Voltammetric Determination of Mercury in Bird Feathers for Biomonitoring Studies

by J. Golimowski and K. Dmowski

Department of Chemistry, University of Warsaw, 02-093 Warsaw, Poland;
*Department of Ecology, University of Warsaw, 00-927 Warsaw, Poland

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Feathers of birds representing higher trophic levels can be a valuable diagnostic material for an estimation of environmental pollution by heavy metals. The contents of mercury in feathers of magpies living in areas of different industrial and agricultural level were investigated. The hermetic method of mineralization in a microwave oven and the UV-digestion were used. Determination of mercury in the digested samples was carried out using voltammetry with a gold disk electrode. The results were compared with those obtained by the ICP-MS technique. As an additional reference material the feathers of white-tailed eagle, in which the amount of mercury was previously measured by AAS, were used. The results were consistent for all examined samples. Relatively high content of mercury was found in the agricultural areas, which may be a result of protecting seed grains with mercury compounds.

Pióra ptaków reprezentujących wyższe poziomy troficzne są cennym materiałem diagnostycznym do oceny skażenia środowiska. Wykorzystując pióra srebrka (Pica pica) uzyskano wcześniej dobrą korelację zawartości ołowiu i kadmum ze skażeniem danego obszaru. W prezentowanych badaniach stwierdzono podwyższone zawartości ręci w pióраch ptaków pochodzących z obszarów rolniczych na Mazurach, co może wskazywać na pozostałości ręci w środowisku na skutek wieloletniego stosowania, zakaźnych dział, impregnatów rolniczych i ziem siewnych. Podwyższone zawartości ręci oznaczono również w pióраch przedstawicieli ptaków rosyjskich (czapli siwych) z tego samego terenu.

Opracowano hermetyczną metodę mineralizacji prób w kuchence mikrowalowej uzupełnioną mineralizacją UV. Do badania zawartości ręci zastosowano woltamperometrię z użyciem elektrody złotej. Metodą porównawczą była ICP-MS. Aby ocenić poprawność wyników oznaczono woltamperometrycznie ręć w pióраch ssaka oznaczenia określonej wcześniej za pomocą ASA, zawartości Hg. Zgodność wyników była zadowalająca.
Mercury compounds belong to a group of the most toxic substances in the environment [1]. Carnivorous species, especially fisheaters or those eating terrestrial vertebrates are particularly exposed to contamination because of the mercury biomagnification at higher levels of the trophic pyramid. From the analytical point of view predatory animals tissues are suitable for biomonitoring studies. Determination of accumulated mercury in the tissue is easier than, for example, in water or air, where there are only trace amounts of this element.

In the sixties, in Sweden the application of mercury compounds to impregnate the seed grains was forbidden as a result of the research focused on the predatory birds and especially on their feathers. It concerned particularly methyl- and other alkyl-derivatives of mercury. The increased number of poisoning of predatory birds and decreased number of hatched nestlings, observed in the previous years (the forties and the fifties) were associated with the use of methylmercury – the substance much more toxic than the inorganic mercury compounds. The retrospective studies of mercury contents in the feathers of birds from museums – the samples from 1840–1960 [2–4], corroborated this idea. In the forties and the fifties the mercury contents in this material increased 10–20 times. This research was possible because of high stability of mercury compounds with keratin – the main component of feathers. The UV-irradiation, heating, ageing of feathers do not change the amount of mercury [5]. The detected losses did not exceed 10%. So, bird feathers can be a good retrospective material from the past, when the samples were not collected in the framework of the environmental specimen banking.

In the recent years, in Europe, the studies concerning the application of magpie (Pica pica) as a bioindicative species for determination of environmental pollution by heavy metals have been conducted [6–8]. The main advantages of magpies are: its common presence, small area of penetration (about 50 ha), and resident behaviour. These features of magpies can be used in biomonitoring of the areas, which are difficult to penetrate, such as military objects (e.g. the Soviet Army garrisons in Eastern Europe, a short time ago), for localization of illegal waste dumps, etc. The elements accumulated in the organisms of these birds come from breeding areas, which are also the wintering places. From the ecological point of view the magpies represent the higher level of the consumers, which are near the top of the trophic pyramid.

Studying the correlation between the contents of heavy metals in the magpie feathers and the degree of environmental pollution the use of certain feathers for routine bioindicative research was tested. The tail feathers (rectrices, see Fig.1), especially external Rs and Rs [6,9] proved to be the most convenient analytical material. The contents of metals in external tail feathers from the left and the right side of the tail are (for many metals) similar, hence it is possible to obtain at least four samples from one bird (a possibility of measurement repetition). These feathers are more seldom exposed to mechanical damages.

Hitherto, the cold vapour AAS method and the neutron activation analysis were used for the mercury determination in biological samples. Sipos et al. [10] were first who used the DPASV technique for the mercury determination. This method was previously applied in the analysis of water and was later modified and used for the
analysis of waste water, domestic garbage [11], fish muscle [12] and others. The authors of the cited papers have used the UV-digestion for water samples and for mineralization of solid samples. Before irradiation HNO₃ and HClO₄ were added to the samples. A microwave oven was applied [13–15].

The DPASV technique is sufficiently sensitive to determine mercury in biological material. This method requires, however, total removal of the organic matrix.

The aim of this paper was to apply the voltammetric method for determination of mercury in biomonitoring research, using bird feathers as the bioindicators of the environmental pollution. The feathers of magpies exposed to different levels of mercury compounds, were used as the material for these investigations.

EXPERIMENTAL

Apparatus

A polarograph PAR 174 A (USA) with a XY HI7045 recorder.
The three electrode system: a rotating gold disc electrode of 3 mm diameter or a static gold electrode of 5 mm diameter, a Ag/AgCl reference electrode and the platinum auxiliary electrode.
A voltammetric cell (Metrohm);
a quartz knife;
teflon pressure bombs (own construction);
a commercial microwave oven, power 650 W, frequency 2.45 GHz, (SANYO);
a UV-digestion device with a 125 W high-pressure mercury lamp (MINERAL, Poland).

Reagents

Nitric acid, 65%, hydrochloric acid 30%, perchloric acid, 70% – Suprapur (Merck) (max. contents of Hg 5×10⁻⁷%);
hydrogen peroxide, 30%, analytically pure;
potassium persulfate, purified by crystallization POCH;
Triton X-100, p.a. (Merck);
corundum powders of size 1 and 0.3 μm (Buehler, USA);
water triply distilled in a quartz apparatus;
1 mg ml⁻¹ standard solution of mercury, prepared from an ampoule of Titrisol (Merck)
a solution for activation of the gold electrode and for stripping: 8.5 ml of 0.1 mol l⁻¹ HClO₄ + 0.25 ml of 0.0025 mol l⁻¹ HCl diluted to 1 l

Material for research

The magpies feathers were taken from the birds captured by modified bal-chatri method [9,16]. The individuals were caught in different areas of Poland:
- the area surrounding the “Miasteeczko Śląskie” zinc smelter (the largest in Poland) near Tarnowskie Góry (MSL);
- the area surrounding the steelworks in Nowa Huta near Kraków (2 km from the main block of the plant) (NHiU);
- the area surrounding the largest Polish petroleum refinery near Plock (PLO);
- the villages Staweck (MIK-1) and Ługanie (MIK-2), near Mikolaizki (MIK) (unpolluted area close to the biosphere reserve “LuKNajno Lake”) in the Masurian Lake District.

The external rectrices (R₉) were taken for the analysis. The layer of external contaminants on each feather was removed in an ultrasonic bath using aqueous solution of nonionic surfactant – Triton X-100 for 15 min. The feathers were then rinsed several times with triply distilled water, dried in a laboratory dryer at 60°C and stored in plastic bags. The vanes of the feathers prepared in this way were separated with a quartz knife on a teflon board. The comparative studies were made by the ICP-MS method in another laboratory. The contents of mercury were determined in the external tail feathers of the same individuals.

Additionally a feather of a white-tailed eagle (Haliaeetus albicilla) was used as a comparative material for the described analytical procedure. This feather was placed in a wing next to the feathers, in which the content of mercury was measured by the AAS method [17,18].

Due to the high value of mercury content in the magpie samples from the village Ługanie, the same determinations were done using the feathers of other high level consumers, living in this region. The feathers of grey herons (Ardea cinerea) from the colony placed 3 km from Ługanie (the samples were collected about 1980; this colony does not exist now) were taken for analysis.

Mineralization of the samples

As the first step the feathers were digested in a teflon pressure bomb with a steel coat. This process was carried out at 140°C in a laboratory dryer for 3 h. 2 ml of nitric acid were added to each sample (50–100 mg). The solution obtained by this way was yellow-green, so it had to be subsequently, after diluting, mineralized by UV-irradiation with an addition of hydrogen peroxide. The colorless solution was obtained after 3 h of irradiation.

The next experiments were carried out in a thick-walled teflon bomb using microwave as an energy source. The bomb containing a sample with added oxidant was placed in a polyethylene vessel, to avoid possible emission of nitric acid vapour. The digestion in the microwave oven was carried out for 3 min. When the bomb had cooled down, the solution was transferred into a 25 ml volumetric flask. The solutions were colorless or light yellowish. Better results were obtained using nitric acid with hydrogen peroxide for the mineralization. To a sample of 50 mg feather 1–2 ml of nitric acid and 40–400 µl of hydrogen peroxide were added. Completely digested samples were obtained if the volume of hydrogen peroxide added was larger than 200 µl.

In the course of the experiments the construction of the bomb was perfected by changing the shape of the internal chamber and using a different type of the thread. After these modifications the time of exploitation of the device was longer.

The solutions digested in the microwave oven, even being colorless, contained still some amounts of organic substances, what was confirmed by spectral measurements of organic carbon (absorption at 254 nm). The voltammetric determination of mercury in these samples was difficult. So, the samples, after dilution, were additionally mineralized by UV-irradiation in the presence of hydrogen peroxide or potassium persulfate. Various types of mercury lamps were tested. The best one was the high pressure
mercury lamp of 125 W. It was also learned that potassium persulfate can be applied in the mineralization as well as hydrogen peroxide.

**Preparation and activation of the gold electrode.**

A new or not used for a long time gold electrode was wet polished with corundum powders (size 1 and 0.3 μm). Next the electrode was polarized alternately at the potentials -0.25 and +1.7 V (10 times for 10 s). The activation was conducted in the stripping solution (HClO₄ + HCl); during this process the electrode was rotated.

To attain a satisfactory electric connection between the rotating part of the electrode and the polarograph two types of brush contacts were tested: one – with collected brushes made of graphite and the second – made of silver containing 5% of graphite. Mercury contact was eliminated because of the possibility of contamination of the samples. The perfect contact was obtained by the use of the brushes made of silver and graphite (Degussa).

**Voltammetric determination of mercury**

The analytical cycle consisted of:
- activation of the gold electrode at +1.7 V (1 min) and subsequently at +0.7 V (10 s) – in a HClO₄ + HCl solution;
- accumulation of mercury at -0.2 V for 2–10 min in the sample solution;
- removal of copper at +0.4 V for 10 s, by stirring (rotating the electrode) in the HClO₄ + HCl solution;
- stripping process in the non-stirred HClO₄ + HCl solution;
- recording the signals.

The quantitative determination was done by the double addition of the standard solution.

**RESULTS AND DISCUSSION**

The results of mercury determination in magpie feathers by the voltammetric method were comparable with those obtained by ICP-MS method (Table 1).

**Table 1. Contents of Hg in feathers (μg g⁻¹ d.w.)**

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>DPASV</th>
<th>ICP-MS</th>
<th>AAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Magpie</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miasteczko Śląskie (MSL)</td>
<td>0.20 ± 0.20</td>
<td>0.20</td>
<td>–</td>
</tr>
<tr>
<td>Nowa Huta (NHIU)</td>
<td>0.70 ± 0.10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Płock (PŁO)</td>
<td>0.40 ± 0.2</td>
<td>0.30</td>
<td>–</td>
</tr>
<tr>
<td>Stawek (MIK-1)</td>
<td>0.80 ± 0.10</td>
<td>0.40</td>
<td>–</td>
</tr>
<tr>
<td>Ługanie (MIK-2)</td>
<td>4.70 ± 1.50</td>
<td>2.05</td>
<td>–</td>
</tr>
<tr>
<td>Grey heron</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ługanie (MIK-2)</td>
<td>4.70 ± 0.30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>White-tailed eagle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warszawa-Młociany</td>
<td>24.00 ± 0.60</td>
<td>–</td>
<td>22.00–27.00</td>
</tr>
</tbody>
</table>

The results of voltammetric determination of mercury in the white-tailed eagle feather, were also similar to the results obtained earlier by the AAS method in the neighbouring feathers (Table 1). In both cases the differences between the two analytical methods can result from the fact, that not the same feathers were investigated but the neighbouring ones. These feathers could potentially contain the same amount of mercury because of the similar growth time, similar location, similar time
and way of exposition to the external contamination and so on. But one should always take in to account some individual differences.

A satisfactory agreement of the results obtained by the two independent methods was found for both the samples of low mercury contents, about 0.1–0.2 µg g⁻¹ d.w. and higher mercury contents, about 25 µg g⁻¹ d.w.

The mineralization in the microwave oven, as well as in conventionally heated teflon pressure bomb, proved to be not complete and the samples were subsequently irradiated by an UV-lamp with an addition of hydrogen peroxide. The great advantage of using a microwave oven is short time of the mineralization (2–3 min) compared with the heating time in the dryer (about 3 h).

An investigation of the additional mineralization with the UV-lamp showed that the high pressure lamp is the best for this aim. This was also confirmed by the analysis of the organic substances remaining after the digestion process. The absorbance of the same solution, which was mineralized only in the microwave oven, was about 1.50 (λ=254 nm, l=1 cm) while after an UV-irradiation by a high pressure lamp it was equal to 0.30. Hydrogen peroxide or potassium persulfate were used as the oxidants. Potassium persulfate is equally efficient as hydrogen peroxide is, and may be used in the UV-digestion process.

The application of the new brush contact of rotating electrode, made of silver containing 5% of graphite, gave reproducible peaks. It was found, that for the determination of a higher amount of mercury the stationary gold electrode and stirring the solution by a magnetic bar could be used instead of the more complicated rotating disc electrode. The potential–time programme described previously [11,12] was applied with small changes for the determination of mercury in feathers. However the use of the calibration curve was not possible, because the degree of the mineralization of organic matrix was not always the same.

The previous studies concerning the determination of lead and cadmium in magpie feathers have pointed out that a good correlation between the contents of these metals and a localization of lead and zinc industry exists [9] (Table 2).

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>Hg</th>
<th>Cd [9]</th>
<th>Pb [9]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minsteczko Śląskie (MSL)</td>
<td>0.20 ± 0.20</td>
<td>28.60</td>
<td>1582.0</td>
</tr>
<tr>
<td>Nowa Huta (IIII)</td>
<td>0.70 ± 0.10</td>
<td>4.50</td>
<td>87.0</td>
</tr>
<tr>
<td>Piłcok (PLO)</td>
<td>0.40 ± 0.20</td>
<td>0.40</td>
<td>18.6</td>
</tr>
<tr>
<td>Sławek (MIK-1)</td>
<td>0.80 ± 0.10</td>
<td>&lt;0.05</td>
<td>8.9</td>
</tr>
<tr>
<td>Ługanie (MIK-2)</td>
<td>4.70 ± 1.50</td>
<td>&lt;0.10</td>
<td>12.1</td>
</tr>
</tbody>
</table>

In this study the contents of mercury was found to be higher in agricultural areas than near the industrial plants. This could be an effect of continuous application of mercury compounds in agriculture. It is suspected that there is another cause for relatively high mercury concentration in the feathers of individuals from the region of the village Ługanie (close to biosphere reservation). Within the territory of the magpies there was a fisherman’s house. Near this house the remains of gutted fishes were thrown out. In the livers of fish, especially carnivorous, considerable amounts of mercury can be accumulated, and such a food could be eaten by the birds from
Ługanie. The level of mercury in the feathers of grey herons from this region was also relatively high (Table 1). This area should be carefully studied in the future.

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