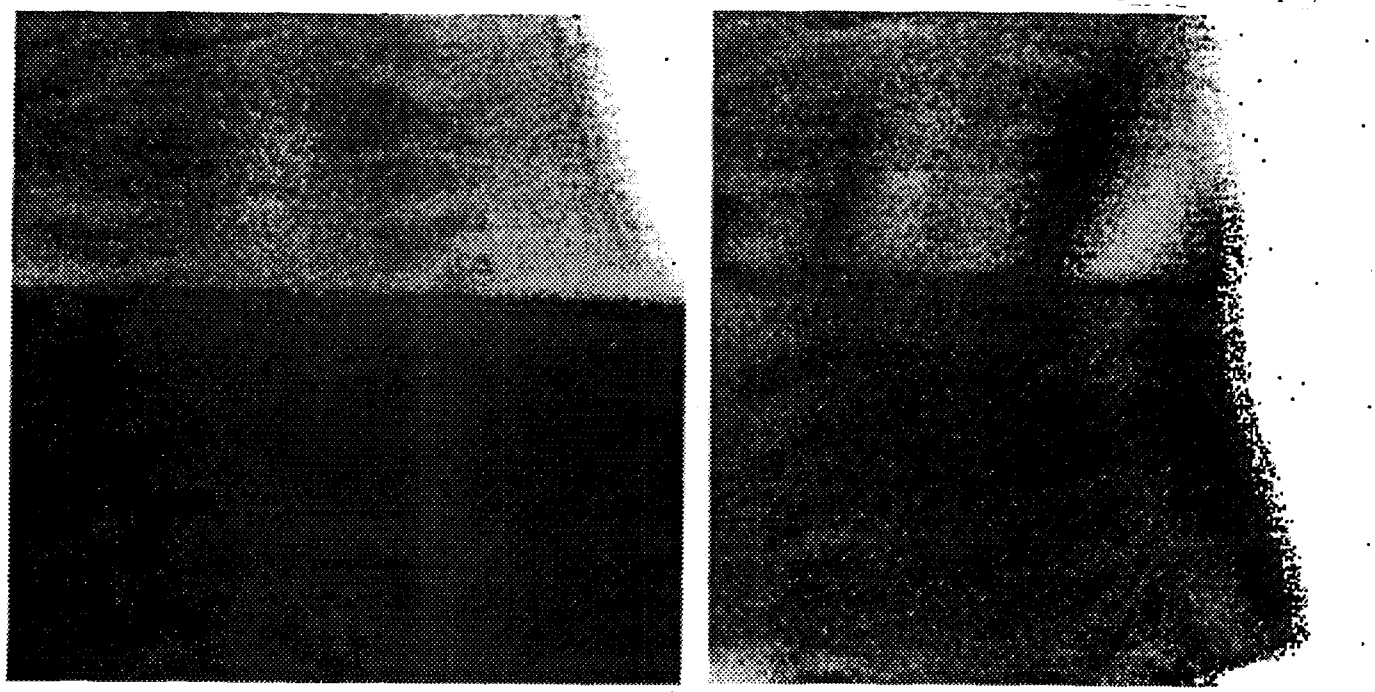


**Fig.3** Bright field STIM image of median energy loss from the specimen before (a) and after (b) PIXE analysis.

collected after PIXE irradiation. As can be seen from these two data sets, the sample experienced severe movements and changes in thickness.

## CONCLUSIONS

When a 3 MeV proton beam was used to collect PIXE data, damage was almost invisible. When a 2 MeV alpha beam was used, damage however was of great concern. The distribution of aluminium proved to be very localized. Some aluminium was found on the lamellae edges but also inside the tissue it could be detected. Especially in the pumpkinseeds, large deposits with an enhanced aluminium concentration were situated inside the secondary lamellae.

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