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Determination of heavy metals in fish scales

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Abstract

The outcomes from measurements of amount of selected elements in the fish scales of common carp are presented. Concentrations in the scales were identified and differences between storage of heavy metals in exposed and covered part of scale were studied. The spatial distribution of elements on the fish scale´s surface layer was measured by Laser Ablation–Inductively Coupled Plasma–Mass Spectrometry (LA–ICP–MS). The average amount of elements in the dissolved scales was quantified by ICP–MS. The fine structure of fish scales was visualized by phase–contrast Synchrotron radiation (SR) microradiography.

Keywords: fish scales, ICP-MS, LA-ICP-MS

Introduction

The information about an elemental distribution in fish scales can give insight to the degree of water pollution in the period of the fish life.

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Fish scales have characteristics that can be found in other similar structures e.g. in bones and teeth. All these materials has as main components collagen type organic ingredients, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) as inorganic components and water (Torres et al. 2008; Onozato et al. 1979). The lattice of hydroxyapatite contains a small amount of sodium, magnesium and carbonate ions. Each fish scale consists of two distinct layers: external, bony layer and inner fibrous layer (Ikoma et al. 2003).

Using LA–ICP–MS the distribution of matrix elements (Ca, Mg, P) and trace elements (Sr, Ba, Zn, Mn, Fe) was monitored. The choice of investigated chemical elements was motivated by the importance related to biological and environmental exposure.

Materials and methods

Measurements were performed at two fish scales from common carp (*Cyprinus carpio)* living in the Brno reservoir in South Moravia, Czech Republic. Before the analysis the samples had to be cleaned of debris, and a thin transparent skin, which secretes mucus, has to be removed. The impurities were relieved using ultrasound machine by submerging the scale in a 5% hydrogen peroxide solution for 5 minutes. All impurities were then removed under a microscope using a toothbrush. The process was repeated until the scale became completely clean.

The thickness of the scales and the individual layers were measured from the scale cross section by Backscattering Electron Microscopy (BSE). The thickness of the external layer changes from 50 to 100 μm, the collagen plate was 70 μm thick.

The samples were further analyzed on the LA–ICP–MS instrument, which is composed of: Nd: YAG laser ablation system UP 213 (New Wave Research, USA) emitting laser light at wavelength 213 nm and ICP–MS spectrometer Agilent 7500ce (Agilent, Japan) in a horizontal arrangement.

The optimized parameters for line scan across the sample were: the laser beam diameter – 40 μm, frequency 10 Hz, energy 8.1 $J.cm^{-2}$ and the speed that the sample moved with – 40 $\mu m.s^{-1}$. In depth profiling measurement the following parameters were set ablation

spot diameter 100 µm, laser frequency 4 Hz, energy 3.4 J.cm^{-2} for 90 s.

Results and Discussion

The following isotopes of elements, with expected uniform distribution across the fish scale were measured: ^{23}Na, ^{26}Mg, ^{31}P, ^{86}Sr and ^{42}Ca. The example of line scans of Ca and P is shown in Fig 1. On the base of measurements of heavy metals isotopes, we found that heavy metals are usually stored in more exposed parts of scales. This can be explained by the fact that these parts are more exposed to the water.

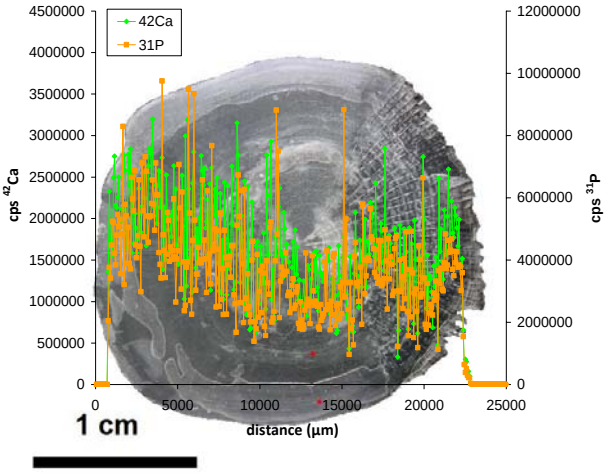

Figure 1: Line scans: intensity of ^{31}P and ^{42}Ca isotopes

Depth profiling, clearly demonstrated the differences on a signal obtained from the hydroxyapatite in collagen layers (Fig 2).

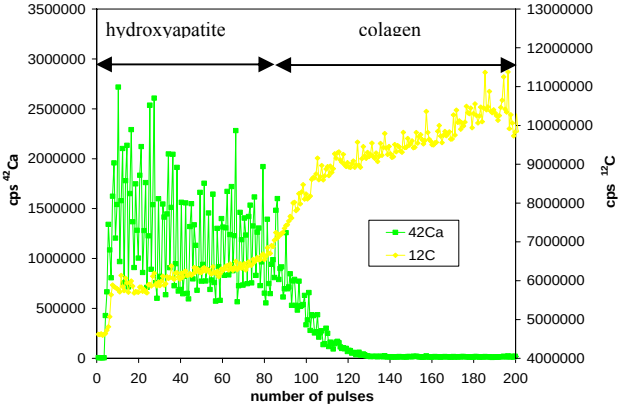

Figure 2: Depth profiling: intensity of ^{42}Ca and ^{12}C isotopes

We also demonstrated the applicability of the SR microradiography for visualization of the fish scales fine structure with high–resolution (Fig 3). This non–destructive method can be used for study of the sample features prior the chemical mapping, line scanning or depth profiling.

Conclusion

Laser assisted techniques are suitable for elemental analysis in fish scales. Combination of different modes can give information about 2D or 3D distribution of elements in such heterogeneous and layered samples.

Line scan measurements confirmed the chemical composition of the upper layers of scales (hydroxyapatite), which contains evenly spaced elements, including calcium, phosphorus, sodium, magnesium, manganese and strontium. The measurements of depth profiles revealed the contents of the lower layers of scales and the collagen layer, which contains mainly carbon.

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References

Ikoma T, Kobayashi H, Tanaka J, Walsh D, Mann S (2003) Microstructure, mechanical and biomimetic properties of fish scales from Pagrus major, Journal of Structural Biology, 142:327–333

Onozato H, Watabe N (1979) Studies on fish scale formation and resorption, Cell Tissue Research 201:409–422

Torres FG, Troncoso OP, Nakamatsu J, et al. (2008) Characterization of nanocomposite laminate structure occurring in fish scales from Arapaima Giga, Materials science & Engineering C–biomimetic and supramolecular systems, 28:1276–1283