ABSTRACT

Upper Silesia is the most transformed by humans of the Polish territories. As a result of mining, urbanization and other industrial activities, irreversible changes have taken place in the environment. In this paper, the method of collecting, preserving, storing and preparing samples from four Silesian rivers was optimized. Silesian sediment and river water samples are a challenge for the analyst, because they have complex matrices and high content of chlorides coming from the discharges of saline water. This study concerned determination of Sb(III), Sb(V), As(III), As(V), MMA, DMA, AB, Cr(III) and Cr(VI) with high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). The following columns were used: Dionex Ion Pack AG7 to determine chromium forms, Hamilton PRP-X100 to separate antimony species and Dionex Ion Pack AS7 to determine five chemical arsenic forms. The methodology used allowed for elimination of interferences during analysis of highly saline and complex samples and ions separation directly without adding any complexing compounds. Depending on the analytes, full separation was obtained within 3–9.5 min. The sediment sample extraction and determination were validated (recovery, limit of detection, repeatability and uncertainty) with the certified reference materials (CRMs). The optimized methodology allowed for study of mobility and seasonality changes of As, Sb and Cr species in the river environment.

Key words | antimony, arsenic, chromium, HPLC-ICP-MS, speciation

INTRODUCTION

The toxicological test results demonstrate that in many cases different speciation forms of a given element, rather than its total content, have a decisive influence on living organisms. In the literature, there have been many examples of using liquid chromatography in speciation analysis (including metal ions in water) in recent years (Ammann 2002; Michalski et al. 2011). The so-called hyphenated techniques are becoming ever more popular. It is necessary to use separation and detection techniques (e.g. high-performance liquid chromatography-inductively coupled plasma-mass spectrometry, HPLC-ICP-MS) simultaneously in the hyphenated methods (Jabłońska-Czapla, Sebastian Szopa).
et al. 2012; Jabłońska-Czapla et al. 2014). Combined techniques allow for determination of different speciation forms of the elements without use of any complex compounds, in a short time and at relatively low cost.

Due to their toxicological characteristics, arsenic, antimony and chromium and their compounds are among the most often researched metals and metalloids in the speciation analysis (Das et al. 2003). Cr(III) plays an important role in living organisms as it participates in metabolic processes. It is thought that Cr(III) compounds positively influence living organisms. They are responsible for correct glucose metabolism in mammals. Additionally, they easily undergo complexation processes with various substances present in environmental samples (Watanabe et al. 2004). On the other hand, the Cr(VI) compounds are highly toxic and constitute a serious threat to the environment due to their mutagenic and genotoxic properties. Their inhalation causes lung inflammation and asthma. Their contact with skin results in allergies and dermatoses (IARC 1990). Cr(VI) species are easily soluble, mobile and more bioavailable than the Cr(III) compounds (Nriagu & Niiboer 1988; Kabata-Pendias & Pendias 1999).

The As(III) compounds are more soluble, mobile and toxic than the inorganic As(V) compounds (Caruso et al. 2000; Nordberg et al. 2007). Arsenic is a toxic metalloid that is common in the environment and various biological systems. The number of its speciation forms is constantly increasing due to economic progress, as the pollution caused by industry has not decreased in recent decades. On the contrary, the arsenic emissions from steel works, industry, animal waste and dust from fossil fuel combustion are rising at present. Arsenic is very mobile. Consequently, it occurs in all the environment elements. Its toxicity depends on the chemical form. Importantly, its inorganic speciation forms are 100 times more toxic than the organic ones (Jabłońska-Czapla 2015). The contact with arsenic can cause various health effects, such as dermatological, inhalatory, cardiologic, genetic, genotoxic or mutagenic lesions (Chou & Rosa 2003).

Antimony occurs at two oxidation states, i.e. Sb(III) and Sb(V). Sb(III) is approximately 10 times more toxic than Sb(V). Its content in rivers is usually lower than 1 mg/L. The antimony concentration in drinking water does not usually exceed 0.5 mg/L (Smichowski 2008). The significant increase in the antimony concentration in water systems observed over recent decades has resulted from the human activity. Its most important anthropogenic sources are the processing industry of the copper ore and coal and municipal waste combustion. As the industry in the Upper Silesian Industrial Region is based on steel and coal excavation, the researchers are intensely interested in the antimony contents in drinking water (Filella et al. 2002).

In the literature, there are many reports on the metal and non-metal ion contents in the river water and sediments (Melaku et al. 2005; Aleksander-Kwaterczak et al. 2006; Prasad et al. 2006; Purushothaman & Chakrapani 2007). Unfortunately, the investigations do not focus on Polish rivers, particularly the highly polluted rivers in Upper Silesia. They mainly concern either determining total contents of analytes (atomic absorption spectrometry) or their fractionation (Evans 2000; Frankowski et al. 2010; Relic et al. 2010).

The Kłodnica, Bytomka, Biała Przemsza and Rawa Rivers flow through one of the most industrialized regions of Poland. Industrial facilities, dense residential land development and heavy industries are responsible for the serious surface water pollution. For many years, there has been a gradual decrease in the groundwater level. Moreover, there are often droughts in summer. Consequently, more than 90% of these river flows is often constituted by various wastewater types. Due to long-term neglect, Upper Silesia is still ‘an environmental bomb’, and its rivers are a potential pollution source for other Polish regions. The rivers selected for the research (i.e. the Kłodnica, Bytomka, Biała Przemsza and Rawa Rivers) are among the most polluted rivers in Poland. As they are tributaries to the main rivers (the Vistula and the Oder), they cause the pollution to spread over a large area of Poland. What is more, these relatively small rivers pose a severe threat for the inhabitants during floods that are often serious and cause permanent pollution of the flooded areas (Czaja 1999; Nocoń & Kostecki 2005; Nocoń 2006).

The aim of this study was to optimize the preparation of samples and determination of two antimony speciation forms (Sb(III), Sb(V)), five arsenic speciation forms (As(III), As(V), MMA, DMA, AB) and two chromium speciation forms (Cr(III) and Cr(VI)). The collection, preservation, storage and preparation of the water and sediment samples from the four researched rivers were optimized in the study. The samples had complex matrices (high pollution...
of the Upper Silesian rivers) and high contents of chlorides coming from the discharges of saline water from the mines. This study is one of the stages of the MoSpeSil research project (2013–2015) performed within the Polish–Norwegian cooperation (Small Grant Scheme 2012; Polish–Norwegian Research Programme). The project will provide knowledge about the redox and speciation changes in the arsenic, antimony and chromium ionic forms in selected river ecosystems of Upper Silesia (the Kłodnica, Bytomka, Rawa and Biała Przemsza Rivers). Such a study has never been conducted in this region of Poland. The aim of the project was to determine the mobility and seasonal changes of the arsenic, antimony and chromium speciation forms in the sediments, water and plants at the riverbanks.

MATERIALS AND METHODS

Sample collection

The research consisted in elaborating the entire methodology (from sampling to quantitative determination) for the speciation forms of arsenic, antimony and chromium in water and sediments. Water samples were taken from the middle part of the river current, by dipping the sampler below the water surface. Both water and bottom sediments were collected always at the same sampling points. Sediments were collected from the layer thickness of 0–5 cm. The in situ physicochemical data (temperature, pH, electrolytic conductivity (EC), redox potential (Eh)) were collected using a CX-401 multi-parameter meter (Elmetron, Poland) equipped with the ERH 111 glass electrode (Hydromet, Poland), ERPt-111 platinum electrode (Hydromet, Poland) and CD-2 conductometric sensor (Hydromet, Poland) with a built-in thermometer. Each water sample was divided into four parts to determine total content of arsenic, antimony and chromium; and to determine the content of the arsenic, antimony and chromium speciation forms. Directly after the sampling, 20 μl of the saturated Na₂EDTA solution was added to preserve the water samples in order to determine the antimony speciation forms. The samples dedicated to determining the arsenic and antimony speciation forms were not preserved. The samples used to determine the total analyte contents were acidified with ultra-pure HNO₃ (Merck, Germany). The sediment samples were also divided into parts. Three of them were frozen for later extraction and determination of the arsenic, antimony and chromium speciation forms. Part of the sediment was air-dried. The air-dried sediment samples were sieved through a sieve (2-mm mesh) and were used for the microwave mineralization to analyse the total analyte content in the sample. The water and sediment samples for speciation analyses were stored at −22°C in a freezer (not longer than for a month).

Sample preparation

The water samples were filtered through an injection PES filter (0.22 μm) just before the analysis. Afterwards, the samples were analysed with the ICP-MS spectrometer to determine total analyte content and with HPLC-ICP-MS for speciation analyses.

The first stage of the sediment sample preparation consisted in defrosting, centrifuging and extracting each sample. The aim of the extraction was to leach the easily leachable arsenic, antimony and chromium fractions from the sediment samples. The certified reference material (CRM) for sediments (NCS DC 73309 Stream Sediment; China National Analysis Centre for Iron and Steel) was selected to optimize the best possible extraction method for the easily leachable forms of arsenic, antimony and chromium. From the biological viewpoint, the easily leachable fraction has the biggest importance for living organisms. For this reason, two extraction methods were used, i.e. the extraction with the phosphate buffer in an ultrasonic bath (Jabłońska-Czapla et al. 2014) for arsenic and chromium speciation forms, and extraction with 20 mmol/l Na₂EDTA with 2-h shaking at room temperature for the antimony speciation forms. The extraction with the phosphate buffer was not possible for the antimony speciation forms as there was a very high antimony background coming from the very extractant. It was also found that Sb(III) oxidized to Sb(V) under the phosphate buffer influence and excessively drastic conditions. No such effects were obtained during the extraction with deionized water. However, the best results were achieved when the antimony speciation forms were extracted with a weak Na₂EDTA solution. Additionally, it stabilized and preserved the sample. The 1-g freshly
defrosted sediment samples were first centrifuged (20,000 rotations/min for 5 min). Afterwards, 10 ml of the phosphate buffer (5 mmol/L Na$_2$HPO$_4$ and 50 mmol/L KH$_2$PO$_4$ pH = 6.0 ± 0.2) was added for the arsenic and chromium extraction. 10 ml of 20 mmol/L Na$_2$EDTA was added for the extraction of the antimony ionic forms. Afterwards, each sediment sample was shaken in a shaker (165 rotations/min) for 2 h. After the obtained eluate was filtered through a 0.22-μm injection filter, the arsenic, antimony and chromium speciation forms were quantitatively determined for each analyte separately. The individual separation conditions were established and optimized.

**Reagents and standard solutions**

The following substances were used for analyses: ultrapure ammonium nitrate (Merck, Germany), ultrapure potassium dichromate (Merck, Germany), 1,000-mg/L Sb(III) standard solution for ICP (Merck, Germany), 1,000-mg/L Cr(III) standard solution for ICP (Merck, Germany), dihydro sodium arsenate heptahydrate ACS reagent (Sigma-Aldrich, Spain), sodium arsenite purum p. a. ≥99% (Sigma-Aldrich, Sweden), disodium methyl arsenate analytical standard (Supelco, USA), arsenobetaine ≥95% NMR (Fluka), dimethylarsinic (Supelco, USA), potassium hexahydroantimonate(V) (Sigma-Aldrich, Czech Republic), phthalic acid p. a. (POCH, Poland), ultrapure nitric acid (65%, Merck, Germany), disodium ethylenediaminetetraacetate (Na$_2$EDTA) for electrophoresis and molecular biology (titration) (Sigma-Aldrich, USA), analytically pure dihydrogen potassium phosphate (POCH, Poland), analytically pure disodium phosphate (POCH, Poland) and ultrapure ammonium nitrate (Merck, Germany). The calibration solutions were prepared each time through diluting suitable standard solutions on an analytical balance. The multi-elemental standards no. XXI and VI (Merck, Germany) were used when determining total arsenic, antimony and chromium with ICP-MS. The ICP-MS spectrometer was optimized daily with a 10-g/L solution (Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, U) in 1% HNO$_3$ Elan 6100 Setup/Stab./Masscal. Solution (Perkin-Elmer). All solutions and standards were prepared with the Milli-Q-Gradient ultrapure deionized water (Millipore, Merc, Germany), whose electrolytic conductivity was <0.05 μS/cm. The method validation was based on the certified reference material (NIST 1643e Trace elements in water).

**Analytical method applied**

Within the project realization framework, quantitative determinations of the total arsenic, antimony and chromium contents in water and sediments (after complete mineralization in the microwave mineralizer) were carried out. The total contents of arsenic, antimony and chromium were determined with the ICP-MS spectrometer (Elan 6100 DRC-e Perkin Elmer). The device was equipped with a standard ICP quartz torch, cross-flow nebulizer, and nickel cones. The samples and standards were fed with a peristaltic pump. $^{103}$Rh was used as an internal standard. The spectrometer was optimized daily. The ICP-MS spectrometer parameters were the same as in the previous study (Jabłońska-Czapla et al. 2014).

Analysis of the arsenic (AB, As(III), MMA, DMA, As(V)), antimony (Sb(III), Sb(V)) and chromium (Cr(III), Cr(VI)) forms in the water and sediment samples were performed in the HPLC-ICP-MS system. The water samples were analysed directly after filtering, whereas the procedure for the sediments was performed after the extraction.

For HPLC-ICP-MS, a 200 LC series speciation system was used. It contained a high-performance Perkin-Elmer liquid chromatograph (Series 200 LC Peltier oven, Series 200 LC autosampler, Series 200 LC gradient pump). The optimized parameters for the separation of the researched speciation forms, together with the retention times, are given in Table 1.

**RESULTS AND DISCUSSION**

**Methodology validation**

The extraction methodology allows for the elution of most analytes related with the easily leachable fractions. To check the performance of the proposed extraction procedures, they were compared with the results obtained for a Community Bureau of Reference (BCR) extraction procedure (Tokalioglu et al. 2005) for the same samples (Table 2). The determination method for arsenic, antimony and chromium speciation forms was validated with certified reference materials for water and sediments (NCS DC 73309 and NIST 1643-e). To check the recovery of the
The proposed extraction procedure, the standard addition method was used (Table 3). The sediment standards underwent extraction.

The calibration curves were obtained through measuring the standard solutions (concentrations of 1, 5 and 10 μg/L) for five speciation arsenic forms, two chromium speciation forms and two antimony speciation forms. The linear model of the concentration dependence on total analyte counts was selected. The obtained curves had good compatibility. Using the numerous determinations of the calibration curves, they also helped to calculate the limit of detection (LOD) for the inorganic arsenic speciation forms. The LOD calculation was based on the following dependence:

$$\text{LOD} = \frac{3s}{b}$$

where $s$ – standard deviation value; $b$ – slope of a straight calibration line.

The standard deviation value could be determined as the standard deviation of the offset of the obtained calibration curve. The limits of quantification are given in Table 3.

The optimized method is selective and demonstrates low detection limits. The repeatability, intermediate precision...
and accuracy allow for its use in the trace analysis of environmental samples.

Elimination of the matrix interferences

Under typical chromatographic separation conditions, Cr(VI) was leached as the first form with the A mobile phase (Table 1). Cr(III) was leached with the B mobile form as the second form. Two main problems related to the chromium speciation, i.e. Cr(III) precipitation and Cr(VI) reduction to Cr(III) in the presence of the reducing agent, were not observed for this separation method. Despite using ultra-pure reagents, the contamination of the mobile phase with chromium cannot be ignored. When the chromium speciation forms were determined, there was a peak coming from the eluent contaminants. Nevertheless, the multiple measurement of the blank samples showed that the height and retention time of this peak were stable and did not have any significant influence on the analysis course.

Chlorine is a commonly found element in the environmental samples. The polyatomic individuals of $^{35}\text{Cl}^{16}\text{O}^-$ and $^{37}\text{Cl}^{16}\text{O}^-$ cause the creation of the peaks interfering with $^{52}\text{Cr}^+$ and with $^{53}\text{Cr}^+$, respectively. Nevertheless, due to using the separation parameters enlisted in Table 2, $\text{Cl}^-$ was completely leached from the column before the chromium speciation forms. In Figure 1 there is a chromatogram obtained from the measurement of the real sample from the Klodnica River and a chromatogram of the standard sample containing both chromium speciation forms. There is a visible peak coming from the chloride ($^{37}\text{Cl}^{16}\text{O}^-$) interference with the retention time of approximately 0.3 min. However, it had no influence on the peaks coming from the chromium forms, i.e. Cr(VI) (approximately 1 min) and Cr(III) (approximately 3 min).

The carbon interference in the form of the polyatomic $^{40}\text{Ar}^{12}\text{C}^-$ ion is also a strong interfering agent during the $^{52}\text{Cr}$ analysis. For this reason, the chromium determination was performed with the $^{53}\text{Cr}$ isotope, whose isotopic $^{40}\text{Ar}^{13}\text{C}^-$ interference is less intensive due to the lower abundance of the $^{13}\text{C}$ isotope.

The common chloride interference of $^{40}\text{Ar}^{35}\text{Cl}$ may pose a basic analytical problem in arsenic detection using ICP-MS. This interference had a great significance for the real

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Validation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>0.19</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>0.37</td>
</tr>
<tr>
<td>Sb(III)</td>
<td>0.009</td>
</tr>
<tr>
<td>Sb(V)</td>
<td>0.012</td>
</tr>
<tr>
<td>As(III)</td>
<td>0.08</td>
</tr>
<tr>
<td>As(V)</td>
<td>0.12</td>
</tr>
<tr>
<td>AB</td>
<td>0.16</td>
</tr>
<tr>
<td>MMA</td>
<td>0.08</td>
</tr>
<tr>
<td>DMA</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Figure 1 | Chromatogram of the real Klodnica River water sample and chromatogram of the chromium speciation form standards (K2 – Ruda Śląska sampling point, August 2014).
samples from the highly saline Upper Silesian rivers. Consequently, its elimination was highly important. Silver cartridges for halide removal were used to remove salts from the Kłodnica and Bytomka river water samples. They enabled the partial removal of the chloride ions. Unfortunately, their price was too high for routine analyses of the numerous real water samples. Consequently, the separation conditions for particular speciation forms were selected in such a way that the peak from the chloride interference was placed between the DMA and MMA (arsenic speciation forms) peaks (Figure 2).

CONCLUSIONS

The preceding study focuses on presenting a simple procedure for determining inorganic speciation forms of chromium, organic and inorganic speciation forms of arsenic, and inorganic speciation forms of antimony at trace levels with the HPLC-ICP-MS technique.

The anion-exchange Dionex Ion Pack AG7 pre-column was used to separate the cationic Cr\(^{3+}\) and anionic Cr\(^{6+}\) forms. The anion-exchange Hamilton PRP-X100 column was used for the five arsenic speciation forms, whereas Sb(III) and Sb(V) were separated with the anion-exchange Dionex Ion Pack AS7 column.

The ions were separated directly without adding any complexing compounds, which was very important for the trace analysis as the sample contamination was limited. The full separation was achieved within 3–9.5 min, depending on the analyte. Such a situation had great importance in the routine analyses of many samples. The careful selection of the separation conditions for five arsenic speciation forms helped to eliminate strong chloride matrix interference (for the highly saline water). The optimization of the sediment extraction procedure enabled determination of the contents of specific ionic forms of arsenic, antimony and chromium without changing their oxidation states.

ACKNOWLEDGEMENTS

The MoSpeSil project (2013–2015) entitled ‘Mobility of arsenic, antimony and chromium speciation forms in selected river ecosystems of Upper Silesia’ is funded by Norway Grants as a part of the Polish–Norwegian Research Programme carried out by the (Polish) National Centre for Research and Development. This paper was presented as a poster at the 38th International Symposium on Environmental Analytical Chemistry in Lausanne (Switzerland).

Conflict of interest statement: The authors declare no financial/commercial conflict of interest.
REFERENCES


Ammann, A. A. 2002 Speciation of heavy metals in environmental water by ion chromatography coupled to ICP-MS. Analytical and Bioanalytical Chemistry 372, 448–452.


Czaja, S. 1999 Changes in the Water Under the Conditions of a Strong Anthropopressure (for example, Katowice Conurbation). Wydawnictwo UŚ, Katowice.


Michalski, R., Jabłońska, M., Szopa, S. & Lyko, A. 2011 Application of ion chromatography with ICP-MS or MS detection to the determination of selected halides and metal/metalloids species. Critical Reviews in Analytical Chemistry 41, 133–150.


First received 23 March 2015; accepted in revised form 14 September 2015. Available online 25 September 2015