Journal of Animal and Veterinary Advances 13 (6): 372-376, 2014
ISSN: 1680-5593


# Examination of Heavy Metal Accumulation in Fish Scale Samples of *Aspius aspius* L. By Micro-PIXE Analytical Method

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**Abstract:** Now a days scientific press pays increasing attention to the risk of environmental pollution from anthropogenic sources. Heavy metal load of the environment increases constantly and pollutants of civilisation origin endanger the flora and the fauna. Measurement of the accumulation of various environmental pollutants including heavy metals in living organisms is increasingly utilized to monitor and detect intoxication. Heavy metals are considered to be the most dangerous inorganic micro-pollutants presently. Since, these elements can be accumulated in living organisms and can be transferred by the food-chain they may be dangerous for humans, too.

**Key words:** Fish scale, heavy metal accumulation, intoxication, PIXE Method, pollutants

## INTRODUCTION

In aquatic ecosystems water contamination by trace metals is one of the main types of pollution that may stress the biotic community. Heavy metals are commonly found in natural waters and some are essential to living organisms yet they may become highly toxic when present in high concentrations (Eneji *et al*., 2011). Heavy metals are considered as the most dangerous inorganic micro-pollutants presently (Czedli *et al*., 2012). They can accumulate in different living organism: an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during the course of their mobility (Olajire and Imeokparia, 2000).

The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Velez and Montoro, 1998; Conacher *et al*., 1993). Heavy metals include lead (Pb), Cadmium (Cd), Zinc (Zn), mercury (Hg), Arsenic (As), silver (Ag), Chromium (Cr), Copper (Cu), iron (Fe) and the platinum group elements. A pollutant is any substance in the environment which causes objectionable effects, impairing the welfare of the environment, reducing the quality of life (Duruibe *et al*., 2007). During mining processes, some metals are left behind as tailings scattered in open and partially covered pits, some are transported through wind and flood, creating various environmental problems (Habashi, 1992). Water ecosystems all over the world are exposed to different pollutants, among them toxic chemicals. The exposure to toxins results in structural and functional changes of certain communities or in changes to the whole ecosystem (Schindler, 1987). Water can be polluted by heavy metals which are accumulated and concentrated by fish therefore they show the degree of environmental pollution (Matasin *et al*., 2011a).

Several ecological disasters proved that heavy metals are among the most dangerous pollutants. One of these disasters was the widespread heavy metal pollution of Tisza River in 2000. There was a widespread pollution in the Tisza River in 2000.

After this pollution a damage assessment has been started. The reports concluded a massive destruction of fish stocks and phyto as well as zooplankton. A very interesting problem has been emerged namely how to reconstruct the spreading of the pollution in time by analysing fish scales. More precisely, analysis of heavy metal distribution of fish scales in radial direction may

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give indication for the time dependence of the pollution. A combined study by the Ions Source Analytical Group of ATOMKI (Institute for Nuclear Research of the Hungarian Academy of Sciences, Debrecen, Hungary) and University of Debrecen Department of Hydrobiology has been initiated to answer these questions.

## MATERIALS AND METHODS

Early studies of fish scales were motivated that they could be used these for species classification (Goodrich, 1907; Van Oosten, 1957). Ichthyologists realized that the scales of temperate-zone fishes have year marks created by seasonal and temperature changes and these marks allow age determination (Waterman, 1970; Fagade, 1973).

The recent research, researchers want on the study of trace elements in fish scales, relative concentrations of trace elements in scales, investigate fish scales can potentially be used as a monitor of the environment. The fish (*Aspius aspius* L.) was collected from the Tisza River. The fish used for the study of the teleost group. The teleost scales are in two forms, cycloid and ctenoid. They consist of a layer of acellular bone and an underlying fibrilary plate of collagenous connective tissue. The main constituent of the bony layer is hydroxyapatite crystal comprising chiefly of calcium phosphate (Harder, 1975). The lower fibrillar layer has a non-mineralised matrix comprising mainly of collagen and also a special protein, isopedine or ichthylepidin (Whitear, 1986). The washing of the scales was in distilled water. After washing the scales researchers clamped tightly between two metal frames. This procedure is necessary, fish scales curl up when they are dry. The scale samples were coated with a thin film of carbons this can minimize the effect of charge accumulation during irradiation (Tang *et al*., 1997). Mapping of element distribution in fish scales shows pollutant rings similarly to the annual rings in trees. Measurement of element distribution on one scalp is rather time consuming and takes 16-20 h for one sample so analysis of large number of samples is not possible. To circumwent this problem a fast, non-destructive and cheap analytical method capable to determine the total heavy metal content of the scale was choosen to preselect specimens with the highest heavy metal content and detailed (μ-PIXE) studies were performed only on selected samples.

Particle Induced X-ray Emission (μ-PIXE) Methods are adequate for the determination of heavy elements distribution in fish scalps in radial direction (Fig. 1).

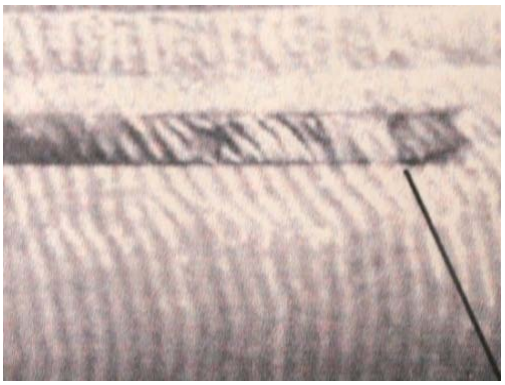

Fig. 1: The scalps of *Aspius aspius* L.

Minimum Detection Limits (MDL) were determined for individual elements by means of all methods. Such method is the best for the preselection which gave the smallest MDL value. Results of PIXE studies as well as their detailed protocolls are presented. Finally, on the basis of the minimal detection limits the best method of preselection was choosen for each heavy metal elements. In worldwide laboratories PIXE is routinely applied for several interdisciplinary problems in environmental science biology, geology, etc. (Ene *et al*., 2009). The question whether the change of heavy metal contamination in time in a given water area could be reconstructed by the subsequent analysis of the squama of fish living in the area (whether the pattern of the contamination in time could be determined from the radius orientated distribution of heavy metal contaminants incorporated into the growing squama) or not was raised in the course of the research. For the bream, (*Aspius aspius* L.) squama investigations the μ-PIXE (Particle Induced X-ray Emission) Method used in the Institute for Nuclear research was applied which is excellent for identifying squama radius orientated element distribution. Since, the measurement of such element distribution is time consuming pre-selection measurements were made using X-ray fluorescence analyses and brought out and inner beam PIXE Method. Minimum Detection Limits (MDL) achievable by the methods applied for each element were determined. Based on the results, the optimal measurement methods were selected for each element. Apart from the element analytical investigation of the squama a macro and a micro-element (calcium and zinc) distribution analyses were also made using the PIXE Method.

Element analysis of bream fish-scale was performed using ED-XRF (Matasin *et al*., 2011b) and PIXE Methods. Metal content accumulated in the scale was studied by X-ray fluorescent analyses by the 60 keV gamma ray of

$^{241}$Am as a selection analysis then in secondary target setting as well. Following these the detection of heavy metals was performed using the PIXE Method with brought out beam and with inner beam. Researchers made measurements under vacuum and also in atmospheric air. The exit window between vacuum and air was a 8 μm thick Kapton foil. The energy loss of the beam in this window was 146.6 keV while in the 2 cm thick air layer between the window and the target 321 keV. The beam energy was adjusted to be 2.5 MeV thus the energy of protons that hit the target was about 2 MeV. This incident energy is ideal for multi elemental PIXE analysis. In case of measurements under vacuum the elapsed time and accumulated charge were 5407 sec and 3900 nC, respectively while in atmospheric air the elapsed time was 4570 sec and the incident charge was not measured. The X-rays that were emitted from the target were detected with a Canberra Si (Li) detector.

## RESULTS AND DISCUSSION

The scales were analyzed with a 2 MeV proton beam with Proton Induced X-ray Emission (PIXE) Method. The PIXE spectra were analyzed by the computer program PIXYKLM developed by Szabo *et al.* (2005). It was found that in the case of element number Z<31 the application of the inner beam PIXE Method (Kertesz *et al.*, 2005) is practical while in the case of element number Z>31 the X-ray fluorescent analysis is the more effective pre-selection method. In the case of brought out beam setting, X-ray peaks associated with lighter elements (P, Ca) are more while peaks associated with heavier elements (Zn, As, Se, Br, Sr) are less intense (Czedli *et al.*, 2012). Macroelements, microelements, transitional metals and heavy metals have been detected in the fish-scale (Fig. 2 and 3). Figure 2 shows the PIXE spectra obtained from the bony side as well as the collagen side of an *Aspius aspius* L. scale in atmospheric air. Figure 3 shows

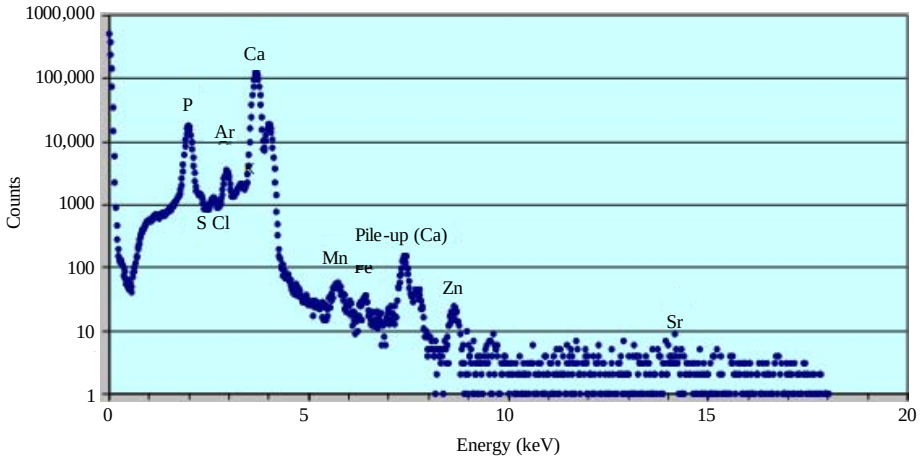


Fig. 2: PIXE spectra obtained from a *Aspius aspius* L. scale (in atmospheric air)

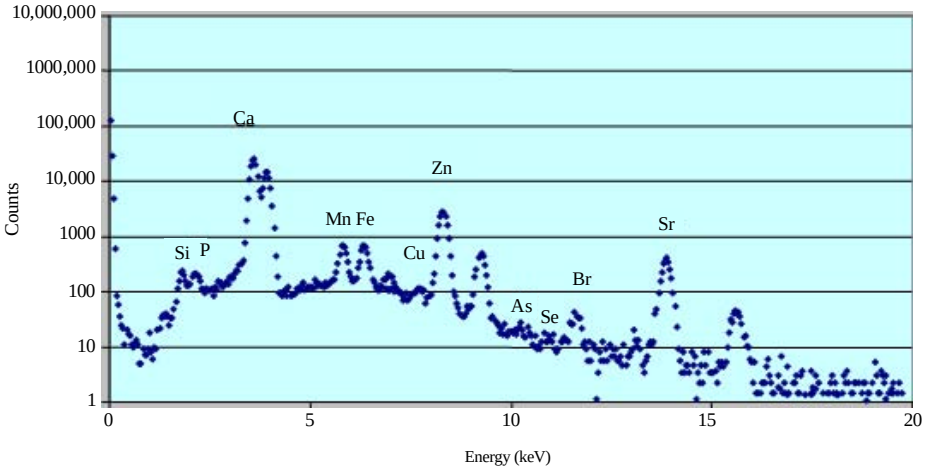


Fig. 3: PIXE spectra obtained from a *Aspius aspius* L. scale (under vacuum)

the PIXE spectra under vacuum. Local accumulations can be observed in the distribution curves taken along the line in the case of Ti, V, Fe, Cu, Zn and Pb. In case Zn concentration is high, Fe, Pb, Cu and Ti are present also in higher concentration. These maximum values can be associated to outer micro-pollutant load. Heavy metal contamination seldom means contamination with only one element, mostly a group of chemical elements are responsible for the pollution. In the course of the determination of the surface distribution of Ca and Zn using the PIXE Method calcium distribution was detected corresponding to the grade of calcification therefore based on the Zn distribution and fish-scale growth intensity the time at which the detected metal load affected the fish can be given.

Fish species can be used well in the course of the studies aiming to prove heavy metal contamination as fish indicate the change in water quality. Moreover, fish accumulate heavy metals therefore analysing them may reveal the way of heavy metals enter the organization. Element enrichments associated with the growth-zone of scales explain the changes of heavy metal contamination in time and by analysing the fish-scale samples of a given fish fauna the grade of heavy metal contamination can be concluded. Connecting scientific and applied research in the framework of a well established interdisciplinary project the measurement of heavy metals accumulating in fish contributes to trace and understand the consequences of contamination in rivers and other waters. As a result the development of highly sensible element analysis methods applicable in the simultaneous, wide range analysis of the element composition of biological samples.

The μ-PIXE is a good analytical method to determine heavy metal content in low concentrations. With the help of the utilized methods and analysis by PIXE it was proven that fish are suitable indicator organisms for heavy metal pollution.

## CONCLUSION

In this study, researchers determined the heavy-metal content of the scale of *Aspius aspius* L. with micro-PIXE Method. It is a good analytical method to determine heavy metal content in low concentrations. This present study is an example for a successful multidisciplinary co-operation.

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