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Laser ablation-ICP-MS in search of element pattern in feathers

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ABSTRACT

The laser ablation-ICP-MS method was used for a quantitative assessment of the distribution of elements Al, Ba, Ca, Cu, Mg, Mn, Pb, Sr, and Zn in feathers. We analysed the rectrices of first-year red-breasted flycatchers characterized by a black-and-white colouration, which gave the opportunity to examine the content of elements in two aspects: (i) quantitative distribution of elements depending on the analysed part of the feathers; and (ii) direct imaging of elements in the context of variation in feather colouration (black – eumelanin pigment and white – free of pigmentation). The results show that distributions of Ca, Cu, Mg, and Zn in the feather shaft were related to melanin pigmentation – concentrations of these elements were significantly higher within black parts. Furthermore, the highest concentrations of Al, Ba, Mn, Pb and Sr were recorded at the end of a shaft – the oldest part of the feathers analysed – which may be connected with the time and dynamic of nestling plumage development.

1. Introduction

Study of the content of elements in living organisms is essential for understanding their structure and mechanisms of functioning [1]. Elements, depending on the requirements of the organisms, can be considered primarily as toxic or physiological. For example As, Cd, Pb and Hg are considered to be toxic and unnecessary for the organisms. The toxicity of these elements depends on many factors, for example, Cd is highly toxic because of its high mobility, bioavailability and physicochemical properties [2]. Physiological elements, on the other hand such as Cu, Zn, Ca, Mg, Mn and Fe are necessary for the proper functioning of organisms. Although physiological elements are essential, above a certain threshold may be toxic or become toxic upon bioaccumulation [3,4]. Therefore, elemental contamination is extremely harmful to the physiology of living organisms, affecting their ability to reproduce and survive [5–8].

One group of animals widely used to study the content of toxic elements in the environment are birds, which are considered to be one of the best bioindicators [9–12]. Their ability to accumulate many harmful or toxic substances including heavy metals in different tissues such as kidneys, liver, lungs, brain, heart and muscles has been repeatedly demonstrated [13,14]. However, the least invasive way to study the content of toxic elements accumulated in birds is the analysis of their feathers. This method has been used extensively in research for many systematic groups of birds [9,15–18]. Toxic elements accumulate in feather structures only during growth or may be found on their surface [19,20], but what is the most important is that birds regularly moult and thereby eliminate harmful elements from the body. This makes feathers the most distinctive metal sink with the highest recorded metal concentration [21–23].

One component of feathers is melanin which is the most common pigment in animal integuments. Melanin is responsible not only for pigmentation but also for binding toxic metal ions with carboxyl functional groups that serve as cation chelators which may allow some body detoxification [24,25]. Therefore, there is a significant increase in the concentration of chemical elements in melanised feather parts compared to non-pigmented parts [26]. Elements are also involved in the synthesis of melanin pigments responsible for the colour of the feathers [24]. Studies on different systematic avian groups have been conducted to investigate a possible correlation between element concentrations and feather pigmentation. They have shown that elements such as Ca, Fe, Cu, Zn and Mn become embedded in the melanin structure of feathers [24,26–28]. Considering the latter, analysis of the feathers, by means of advanced analytical methods could be a very effective means of making a quantitative assessment of environmental pollution, and may contribute to determine the metal contents within their cross-sections. Furthermore, analyses of element concentrations in feathers are important as they may be able to shed light on the role of different elements in melanin synthesis and the function of feather pigmentation in the form of melanin in binding toxic metal ions in keratin products of the skin. Keratin fiber has the property of binding ions of metals such as Cu, Pb, Hg, Cr and U [29]. The complex structure of polypeptide chain in keratin is supported by disulfide, electrostatic and hydrogen

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bonds, hydrophobic interactions and dipole attraction which all have their share in interaction with metal cations. However, the metal uptake is difficult from aqueous solutions due to the hydrophobicity of keratin caused by cross-linking of keratin molecules by double sulfide bonds of cystine [29,30].

Following acid digestion of the samples, several methods are commonly used to carry out analysis of the metal content in the feathers, such as FAAS, ETAAS, ICP-OES, and ICP-MS [31–33]. Alternatively, the elemental composition of the feather samples can be determined by methods based on the direct analysis of solids such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [31], energy-dispersive X-ray fluorescence spectrometry (EDXRF) or Synchrotron Rapid Scanning X-ray fluorescence (SRS-XRF) [34,35]. These techniques have the potential to provide images of the elemental distribution in the feathers in comparison with other techniques after digestion.

LA-ICP-MS has been used for direct imaging of essential, beneficial and toxic metal distribution in biological samples such as feathers. Analysis of samples with natural heterogeneous distribution of elements requires techniques with good spatial resolution and high signal/noise ratio (low detection limits). Laser ablation inductively coupled plasma mass spectrometry is a powerful analytical technique for mapping the distribution of elements in environmental and biological solid samples [31,36–39]. Multi-elemental analysis, high sensitivity and easy sample preparation are among its main advantages; however, quantitative analysis is probably the most challenging step. The development of suitable calibration standards, internal standards and improvement of reproducibility and accuracy are still under investigation [40–43]. LA-ICP-MS presumably has the potential to provide a more detailed picture of elemental distribution in the feathers than any other techniques.

The aim of the presented work was to develop a method of quantitative analysis of elements Al, Ba, Ca, Cu, Mg, Mn, Na, Pb, Sr, and Zn along the tail feathers of the red-breasted flycatcher by LA-ICP-MS and to examine the distribution of the elements in the quill and different parts of the shaft varying in colour (black – eumelanin pigment and white – free of pigmentation). The following issues will be discussed: (1) preparation of solid standards and calibration strategy; (2) examination of validation parameters; and (3) application of the calibration method, dependent on the colour and part of the feather, to imaging and quantification of elements in the real sample.

2. Experimental

2.1. Material and sample preparation

The research material consisted of 13 samples of feathers (two rectrices T3 – third feathers numbered from the central pair, which are black with a white spot at half of its length; see Fig. 1a taken from first-year red-breasted flycatchers (*Ficedula parva*, order Passeriformes) caught during autumn migration in the year 2015 in Kızılırmak delta (northern Turkey; 41°38′N, 36°05′E) and stored in zip-lock bags in darkness at −18 °C until further analysis [44]. To remove any external contamination, feathers were washed vigorously in deionized water (Smart 2 Pure, TKA, Germany) alternated with 1 mol L^{-1} acetone (99.5% pure p.a. basic, POCH, Poland) after which they were rinsed with deionized water. Later, samples were dried at room temperature for 48 h [44,45], until stabile mass was achieved, and stored in a sterile 15 mL capped polypropylene centrifuge test tube (VWR International, Poland).

In order to be analysed by LA-ICP-MS, the clean and dry feather samples were placed on polyethylene terephthalate (PET) slides. The length and width of the samples was approximately 60 × 2 mm. Each sample was analysed along the shaft (from quill to tip of feather) using the single-line scan method (Table 1). In these parts of the feather isotopes of the following elements ^{27}Al, ^{138}Ba, ^{43}Ca, ^{65}Cu, ^{26}Mg, ^{55}Mn, ^{208}Pb, ^{88}Sr, and ^{66}Zn were determined.

The comparative quantitative analysis was done using the ICP-MS technique. For this purpose, the feather samples were decomposed in a microwave system (ETHOS One Milestone Inc., Italy) according to the following procedure: 200 mg of feather samples was dried for about 6 h at 25 °C until achieving constant weight. Dried material of feather samples was accurately weighed and decomposed in closed Teflon vessels with 2.0 mL of 60% (*v*/*v*) HNO_3 (Suprapur, Merck, Germany). A two-step heating program was used in operating the oven in two steps: 1) ramp time was 20 min for 1200 W at a maximum temperature of 240 °C and a pressure of 80 PSI and 2) held for 15 min and then 20 min vent. After decomposition, the microwave vessel was cooled, the digest was transferred into a 10 mL volumetric flask and adjusted with demineralized water to the proper volume. Prepared samples were analysed by the ICP-MS technique. Blanks for control of the digestion process were prepared in a similar manner. All samples were analysed in batches with certified reference materials and blanks. Analytical accuracy was ensured by measuring certified reference material NCS ZC 81002b Human Hair (China National Analysis Center for Iron and Steel, China).

2.2. Apparatus and measurement conditions

In this analysis, quadrupole ICP-MS instrumentation (Elan DRC II, Perkin Elmer SCIEX, Canada) was used. For solid sampling, a commercially available laser ablation system (LSX 500, CETAC, USA) equipped with a 266 nm Nd:YAG laser was used. The optimization of the LA-ICP-MS conditions was performed by ablating NIST 610 trace metals in glass standard (National Institute of Standards and Technologies, Gaithersburg, MD, USA) for maximum $^{24}Mg^+$, $^{115}In^+$, and $^{238}U^+$ signals and to set the ratio of oxide $^{232}Th^{16}O^+/^{232}Th^+ < 0.2\%$ and doubly charged ions $^{42}Ca^{2+}/^{42}Ca^+ < 0.5\%$ signal before every experiment. The optimization of laser conditions was performed by ablating a pellet of matrix-matched solid standard using the single variable method. The parameters for optimization were energy of laser beam, spot size, shot frequency and scanning speed. Factors influencing the choice of laser parameters were the highest intensity of the signal of an internal standard taking into account the lowest value of relative standard deviation (RSD). The parameters were set so as to obtain a compromise between the sufficient intensity of analytical signals and the spatial resolution of images generated from acquired analytical signals. Apparatus parameters used for the sample measurements are shown in Table 1.

Solution nebulization for ICP-MS measurements was carried out using a cyclonic spray chamber equipped with a concentric quartz glass nebulizer for sample introduction. While tuning the ICP-MS, compromise conditions for maximum signal intensity of the analyte ($^{24}Mg^+$, $^{115}In^+$, and $^{238}U^+$) and minimum ratio of oxide ($^{140}Ce^{16}O^+/^{140}Ce^+ < 3\%$) and doubly charged ions ($^{138}Ba^{2+}/^{138}Ba^+ < 3\%$) were found. DRC was employed to remove spectral interferences. The internal standards, ^{45}Sc and ^{103}Rh at a concentration of 10 μg L^{-1}, allowed correction to be made for matrix-induced variation and instrumental drift.

3. Development of a calibration method

In the literature, many innovative strategies for calibration of LA-ICP-MS have been used in the analysis of biological tissues, although not feathers [40,46–48] in an attempt to overcome limitations resulting from the lack of commercially available solid standards. To our knowledge, we are the first to use this technique for imaging and quantification of elements in feathers. Therefore, we had to develop a procedure for a quantitative analysis using LA-ICP-MS, which was associated with the development of experimental calibration strategy. Solid standards with a matrix similar to that of bird feathers are not commercially available, hence it was necessary to prepare matrix-matched laboratory standards which were used to prepare the calibration curve. Feathers, like hair, bristle and hooves are considered among the keratin materials,