

dr Artur Kowalski
Adam Mickiewicz University in Poznań
Faculty of Chemistry
Department Water and Soil Analysis
Umultowska 89b
61-614 Poznań

Summary of Professional Accomplishments

**“Mercury in different sample matrix, analytical,
environmental and health aspects”**

Documentation supporting the application for the initiation of habilitation procedure



**ADAM MICKIEWICZ
UNIVERSITY
IN POZNAŃ**

Poznań, 2016

1. Education:

- 1989 – 1994** Secondary Technical School of Construction no. 1 in Poznań
Profile: General construction technician
- 1994 – 1996** Post-Secondary School in the Chemical Schools Complex in Poznań,
Profile: Environmental protection
- 1996 – 1999** Bachelor's degree course – Faculty of Geography and Geology, Adam Mickiewicz University in Poznań,
bachelor's degree at the Faculty of Chemistry, speciality: Environmental protection
- 1999 – 2001** Master's degree course – Faculty of Chemistry, Adam Mickiewicz University in Poznań, master's degree in chemistry, speciality: Environmental protection
- 2001 – 2006** Doctoral degree course – Faculty of Chemistry, Adam Mickiewicz University in Poznań, doctor of chemical sciences in the field of chemistry

2. Employment:

1/10/2006 – present: assistant professor at the Department Water and Soil Analysis, Faculty of Chemistry, Adam Mickiewicz University in Poznań

3. The scientific field, discipline and speciality:

Field: Chemical science

Discipline: Environmental protection

Scientific speciality: environmental protection, environmental chemistry, environmental analysis

4. Bibliography parameters

List of all publications (including publications being part of the habilitation dissertation), IF as of the year of publishing

The number of publications

Before the doctorate – 24

- IF before obtaining the doctoral degree as of the year of publication: **5.904**

After the doctorate – 35

IF after obtaining the doctoral degree as of the year of publication: **20.055**

- IF of the publications included in the habilitation dissertation: **11.781**

Total IF – 25.955

Scientific publications: **22**

Popular science publications: **37**

Conference papers:

– conferences in Poland: **11**

– conferences abroad: **4**

Monographs: **1**

Book chapters: **10**

Total number of MNiSW (Ministry of Science and Higher Education) points: **460**

Number of citations: **266**

H-index: **9**

5. Description of the works constituting the scientific and research achievement

The main research interest after obtaining the doctoral degree was to determine total mercury and mercury fractions in complex matrix samples using optimized techniques of determination of the studied material. In the work, attention was also given to the aspects of collecting and preparing samples for the analysis. The study focused on the occurrence, possible mobility and bioavailability of mercury in different elements of the environment, and potential effects of mercury on human health. Mercury concentration in complex matrix

samples was determined using a cold-vapour atomic fluorescence spectrometer (CV-AFS) Millennium Merlin Analyzer 10.025 (PSAnalytical, England). The obtained study results were presented in a series of eleven publications devoted to the same subject (**H1-H11**), being part of the scientific achievement referred to in Article 16 section 2 of the Act of 14 March 2003 on Academic Degrees and Title and Degrees and Title in the Arts (Journal of Laws Dz.U. No. 65, item 595 as amended). The remaining research results were published in peer reviewed journals other than the “Philadelphia List”, peer reviewed conference materials, book chapters, and a scientific monograph (Appendix 3).

A. Title of the scientific achievement

“Mercury in different sample matrix, analytical, environmental and health aspects”

B. List of publications being part of the research scientific achievement

H1. Kowalski A., Boszke L., Siepak M. (2007) Mercury Contamination of Surface and Ground Waters of Poznań City, Poland. Polish Journal Environmental Studies 16 (1):67-74

H2. Boszke L., Kowalski A. (2007) Total Mercury in Floodplain Soils of the Warta River, Poland. Polish Journal Environmental Studies 16 (4):517-523

H3. Kowalski A., Siepak M., Frankowski M., Ziola A., Siepak J. (2007) Determination of mercury in sedimentary rock samples using cold vapour atomic fluorescence spectrometry Oceanological and Hydrobiological Studies XXXVI (3):143-153

H4. Boszke L., Kowalski A., Siepak J. (2007) Fractionation of mercury in sediments of the Warta River (Poland) Environmental Engineering – Pawlowski, Dudzińska & Pawlowski (eds). Taylor & Francis Group, London

H5. Boszke L., Kowalski A., Szczuciński W., Rachlewicz G., Lorenc S., Siepak J. (2006) Assessment of mercury mobility and bioavailability by fractionation method in sediments from coastal zone inundated by the 26 December 2004 tsunami in Thailand. Environ Geol 51:527-536

H6. Boszke L., Kowalski A. (2007) Mercury fractionation in sediments of the Lower Vistula River. Oceanological and Hydrobiological Studies XXXVI (3):79-99

H7. Boszke L., Kowalski A. (2008) Mercury fractionation in floodplain soils of the Warta River, Poland Oceanological and Hydrobiological Studies XXXVII, Suppl 1: 21-33

H8. Boszke L., Kowalski A., Astel A., Barański A., Gworek B., Siepak J. (2008) Mercury mobility and bioavailability in soil from contaminated area. Environmental Geology 55:1075-1087

H9. Kowalski A., Frankowski M., Ziola-Frankowska A., Mocek-Płóciniak A., Siepak, J. (2012) Variability of Mercury Concentrations in Soil and Leaves of *Acer plantanoides* and *Tilia platyphyllos* in Poznań City, Poland. Soil and Sediment Contamination vol. 21, 8:1022-1031

H10. Kowalski A., Frankowski M. (2015) Levels and potential health risks of mercury in prescription, non-prescription medicines and dietary supplements in Poland. *Regulatory Toxicology and Pharmacology* 73:396-400

H11. Kowalski A., Frankowski M. (2016) Seasonal variability of mercury concentration in soils, buds and leaves of *Acer platanoides* and *Tilia platyphyllos* in central Poland. *Environ Sci Pollut Res.* 23:9614–9624

C. Description of the works constituting the habilitation dissertation.

Introduction

Mercury and its compounds are considered to be among the most dangerous substances able to migrate in the natural environment. The circulation of mercury in the environment is a very complex process, because it undergoes constant transformations in individual components of the environment: air, soils, waters, bottom sediments, plants, or living organisms. Due to its high toxicity, the ability to bioaccumulate and biomagnify in living organisms, mercury has been in the center of interest of researchers and the public opinion for many years.

Deadly poisonings with mercury compounds that occurred at the Minamata Bay in Japan in the 1950s attracted the public opinion to the danger resulting from excessive concentration of this metal in the environment. The Japanese tragedy led to the development of new selective analytical methods that help analysts learn more and more advanced and complicated processes connected with mercury circulation in human's natural environment. However, it is difficult to obtain reliable results of mercury determinations in complex matrix samples. It depends on many factors: the appropriate way of sample collection and transport, sample storage and preparation, and the selection of the optimum analytical procedure. Taking into consideration very low mercury content e.g. in mobile fractions, the analyst's appropriate "workshop" must be ensured. Errors that may be generated at this stage of the work significantly affect the final outcome of the assays, making them an unreliable source of information about mercury content in human's natural environment.

The most popular methods of determination of total mercury content at very low levels (ng, pg) in environmental samples are: cold vapour atomic fluorescence spectroscopy (CV-AFS) and cold vapour atomic absorption spectroscopy (CV-AAS), with single or even double amalgamation. However, information on total mercury content is not enough to know the toxicity of the element, its bioavailability, and potential migration in human's natural

environment. Therefore, fractioning and speciation analysis is often used in analysis. Only this analytical approach can enable a full assessment of the effects of mercury on natural environment. In addition, measurements of e.g. fractions of mercury may be useful for the determination of ecological changes and effects on health caused by the occurrence of mercury in the ecosystem.

The goal of the research carried out as part of the habilitation dissertation was:

1. Elaboration of procedures and new analytical methods for trace mercury determination by single- and multi-step extraction in different matrix samples.
2. Comprehensive determination of total mercury content in different components of the environment of Poznań agglomeration
3. Determination relations of mercury migration over the soil – tree leaves route
4. Measurement of mercury content in medicines and dietary supplements using pressure microwave-assisted mineralization. Estimation of potential threat to the health of people taking medications.

Development of a new analytical procedure to ensure trace amounts of mercury in complex matrix samples

Mercury analysis is quite a well known issue in analytical chemistry. A very important issue affecting the final analytical result is the proper selection of the sampling method, preliminary processing and storage of the samples, as well as isolation and/or doping of mercury compounds and their quantitative determination.

In my study of mercury content in complex matrix samples I used the newly developed analytical procedure aimed at eliminating errors originating in the stage of preparation of the sample and the analysis, which may influence the final evaluation of mercury content in the investigated material. The first issue of key importance at the determination of mercury in samples with very low contents of that metal was to ensure reagents with appropriate purity. I often checked several manufacturers and the batch of reagents in terms of mercury concentration. Another very important aspect I highlighted was to develop a procedure of preparation of deionized water with appropriate purity. In order to remove the trace amounts of mercury from water, I placed the water in a glass bottle and then purified it with a stream of argon for 12 hours.

The next stage of the procedure was to prepare clean containers and laboratory glassware for mercury analyses. In the analytical process, this stage is often neglected by many analysts dealing with this issue. For this purpose, I developed a procedure of glassware washing that involves washing the glass with deionized purified water (WRO), etching the glassware with 10% HNO₃ for 48 hours, washing it with deionized purified water, and etching again with 5% HNO₃ for 24 hours. After that time, I washed the glass (WRO) and filled it with deionized purified water. If a laboratory glass was not used immediately for assays, I stored it in plastic bags. If filters were used to prepare the samples, I washed them with 450 ml of deionized purified water.

Checking the purity of reagents, water and laboratory glassware or appropriate preparation of filters for the subsequent stages of sample preparation procedure confirms how important analytical purity is. Having the analytical procedure checked, I could carry out the research activities involved in the scientific achievement (**H1-H11**).

Comprehensive determination of total mercury content in different components of the environment of Poznań agglomeration

The aim of the study was the determination of total mercury in different components of the environment and recognition of the mercury sources in the Poznan agglomeration. In the first work included in the habilitation dissertation (**H1**) I presented the results of spatial distribution of mercury in surface water and ground water samples collected from the city of Poznań. In order to carry out the research tasks presented in the publication (**H1**), I collected samples from all the lakes, ponds and rivers within the city of Poznań. Moreover, I collected ground water samples from randomly selected emergency wells drilled mostly in shallow Quaternary permeable formations (at the depth of 6-25 metres). It must be mentioned that it was the first comprehensive study in this area, both for surface waters and ground waters. On the basis of the obtained results, I concluded that the spatial distribution of mercury in surface water samples collected from different locations in Poznań is relatively uniform. The highest mean concentration was found in samples collected from the Warta River, $27 \pm 7 \text{ ng}\cdot\text{L}^{-1}$ (range 19.6-36.0 $\text{ng}\cdot\text{L}^{-1}$). In the other rivers and streams, the value was $23 \pm 12 \text{ ng}\cdot\text{L}^{-1}$ (range 11.7-39.5 $\text{ng}\cdot\text{L}^{-1}$), and in ponds it was $20 \pm 12 \text{ ng}\cdot\text{L}^{-1}$ (range 16.2-31.3 $\text{ng}\cdot\text{L}^{-1}$). The lowest mean concentration of mercury was found in lakes, $15 \pm 5 \text{ ng}\cdot\text{L}^{-1}$ (range 7.6-23.8 $\text{ng}\cdot\text{L}^{-1}$). The conducted ANOVA only showed statistically significant differences between the concentrations of mercury determined in samples of water collected from the Warta river and from lakes ($p = 0.0354$). The obtained results of mercury content in surface waters (Fig. 1) are

typical of industrial areas and do not differ significantly from the concentrations described by other authors [23].

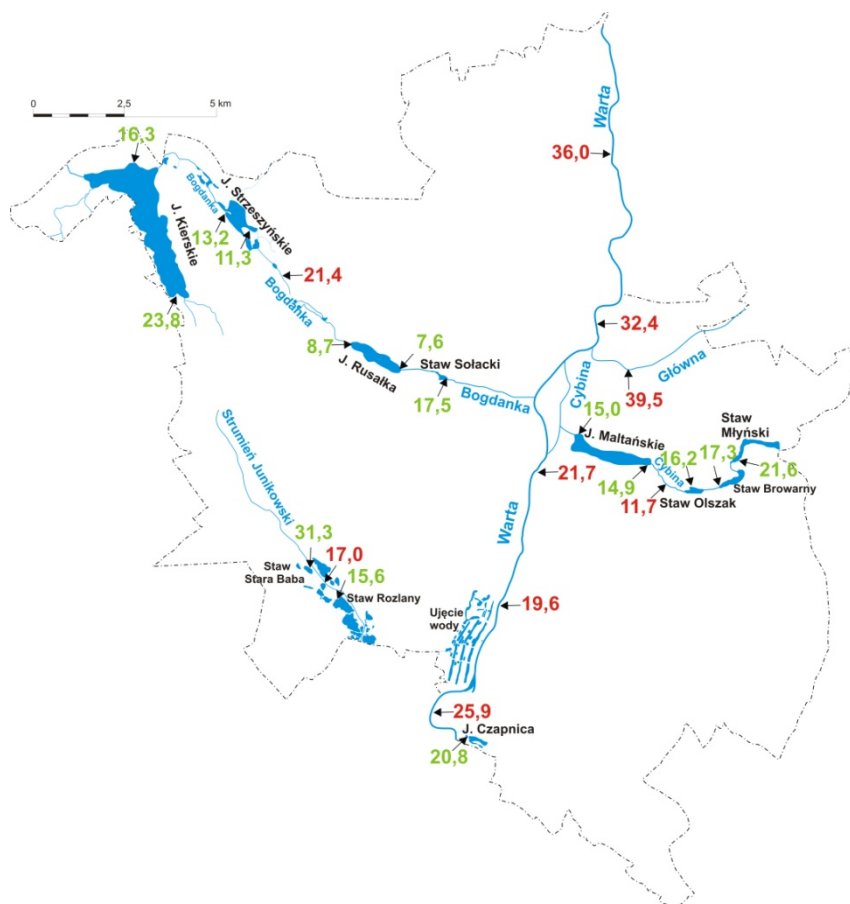


Fig. 1. Mercury concentration in surface waters of the city of Poznań (ng·L⁻¹)

The results of the research presented above made it possible to propose a hypothesis that mercury contained in surface waters mostly originated from the re-emission of atmospheric mercury and was connected with wet and dry precipitation [28]. The investigated area is constantly under the influence of different industrial and urban emission sources, mainly including: coal thermal power stations ZEC Karolin and ZEC Garbary, local boiler rooms and domestic stoves with coal as the basic source of heat. Other sources are: a municipal and hospital waste stockpile, cement plants, wastewater treatment plants, factories using high temperature in industrial processes, and traffic. The reason for slightly increased concentrations found in rivers in comparison with standing surface waters might have been the mainstream carrying greater amounts of organic matter in the form of a suspension on which mercury was adsorbed [6]. I concluded that the other factor increasing mercury concentration in rivers might be illegal wastewater discharge.

Although Poznań is regarded as an industrial town, I did not observe high mercury concentrations in ground waters. The mean value was 1.3 ± 0.7 ng·L⁻¹ (range 0.8-4.1 ng·L⁻¹).

These concentrations were low, typical of background value [22]. Comparing the mean concentrations of mercury for surface waters ($20.0 \pm 8.0 \text{ ng}\cdot\text{L}^{-1}$, range 7.6-39.5 $\text{ng}\cdot\text{L}^{-1}$) with the results for ground waters, I found that the samples of ground waters had much lower concentrations of that metal.

I also noticed that the obtained results of mercury concentrations in ground waters displayed uniform spatial distribution, just like in the case of surface water concentrations. I did not find any considerable difference between the extreme values of mercury in ground waters.

The research described in the **H1** publication was continued in works **H2 and H4**. They were the development and extension of research interest with another element of human's natural environment. I determined mercury concentrations in samples of soils and bottom sediments collected from the floodplain of the middle part of the Warta river before, in and after the Poznań agglomeration (**H2,H4**). The results obtained in a study of total mercury content gave me the information on the variability of concentration of this element in bottom sediments and in soils depending on the place of sampling. On the basis of Kruskal-Wallis test, I found that there were no statistical differences between the mercury contents in the samples of soil collected from Poznań and those before or after Poznań ($p = 0.283$). However, I found the highest values in soils both below Poznań (median 300 $\text{ng}\cdot\text{g}^{-1}$, range 75-884 $\text{ng}\cdot\text{g}^{-1}$) and above Poznań (median 228 $\text{ng}\cdot\text{g}^{-1}$, range 54-774 $\text{ng}\cdot\text{g}^{-1}$) that in samples collected from Poznań (median 183 $\text{ng}\cdot\text{g}^{-1}$, range 72-303 $\text{ng}\cdot\text{g}^{-1}$). The observed higher mercury concentrations in soil and bottom sediment samples (**H2,H4**) collected after the city of Poznań are typical of urbanized areas and such a relation is usually found behind a city agglomeration [70]. I also noticed higher values of mercury in floodplain soils after Poznań than before it (**H2**). The analyzed section of the Warta river below Poznań mostly goes through agricultural areas. In agricultural areas mercury may be remobilized, because mercury compounds were commonly used in the previous century as fungicides [48]. Another cause of higher concentrations in floodplain soils both before and below Poznań may be the fact that samples were collected after spring melt and the subsiding of high water levels in the Warta river. My observations during sample collection show that the banks of the Warta river in Poznań are regulated, but in other places the river floods the floodplains if the water level is high. When the water table lowers, a suspension containing high amounts of organic matter is deposited in soils, and as my study demonstrated, this causes elevated mercury values in these places.

The Kruskal-Wallis test also showed that there are no statistically significant differences in mercury content in the samples of soil collected at different distances from the

river channel ($p = 0.812$). The mean mercury content in soil samples collected 1 meter away from the main stream was $228 \text{ ng}\cdot\text{g}^{-1}$ (range $7\text{-}637 \text{ ng}\cdot\text{g}^{-1}$), in those collected 10 m away, $185 \text{ ng}\cdot\text{g}^{-1}$ (range $80\text{-}754 \text{ ng}\cdot\text{g}^{-1}$), and 50 m away from the river, $190 \text{ ng}\cdot\text{g}^{-1}$ (range $54\text{-}884 \text{ ng}\cdot\text{g}^{-1}$) (**H2**). I found out that the soils of the Warta river floodplain had higher mercury contents (median $198 \text{ ng}\cdot\text{g}^{-1}$, range $54\text{-}884 \text{ ng}\cdot\text{g}^{-1}$) (**H2**) than the river sediments (median $121 \text{ ng}\cdot\text{g}^{-1}$, range $47\text{-}340 \text{ ng}\cdot\text{g}^{-1}$) (**H4**). A similar relation was described by other authors, too [70]. Higher contents of mercury in floodplain soils can be explained with the fact that these samples contained higher amounts of organic matter (mean 6.58% , range $1.2\text{-}28.4\%$) (**H2**) than did river sediments (mean 2.19% , range $1.0\text{-}4.4\%$) (**H4**). As demonstrated by many authors, mercury in soils with weakly acidic to alkaline reaction is strongly bound to macromolecular humus substances [9, 46, 57, 71]. The obtained results of mercury content in floodplain soils (**H2**) also show higher mercury values than do the results obtained by Boszke and Kowalski (2006) for the soils within the city of Poznań (mean $146 \pm 130 \text{ ng}\cdot\text{g}^{-1}$, range $17\text{-}746 \text{ ng}\cdot\text{g}^{-1}$).

The conducted study was one of the first measurements of mercury in that area, and the described observations are very important for environmental protection. The determined high mercury contents in soils of the Warta river floodplain may have some consequences in the case of copious precipitation and increased surface run-off or high water levels during the flood. The mercury deposited in soils may be mobilized and transported to the river, causing secondary water contamination.

Development of new analytical methodologies of mercury determination using single- and multiple-stage extraction and applying them in the determination of mercury fractions in complex matrix samples

In environmental analysis, the determination of total mercury content is very important but does not give the full information on the toxicity of the element, its bioavailability, and potential migration in the environment. Full knowledge on the influence of mercury on the environment may result from the determination of mercury fractions using sequential extraction. However, such determinations are very complicated, expensive and time-consuming, and the applied analytical methodologies can only be used for mercury determination.

The main research task was to create a methodology of single-stage extraction that can be used to determine the mobility of mercury and heavy metals (**H3,A58**) in samples of sedimentary rocks collected from the drilling profile in the Warta valley. In this region, there is the Mosina-Krajkowo intake of Quaternary ground waters. In the work, I evaluated the

content of mercury depending on the granulometric fraction in the S9 profile, taking into account the changing rock age series and the type of rock (sand, gravel, slime, clay, or loam) **(H3)**.

The first stage of the study was the granulometric analysis of sedimentary rocks in accordance with the guidelines of PN-ISO 565:2000 and PN-ISO 3310-1:2000. I identified granulometric fractions with grain sizes: >2.0; 2.0-1.0; 1.0-0.5; 0.5-0.25; 0.25-0.1; 0.1-0.063; < 0.063 mm. On the basis of the results of analysis, I found that the fraction with 0.5-0.25 mm grain size is the most common (mean 23.9%; range 0.94-68.2%), followed by 0.25-0.1 mm (mean 22.2%; range 0.6-78.1%) and 1.0-0.5 mm (mean 20.0%; range 0.18-49.3%) and the 0.1-0.063 mm fraction has the lowest share (mean 5.7%; range 0.13-37%). The obtained grain size results are connected with the geological formation of the study area, mainly composed of: fine- and medium-grain sediments of the contemporary Warta river (fH), gravel, sand and gravel mix, and sand of the Baltic glaciation (fB), as well as fine- and medium-grain sand connected with the Holstein interglacial (N₂).

The most important stage of the work was the choice of the optimum extractant. When choosing the extractant, I wanted it to be universal, to be able to use it for the single-stage extraction, not only of mercury but also of other heavy metals from sedimentary rock samples **(H3,A58)**. Heavy metal determination using single-stage extraction has been the subject of many studies, while diluted HCl as an extractant has numerous applications, e.g., to investigate the mobility of heavy metals from soils and sediments [17,24,32,55,56]. In my study of the mobility of mercury from sedimentary rocks, I used 3 mol·L⁻¹ hydrochloric acid as the extractant. The accuracy of the applied analytical technique had to be verified; for this purpose, I used the SRM 2709 reference material (National Institute of Standards and Technology, USA). I mineralized the reference material with aqua regia and performed single-stage extraction using 3 mol·L⁻¹ HCl. When using aqua regia, I had the recovery ratio of 102.5 ± 2.9% (accuracy 2.5%), and for 3 mol·L⁻¹ HCl the recovery ratio was 59 ± 3.2%. Then, so as to check the proposed method of preparation of sedimentary rocks, I performed single-stage extraction and mineralization in aqua regia as the reference method. The comparative study was performed for the most common granulometric fraction of 0.25-0.1 mm from the S9 fraction. The obtained mean ration of recovery in comparison to the total value in the samples from the whole S9 profile was lower than in the certified material and amounted to 39.9% (range 15.5-70.1%), which was connected with different area of contact between the sample and the acid **(H3)**.

The study of mobile forms of mercury in granulometric fractions was an innovative analytical approach. Analyzing the variability of concentration of labile forms of mercury depending on the size of granulometric fraction for the S9 drilling profile, I found out that mercury concentration decreased from the largest fractions >2.0 and $2.0-1.0$ mm to the fractions of $1.0-0.5$ mm grain size, where the lowest concentrations were determined (mean $24.4 \text{ ng}\cdot\text{g}^{-1}$, range $1.0-178 \text{ ng}\cdot\text{g}^{-1}$). Then, I observed an increase of mercury concentration along with the decreasing grain size. I observed the highest values in the fraction <0.063 mm, where the mean mercury concentration was $93.5 \text{ ng}\cdot\text{g}^{-1}$ (range $4.7-250 \text{ ng}\cdot\text{g}^{-1}$). However, this fraction was not isolated in each sample. The concentrations of mercury were also quite high in the fraction over 2 mm (mean $37.1 \text{ ng}\cdot\text{g}^{-1}$, range $1.0-199.5 \text{ ng}\cdot\text{g}^{-1}$). According to the recommendations of PN-ISO 11464 and PN-ISO 11466, this fraction is ignored when preparing the samples and not determined.

With the use of single-stage extraction, I performed a study of the variability of mercury concentration depending on the decreasing depth in the vertical S9 profile. In Figure 2, I presented the results of geochemical investigation with consideration of the age series of the rocks and the depth of the layer, type of rock (sand, gravel, slime, clay, or loam).

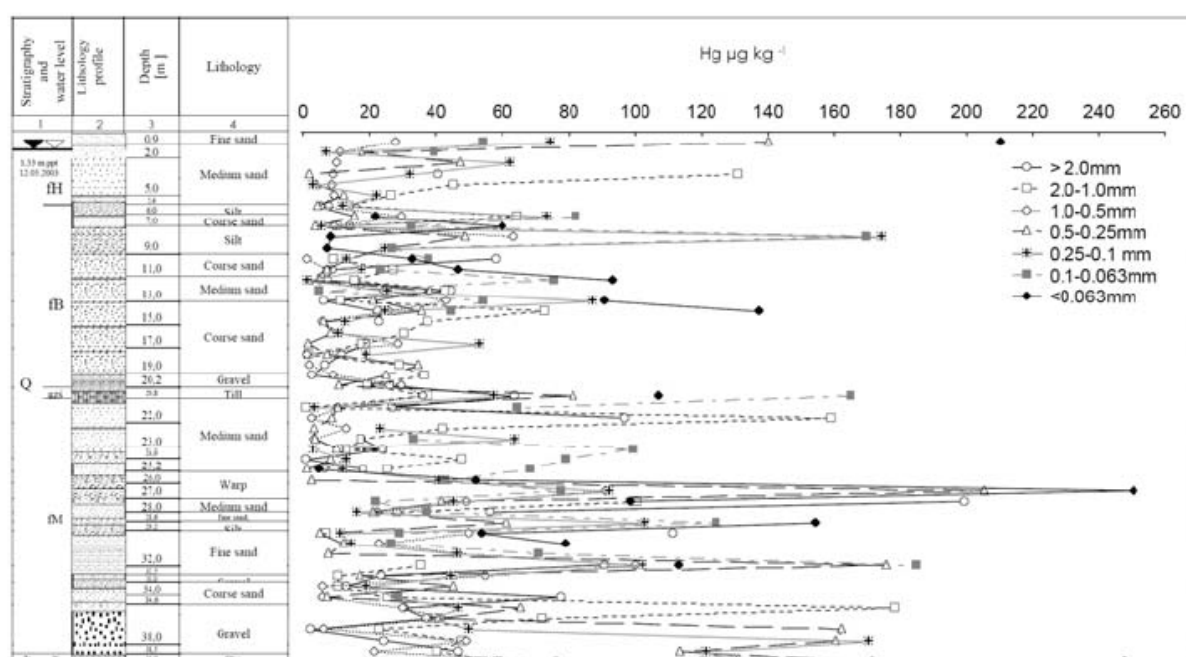


Fig. 2. Change of mercury concentration along with the depth and age of sedimentary rocks in S9 drilling profiles at the Mosina-Krajkowo intake of ground water ($\text{ng}\cdot\text{g}^{-1}$). (fH) sediments of the contemporary Warta river, (fB) Baltic glaciation sediments, (gzs) Riss glaciation sediments, (fM) Holstein interglacial sediments, N₂ loam of the Poznań Upper Miocene series, Q quaternary

In the sediments of the contemporary Warta river valley (fH), the highest concentration of $210 \text{ ng}\cdot\text{g}^{-1}$ was found in the < 0.063 mm granulometric fraction, in the surface loam layer. Next, the concentration decreased up to the depth of 4 m BGL. The next

increase of concentration occurred in the layer of slime in the Baltic glaciation formations (fB). The highest determined concentration was $174 \text{ ng}\cdot\text{g}^{-1}$ in the 0.25-0.1 mm fraction. In the lower layer of those formations, mainly composed of coarse-grain sand and gravel, I found the decreasing concentration of mercury down to $40 \text{ ng}\cdot\text{g}^{-1}$. Another marked increase of mercury concentration up to $165 \text{ ng}\cdot\text{g}^{-1}$, was determined in the till of the Riss glaciation (gzś) in the 0.1-0.063 mm fraction. The highest mercury concentration $250 \text{ ng}\cdot\text{g}^{-1}$ was found in the $< 0.063 \text{ mm}$ fraction of the Holstein interglacial formations (fM) with slime and mud layers. What is important, ground water is taken from these sediments as drinking water for Poznań residents. I found another rise in mercury concentration in the last layer of the studied profile, in the loam of the Poznań series of Upper Miocene (N_2). The maximum mercury concentration for this age series was $248 \text{ ng}\cdot\text{g}^{-1}$ and it was found in the finest granulometric fraction. Analyzing the results for all the age series of sediments, I concluded that the highest mercury concentrations in all the granulometric fractions occurred in the ceiling rock samples represented by loam of the Poznań series of Upper Miocene (N_2). With regard to the age series of the sediments, mercury concentrations had the following order: $N_2 > \text{gzś} > \text{fM} > \text{fB} > \text{fH}$.

The proposed single-stage extraction procedure may well be used for geochemical tests regarding mobile forms of heavy metals, including mercury. The use of $3 \text{ mol}\cdot\text{L}^{-1}$ HCl extraction provides more information on the potential migration of heavy metals (including mercury) and their bioavailability in the environment. The use of single-stage extraction is recommended for economic reasons (shorter reaction time and a lower number of reagents). The investigations concerning the mobile forms of mercury in granulometric fractions in sedimentary rock samples collected from different depth of drilling profile was an innovative analytical approach described in literature for the first time.

The leading idea of **H4-H8** works was to develop a new sequential extraction procedure to be used for routine determinations of complex matrix mercury fractions. Tessier et al. (1979) were the first researchers to apply sequential extraction; they determined five different metal fractions in bottom sediments. However, due to the specific properties of mercury different from the other heavy metals, the pattern of extraction procedure proposed by Tessier et al. (1979) should not be used in mercury analysis.

In the case of binding mercury with a solid matrix, two groups can be identified in terms of the potential availability of mercury for the environment. The first group is the forms

of mercury that are weakly bound with the solid phase in the natural environment and are the most mobile. This fraction is referred to as: exchangeable, water soluble, or bound to carbonates. The other fraction is the forms of mercury that are strongly bound to the solid phase and can be released from it as a result of changes in conditions of the system. This fraction is bound to hydroxides, organic matter, sulfides, and the residual fraction, and is referred to as the immobile fraction [7].

Source literature describes many schemes of sequential extraction to determine mercury fractions in sediments and soils, differing with the extractants used [3,5,15,30,35,36,42,44,70,72]. However, most of the schemes proposed by different researchers have been mainly used for contaminated samples with high mercury contents, coming from areas strongly transformed by people. My objective when doing the research was to create a universal scheme of sequential extraction to be used for fractioning mercury both in the case of low and high mercury content in the humans' natural environment. In the first stage of the work, I selected the appropriate extractants with increasing eluting power, and the extraction parameters, i.e., the amount and concentration of the reagents and the time of contact with the sample. On the basis of the conducted optimization tests, I received the scheme of sequential extraction (Fig. 3), providing information on five different fractions of mercury. I proposed the following mercury fractions: organic mercury fraction (F1) – extraction with the use of CHCl_3 , fraction washed out with water (FII) – extraction with the use of deionized water, fraction washed out with acids (FIII) – extraction with the use of $0.5 \text{ mol}\cdot\text{L}^{-1}$ HCl, fraction bound to organic matter (FIV) – extraction with the use of $0.2 \text{ mol}\cdot\text{L}^{-1}$ NaOH, and residual fraction (FV) – mineralization with the use of aqua regia.

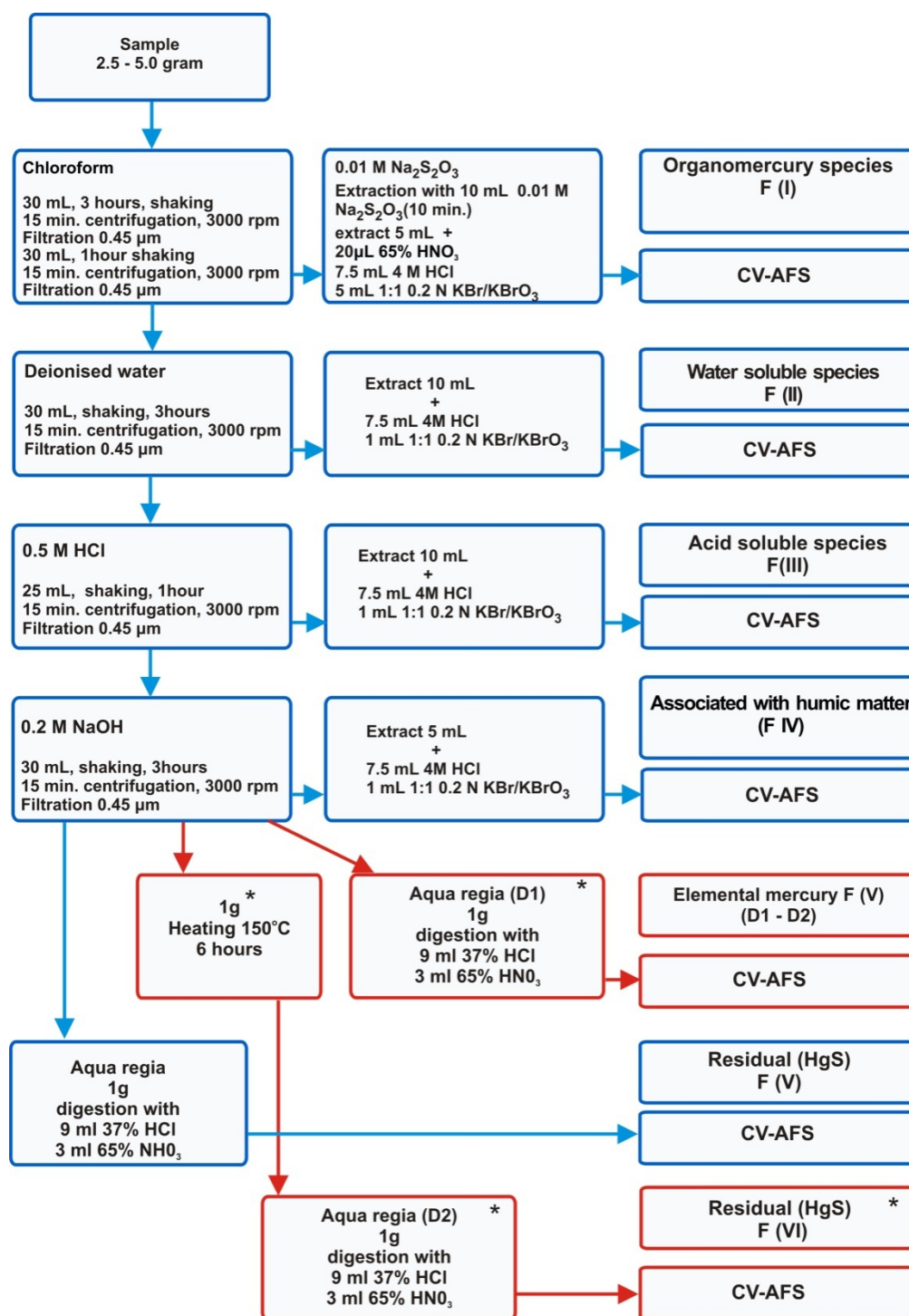


Fig. 3. Sequential extraction diagram (H4-H7). The elementary mercury fraction was marked in red (H8).

The next stage of the research was to check the recovery ration of the developed sequential extraction procedure on a certified reference material LGC 6137 (European Reference Material, Belgium). I mineralized the reference material with aqua regia in accordance with the manufacturer's recommendation and I performed five-stage sequential extraction. In the case of total mercury content, I achieved the recovery ratio of $109.7 \pm 2.0\%$. The percentage share of mercury in each fraction was respectively: (F I) 2.4%, (F II) 0.28% (F

III) 0.19%, (F IV) 5% and (F V) 101%. In a subsequent stage of the research, having an optimized and tested scheme of sequential extraction, I fractioned mercury in complex matrix samples (**H4-H8**). The samples collected for analysis had different origin, composition and degree of contamination. I used in the study the sediments and soils from Warta (**H4, H7**) and Vistula river (**H6**) floodplains, as well as strongly contaminated soils collected from the Areometr plant in Warsaw (**H8**). I was the first researcher ever to perform sequential extraction of mercury in samples of sediment deposited by a tsunami wave in Thailand (**H5**).

The determined contents of total mercury reported in works (**H4-H7**) had normal or slightly increased mercury values, typical of the region the samples were taken from. But I obtained very high values of mercury (mean $147 \pm 107 \mu\text{g}\cdot\text{g}^{-1}$, range $62\text{-}393 \mu\text{g}\cdot\text{g}^{-1}$) in the soils collected from the Areometr plant. Such high values prove strong contamination of soils with mercury coming from technological processes connected with the manufacture of thermometers and aerometers (**H8**).

The conducted sequential extraction of mercury in complex matrix samples gave me information on the fractions of mercury that are most significant from the perspective of environmental analysis (mobile and toxic fractions). The determined first three fractions (FI-FIII) contain most bioavailable forms of mercury, which may be washed out from the sample with little changes of environmental conditions. As for the other mercury fractions bound to organic matter (FIV) and sulfides (FV), they are more permanent in the environment, i.e., less mobile.

The results obtained for complex matrix samples undergoing the sequential extraction procedure (**H4-H8**) are discussed below, for each mercury fraction separately. In Figure 4, I presented an example percentage share of mercury versus total concentration for the samples of sediments deposited by the tsunami wave (**H5**).

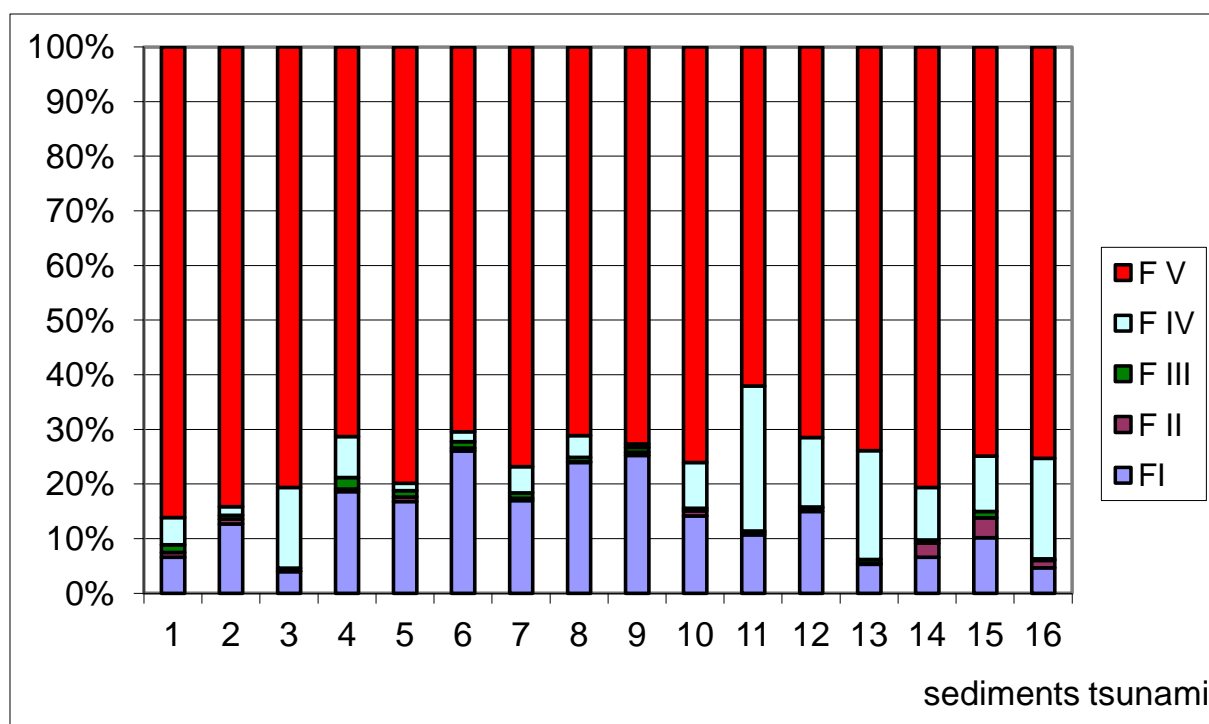


Fig. 4. The share (%) of mercury fractions vs total concentration in the samples of sediments from areas hit by a tsunami. (F I) organic mercury fraction, (F II) fraction washed out with water, (F III) fraction washed out with weak acids, (F IV) fraction bound to organic matter, (F V) fraction bound to sulfides (**H5**).

Discussion of the result of determination of mercury concentration in the organic mercury fraction (F I)

The organic mercury fraction is mainly made up of organic forms of mercury (CH_3Hg^+ , $\text{C}_2\text{H}_5\text{Hg}^+$, $\text{C}_6\text{H}_5\text{Hg}^+$), where mercury is directly bound to a carbon atom. Methylmercury ion may have the greatest share in this fraction. It is the most toxic form of mercury in the natural environment due to its ability to accumulate and biomagnify in a trophic chain (**A4,H4**). Yet, the use of organic extractants, e.g. chloroform or toluene, may cause the extraction of not only methylmercury ion but also a little mercury bound to organic matter [11]. Based on literature data, it can be said that usually the percentage share of mercury in this fraction to the total mercury content is low, not higher than a few % [11,47]. In isolated cases, however, the content may exceed 60% (54). On the basis of the experiments, I found out that the lowest percentage share of mercury in this fraction was in the samples collected from Warta river floodplains: $1.6 \pm 0.8\%$ (range 0.8 – 4.8%) (**H7**). Higher values occurred in soils collected from the Areometr plant in Warsaw $2.3 \pm 2.7\%$ (range 0.1 – 6.5%) (**H8**), Vistula river sediments $6.4 \pm 5.3\%$ (range 0.6 – 13%) (**H6**), sediments from Thailand $14 \pm 7\%$ (range 4 – 27%) (**H5**), and the highest share of mercury in this fraction was found in Warta river sediments $16.6 \pm 18.0\%$ (range 0.03 – 64.5%) (**H4**).

Discussion of the result of determination of mercury concentration in the fraction washed out with water (FII)

The content of mercury in the fraction washed out with water is usually very low, often below the limit of detection of the applied analytical technique [27]. The mean percentage share of mercury in this fraction vs total concentration does not exceed 4-7% [3,5,8].

In the conducted experiment I demonstrated that the lowest percentage share of mercury in the fraction washed out with water was observed in the samples of sediments deposited by the tsunami wave in 2004. The mean share of mercury in this fraction was $0.8 \pm 1.0\%$ (range 0.1 – 3.6%) (**H5**). I found a higher percentage share of mercury in the fraction washed out with water in samples of contaminated soils collected from the Areometr plant $1.0 \pm 0.3\%$ (range 0.6 – 1.7%) (**H8**). I found higher mercury concentrations in the sediments and flood plain soils of the Warta river: $2.1 \pm 0.9\%$ (range 1.1 – 3.8%) (**H4**) and $2.2 \pm 1.8\%$ (range 0.3 – 7.8%) respectively (**H7**). The highest mean concentration of mercury, $6.9 \pm 2.9\%$ (range 3.9 – 13.2%), was determined in the sediments and soils of the Vistula (**H6**).

Discussion of the result of determination of mercury concentration in the fraction washed out with weak acids (FIII)

The percentage share of mercury in this fraction is similar to or slightly lower than the share of mercury contained in fraction (FII) washed out with water and does not exceed a few % [4,5,8]. On the basis of the obtained results of mercury in the fraction washed out with $0.5 \text{ mol}\cdot\text{L}^{-1}$ HCl, I observed comparable values of % share of mercury. For the samples of Warta and Vistula river sediments, the values were respectively: 0.4% (range 0.2 – 1.9%) for the Warta river (**H4**) and 0.4% (range 0.1 – 0.7%) for the Vistula river (**H6**). Slightly higher values of Hg concentration were determined in sediments deposited by tsunami waves $0.9 \pm 0.5\%$ (range 0.2 – 2.1%) (**H5**). The highest concentrations of mercury were obtained in contaminated soils from Warsaw $1.5 \pm 1.3\%$ (range 0.1 – 3.5%) (**H8**) and the samples of soils collected from Warta river floodplains $2.0 \pm 1.7\%$ (range 0.5 – 10.0%) (**H7**).

Discussion of the results of mercury concentration in the fraction bound to organic matter (FIV)

Organic matter plays a very important role in binding mercury occurring both in bottom sediments and in soil. This is one of the most important stages of the majority of known procedures of mercury fractionation [3,5,30,35,44,70,72]. The obtained results

allowed me to conclude that the mercury concentration in this fraction is much higher than in the fractions described above. The highest percentage share of mercury in this fraction was determined in the soils collected from Warta river floodplains: $28.5 \pm 6\%$ (range 16.5 – 42%) (**H7**). Lower values of mercury concentration in the fraction bound to organic matter were found in bottom sediments of the Warta river $23 \pm 9\%$ (range 4 – 36%) (**H4**), in contaminated soils of the Areometr plant $22 \pm 9\%$ (range 11 – 34%) (**H8**), and in Vistula river sediments $19 \pm 10\%$ (range 10.0 – 35.2%) (**H6**). The lowest percentage share of mercury in fraction (FIV) was determined in the samples collected after the tsunami wave: it was $9.2 \pm 7\%$ (range 0.6% to 26.5%) (**H5**). The results described in publications **H4-H8** are comparable to data found in literature [3,5,30,35,44,70,72].

Discussion of the results of mercury concentration in the fraction bound to sulfides (FV)

The determination of mercury bound to sulfides for all the sequential extraction procedures is the last stage of fractioning. The % share of mercury in this fraction, both in soils and in contaminated sediments, is the highest [3,30,47]. For example, the mean percentage share of mercury bound to the residual fraction in sediments from the Gdańsk Bay was 40% (maximum 96%) [3]. A higher percentage share of mercury, amounting to 69%, was determined in soils by Lechler et al. (1997). In the sediments from the Kogushima Bay (Japan), the mean share of mercury in the fraction bound to sulfides was estimated to be 71.4% (range 38.4 – 96.1%) [47].

In the analyzed samples, the % share of mercury in this fraction was the highest, both in soils and in sediments. The highest percentage share of mercury in this fraction was determined in the sediment samples collected after the tsunami wave: $75.5 \pm 6.2\%$ (range 62.0 – 86.1%) (**H7**). Lower mercury concentrations in the fraction bound to sulfides were found in sediments collected from the Vistula river and floodplain soils of the Warta river. The mean percentage share was $68 \pm 11\%$ (range 54.7%-81.6%) for the Vistula river samples (**H6**) and $66 \pm 7\%$ (range 52.0 – 77.5%) (**H7**), respectively. In Warta river sediments, the concentration of mercury in this fraction was $57.7 \pm 13\%$ (range 20%-81%) (**H4**). The lowest percentage share of mercury in this fraction was determined in contaminated soils collected from the Areometr plant: the mean value was $56 \pm 8\%$ (range 45 – 66%) (**H7**).

Since I obtained very high contents of total mercury in soils collected from the Areometr plant, before the mercury fraction bound to sulfides I determined the elementary mercury fraction in those samples. The determination of elementary mercury involved computing the difference between the total mercury content before and after heating the

sample at 150°C for 6 hours (Fig. 3). The obtained results of mean concentration, i.e. $17 \pm 5\%$ (range 8 – 23%), prove very serious contamination of soils with elementary mercury used for the manufacture of mercury thermometers and aerometers (**H8**). The elementary form of mercury is usually not detected in samples that are not contaminated [30,37,44,47]. But in the samples of strongly contaminated soils, the maximum share of this fraction was 82% [30].

The use of sequential extraction allowed me to determine five fractions of mercury (**H4-H7**) and a sixth fraction of elementary mercury in the soils from the Areometr plant (**H8**). In the proposed scheme of sequential extraction (Fig. 3), the first three fractions: mercury extracted with chloroform (FI), washed out with water (FII) and extracted with diluted acid (FIII) included mobile forms of mercury, sensitive to changes of environmental conditions (e.g. floods or low water levels). The forms of mercury contained in those fractions migrate most quickly and are most bioavailable for organisms. The lowest summary share of mercury in the first three fractions (FI-FIII) was 4.9% and I determined it in contaminated soils collected from the Areometr plant (**H8**), while the highest percentage share was found in the samples of sediments collected from the Warta river and amounted to 19.2% (**H4**). Methylmercury ion occurring in fraction (FI) [11,53] is considered to be the most dangerous form for organisms. It is believed that the methylation speed has an impact on the concentration of Hg^{2+} , sulfides and organic matter, as well as the conditions of the reaction. The forms of mercury washed out with water in natural processes (fraction FII), and the forms of mercury washed out by diluted acid (fraction FIII) can be a potential source of mercury in methylation processes [5,36,53,60].

Mercury bound to organic matter is temporarily immobilized and excluded from circulation: it is less mobile. However, as a result of changes occurring in the environment during a flood or low water levels, some amounts of mercury compounds may be released from organic matter or attached to it [53]. In the last fraction (FV), mercury is mostly bound to sulfides, making a form least available for living organisms and least toxic [20]. Still, HgS may be transformed to an organic form, methylmercury, with the participation of sulfide-reducing bacteria and fungi [35,53].

As shown by the results of research published in works **H4-H8**, the developed procedure of sequential extraction may be used to determine the fractions of mercury in soils and bottom sediments collected both from non-contaminated areas and areas transformed by humans. From the point of view of environmental protection, these results give more information on the condition of the human's natural environment.

Determination relations of mercury migration over the soil – tree leaves route

Research on the content of mercury in soils and air regarding the variability of this metal in plants, including leaves, has been carried out for many years [1,12-14,38,40]. Most of the experiments, however, were carried out in laboratory conditions. Model measurements are only focused on the estimation of changes in mercury contents in plants during the experiment, as part of which mercury concentration in soils or air was modified. Seedlings or young plants were used in the research [1,12-14,38]. The use of such research material does not lead to reliable results, because young plants do not have a fully developed root system and are less resistant to contamination. The only relevant study was carried out by Poissant et al. (2008) on perennial trees growing in a natural forest, but in less than 2 months of vegetation period from August to October.

As part of my habilitation dissertation, I determined the spatial distribution of mercury in soils and leaves of 2 tree species: *Tilia platyphyllos* and *Acer platanoides*, as well as mercury bioavailability from soils to leaves (**H9**, **H11**). In the first stage of the research, in the autumn 2008, I collected samples of soils and leaves in different parts of Poznań (subject to different anthropogenic pressure) before shedding leaves. It should be mentioned that perennial trees growing in Poznań have been chosen for the first time for a study on mercury bioavailability from soils to leaves. Leaf samples came from two tree broadleaf species: *Acer platanoides* and *Tilia platyphyllos*, because these trees are very popular in Poland.

The conducted study of mercury concentration demonstrated variabilities in the spatial distribution of mercury content, both in soils and in leaves of two tree species (Fig. 5). I found the highest concentrations both in soils and leaves in the city center. Statistical analysis with the use of t-Student test showed that the obtained mean mercury concentration in maple leaf samples $162 \pm 22 \text{ ng} \cdot \text{g}^{-1}$ (median $165 \text{ ng} \cdot \text{g}^{-1}$) and linden samples $165 \pm 43 \text{ ng} \cdot \text{g}^{-1}$ (median $170 \text{ ng} \cdot \text{g}^{-1}$) did not differ in a statistically significant way ($p = 0.795$). However, I observed a positive correlation between the content of mercury in soils and leaves of linden ($r=0.655$) and maple ($r=0.504$) (**H9**).

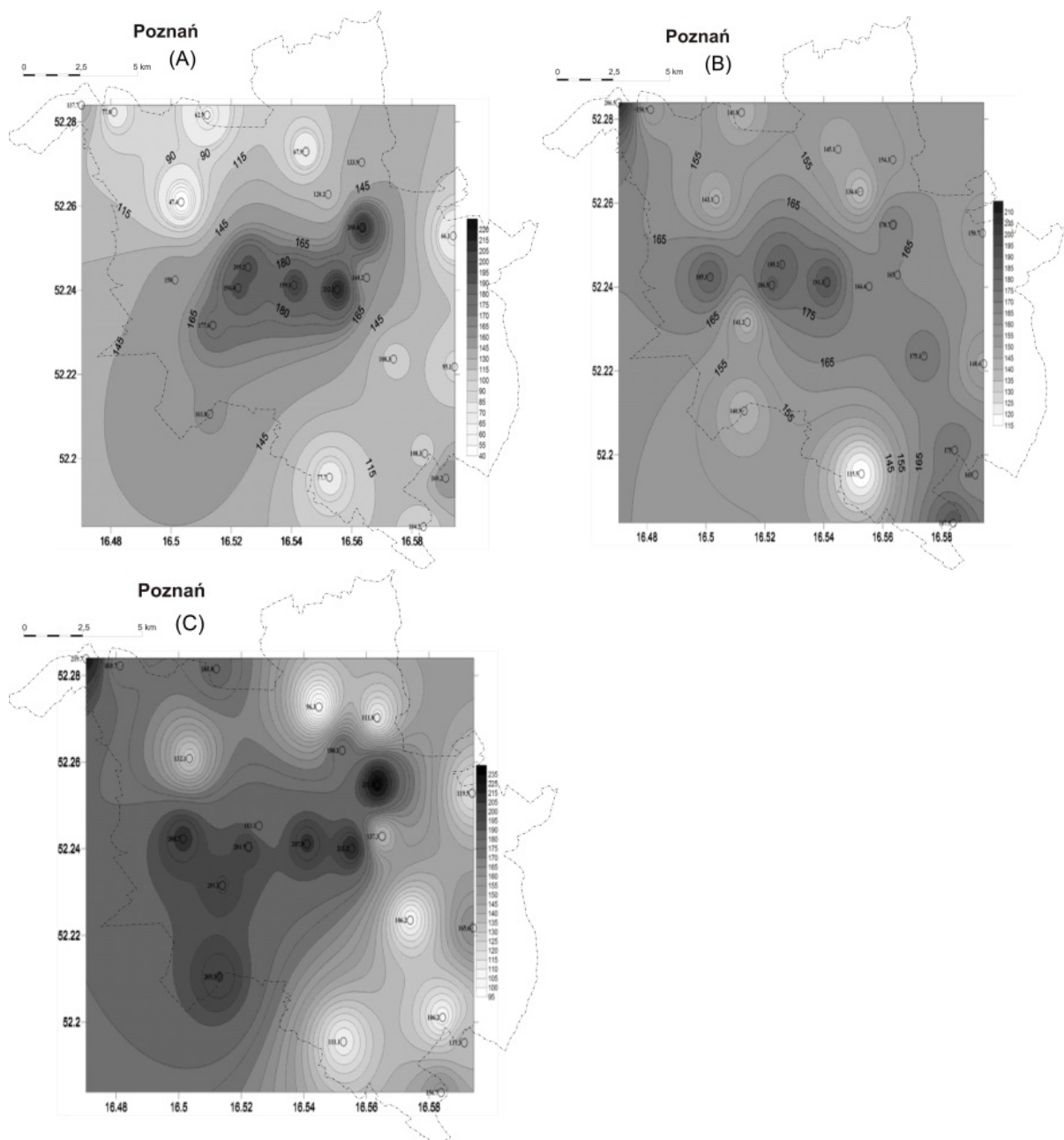


Fig. 5. Maps of mercury contents in soils (A), maple leaves (B), and linden leaves (C) (ng·g⁻¹)

The consequence of the research described in publication (H9) was performing further analyses in 2013 (H11). This was the first study of this kind over the full vegetation period on perennial trees. I carried out the research in the vegetation periods of trees: I collected the samples from April to November at three month intervals. In April, leaf buds were collected first. The study carried out for samples collected at three month intervals allowed me to observe both seasonal and spatial changes occurring in soils and leaves of the two tree species. In the analyzed samples of soils, buds and leaves, the mercury content rose in the first two measurement periods (April-June); then, I observed the decrease of mercury concentration both in soils and in leaf samples (Fig. 6). Previous measurements of mercury

content in plants have shown that mercury accumulates in leaves and its concentration rises with time [13,46,57]. But the authors carrying out laboratory tests did not take into consideration the changing atmospheric conditions, i.e., dry and wet precipitation, changes of the wind direction and speed, changes of temperature, sun exposure, and humidity. The determined highest contents of mercury in soils in June is connected with mercury from atmosphere and mercury released after the mineralization of organic matter e.g. from leaves in the period of external temperature growth in spring [57,71]. The highest determined mercury concentration in maple and linden leaves in June is caused by intensive uptake of gaseous mercury from the atmosphere through the leaf stomas and the adsorption on the leaf surface of mercury bound to aerosol molecules [12,41]. The exchange of mercury through the stomas is less and less intensive at the end of the plant vegetation period, which is connected with leaf ageing [41].

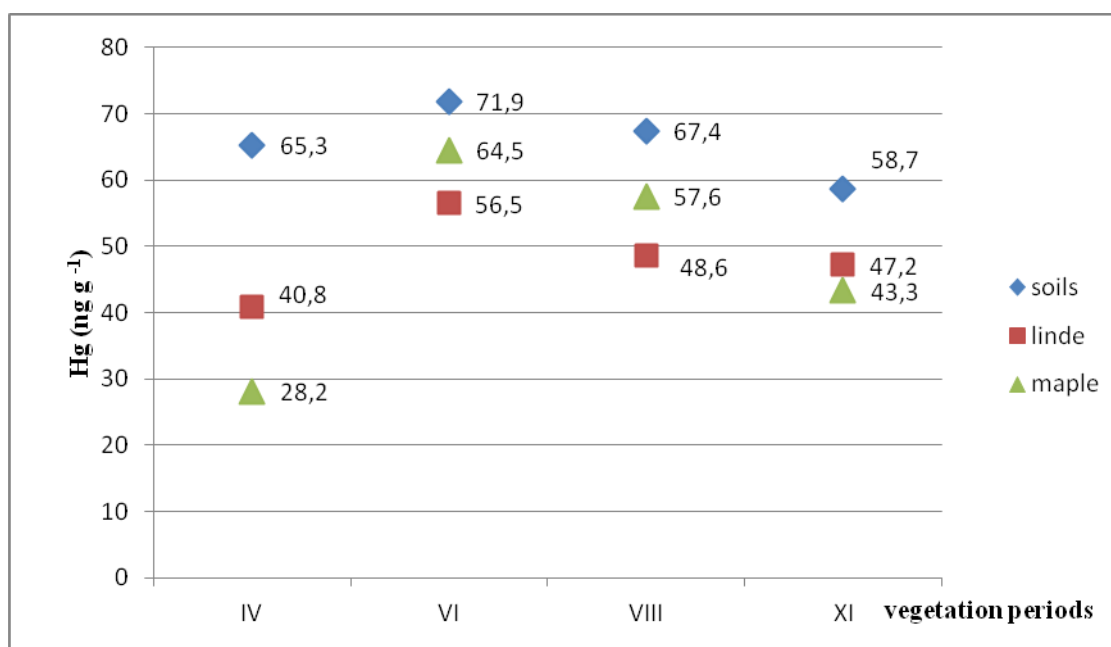


Fig. 6. Mean concentration of mercury (ng·g⁻¹) in soils, linden and maple in different vegetation periods

The conducted research proved that the greatest concentration of mercury in soils was determined in the city center (**H9, H11**). The Mann-Whitney U test showed that the obtained mean value of mercury in soils collected from the city center in four measurement periods (April-November), amounting to 95.5 ± 39.1 ng·g⁻¹ (median 91.4 ng·g⁻¹), was statistically different from the mean value of 48.8 ± 32.9 ng·g⁻¹ (median 39.0 ng·g⁻¹) for the other soil samples. Points located in the center of Poznań have twice as high concentrations as other parts of the city (**H11**). Given that the highest mercury concentrations in soils were determined in points located in the closest proximity of the main Poznań emitters – Karolin

and Garbary thermal power stations, I concluded that they have a negative impact on human's natural environment, especially in soil samples. I also compared the distribution of mercury content in soils with the direction of wind (blowing mainly from North-West) and excluded the impact of other potential emitters, i.e., a municipal waste stockpile, airports or highway on the content of the metal in soils (**H11**). The relation between high mercury concentrations in the leaves of both species in the city center, which I observed in previous studies (**H9**), did not occur in the measurements over the whole vegetation period in 2013 (**H11**).

On the basis of previous studies, I observed that the mean values of mercury concentration in maple and linden leaves were not statistically significantly different (**H9**). The study carried out in 2013 confirms this relation, and the t-Student test for the samples of material collected in the June-November period (VI-XI) from both tree species showed that there are no statistically significant differences ($p = 0.137$) between the mean values in the leaves of linden ($50.7 \pm 15.8 \text{ ng}\cdot\text{g}^{-1}$) and maple ($55.4 \pm 18.1 \text{ ng}\cdot\text{g}^{-1}$) (**H11**). The above-mentioned studies show that mercury content does not depend on the tree species (**H9, H11**). Similar relations were observed by other authors, too [33,43]. In an experiment performed in a full vegetation period, I also confirmed the fact described by Fay and Gustin (2007), Pérez-Sanz et al. (2012), Lodenius (2013) and Amorós et al. (2014), that mercury content in leaves does not depend of the concentration of this element in soils. The Spearman's rank correlation analysis performed for the samples collected in the April-June period (IV-VI) and Pearson's coefficient for the study period (VIII-XI) did not show a mutual relation between mercury contents in soils and the leaves of both tree species. Correlation coefficients were in the range from $r=-0.183$ to $r=0.373$ (**H11**). Another evidence for the fact that the main transfer of mercury from atmosphere to leaves involves dry deposition (of gaseous and molecular mercury) and wet deposition (rains) is that in my study I obtained high values of diffusion coefficient proposed by Klocke et al. (1984). This coefficient describes the relation between concentration of metal contained in the overground part of the plant divided by the concentration of the metal in soil; for mercury, it is within the 0.01-0.1 range. On the basis of previous studies, I concluded that for maple samples the coefficient was 1.43 (range 0.78-3.02), and for linden, 1.40 (range 0.83-2.92) (**H9**). In the case of the analyzed material, in 2013 I obtained a mean diffusion coefficient of 1.04 (range 0.14-2.79) for linden and 1.11 (range 0.15-3.37) for maple (**H11**).

Comparing the results of mercury content obtained over the years I observed a positive trend of lowering concentration of the element both in soils and in leaves. In the case of soils, mean mercury concentration decreased from $146 \pm 130 \text{ ng}\cdot\text{g}^{-1}$ (range 17-746 $\text{ng}\cdot\text{g}^{-1}$) in the year 2003 [7] to $65.8 \pm 41.7 \text{ ng}\cdot\text{g}^{-1}$ (range 14.5-238.9 $\text{ng}\cdot\text{g}^{-1}$) in 2013 (**H11**). I observed

similar relations for leaf samples. Frankowski et al. (2007) in the year 2005 determined mercury in maple and linden leaves, and mean mercury concentrations were: $123 \text{ ng}\cdot\text{g}^{-1}$ (range $10\text{-}544 \text{ ng}\cdot\text{g}^{-1}$) in linden samples, and $89.8 \text{ ng}\cdot\text{g}^{-1}$ (range $6.6\text{-}328 \text{ ng}\cdot\text{g}^{-1}$) in maple leaves. The most recent study of 2013 showed the lowering of mean values of mercury concentration and the maximum contents in the leaves of two tree species. In *Acer platanoides*, mean Hg concentration was $55.4 \pm 18.1 \text{ ng}\cdot\text{g}^{-1}$ (range $26.5\text{-}106.9 \text{ ng}\cdot\text{g}^{-1}$), and in *Tilia platyphyllos*, $50.4 \pm 15.8 \text{ ng}\cdot\text{g}^{-1}$ (range $23.1\text{-}88.7 \text{ ng}\cdot\text{g}^{-1}$) (H11). It is mainly connected with the improvement of atmospheric air quality in Poznań by ecological investments, especially concerning the Thermal Power Station Complex of Poznań – Karolin and Garbary, polluting the environment.

The first measurement of mercury content in medicines and dietary supplements using pressure microwave-assisted mineralization. Estimation of potential threat to the health of people taking medications.

In recent years, the consumption of medicines and dietary supplements has been on the increase in Europe and Northern America [2,10,18,21,73]. Daily and long-term use of medicines and dietary supplements mostly by adults may be a potential source of mercury in their organisms [18]. The assessment of mercury concentration is of primary importance for human health, especially in the case of medicines for which the EU legislation has not specified the highest acceptable concentration of that metal. The acceptable concentration of mercury in dietary supplements should not exceed $100 \text{ ng}\cdot\text{g}^{-1}$ [45].

The goal of my work was to determine the content of mercury in medicines and dietary supplements available in Poland and to estimate their potential threat for the health of people taking certain preparations (H10). In the first stage of the study, I developed the method to decompose solid samples (tablets) through pressure mineralization using a microwave mineralizer. The specific and heterogeneous composition of medicines and dietary supplements made it necessary to optimize the following parameters: the mass of the weighted amount, the amount and composition of mineral acids, the time of preliminary mineralization and the temperature of mineralization with microwaves. A necessary stage of the study was the verification of consistence of the obtained results using the optimized method of sample mineralization. In the case of medicines, there is no reference material with a certified value of mercury. I used in the research SRM 1515 Apple Leaves (National Institute of Standards and Technology, USA).

The next stage of the study was to determine mercury content in the following groups of medicines: painkillers, diuretic, cardiac, antihypertonic, anti-flu, anti-allergy, sedative, antibacterial, and dietary supplements available in Poland (**H10**). I also carried out statistical evaluation of mercury concentration in medicines with consideration of their availability: Rx – prescription medicines, and OTC – non-prescription medicines. The study of mercury content in medicines was the first in Poland and in Europe. The obtained results show different levels of content of this metal in medicines. The highest mercury content was found in prescription medicines Rx 0.9-476,1 ng·g⁻¹ (median 7.4 ng·g⁻¹), lower in over the non-prescription medicines OTC 1.2 – 45.8 ng·g⁻¹ (median 6.0 ng·g⁻¹). Dietary supplements had the lowest content of mercury, amounting to 0.9-16.7 ng·g⁻¹ (median 5.9 ng·g⁻¹) in comparison to Rx and OTC medicines. The results of mercury in groups of medicines and dietary supplements are presented in Figure 7.

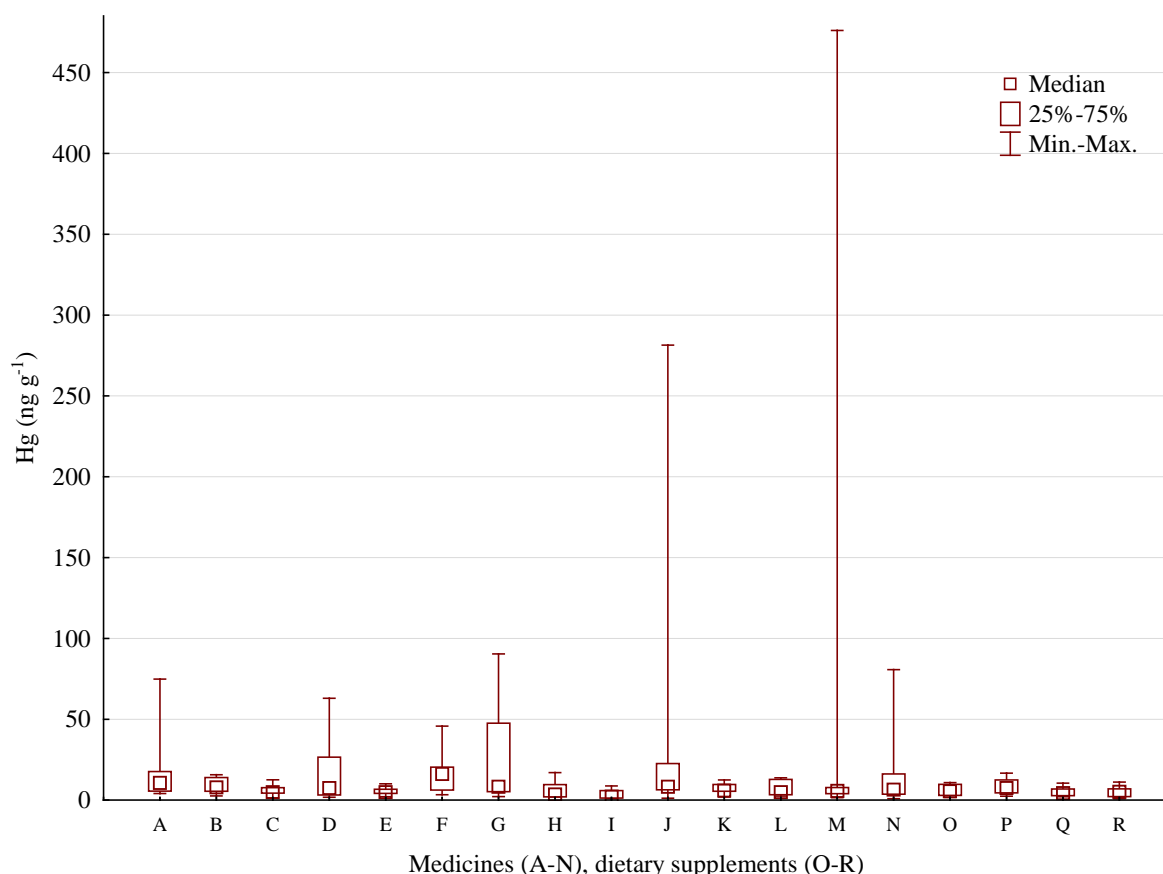


Fig. 7. Mercury concentration in medicines and dietary supplements **Medicines:** A – antihypertonic B – anticoagulants, heart ischemic disease C – painkillers, febrifuges, anti-inflammatories D – painkillers, anti-inflammatories, rheumatic pain E – anti-allergy F – sedatives, soporifics, antidepressants, anxiolytics G supporting urination, diuretics I – antibacterial, antiviral, antifungal, antibiotics J – inhibiting gastric acid secretion K – sore throat medicines L – antidiarrheals M – cardiovascular diseases medicines N – others*. **Dietary supplements:** O – supplementing macro- and microelements P – supplementing vitamins Q – improving skin, hair and nails condition R – others*.*medicines and dietary supplements that cannot be classified in any the the above groups due to their composition and activity.

The obtained values of mercury content in medicines are lower than the results concerning medicines from China, Malaysia or India used in natural medicine [2,21,50]. Medicines from Asia may contain mercury as the main ingredient or an excipient [2,29]. The highest values of mercury in traditional medicines from Asia were determined by Saper et al. (2004,2006) from 24.5 up to 100,400 $\mu\text{g}\cdot\text{g}^{-1}$.

In the case of analyzed dietary supplements, I did not observe mercury concentrations exceeding the values acceptable pursuant to the legislation of the European Union [45] in any of the samples. In 30 supplements available in Polish pharmacies, Socha et al (2013) determined the mean value of mercury at the level of $5.36 \pm 11.3 \text{ ng}\cdot\text{g}^{-1}$ (range 0.1-47.99 $\text{ng}\cdot\text{g}^{-1}$). Higher values (max. 123 $\text{ng}\cdot\text{g}^{-1}$) were observed by other researchers [31,59].

In the work, I also assessed the potential threat for patients' health when taking the medications. On the basis of the obtained results and information necessary for the proper use of the medicine given by the manufacturer in the leaflet, i.e., dosage and frequency of use, I calculated the provisional tolerable weekly intake (PTWI). The PTWI index was established by the FAO/WHO Expert Committee on Food Additives [25] to estimate the intake of mercury with food; for inorganic mercury, it is 4 $\mu\text{g}\cdot\text{kg}^{-1}$ body mass / week. In the case of the highest determined mercury content (474.1 $\text{ng}\cdot\text{g}^{-1}$) in an Rx medicine taken for cardiovascular diseases, one consumes 674 $\text{ng Hg}\cdot 60\text{kg}^{-1}$ b.w. mercury within a week (assuming the average body mass of 60 kg), which means 0.28% PTWI. For the OTC medicine with the highest content of 45.8 $\text{ng}\cdot\text{g}^{-1}$, taken for anxiety disorders, the weekly intake is 495.6 $\text{Hg}\cdot 60\text{kg}^{-1}$ b.w., which means 0.21% PTWI. As for a dietary supplement with the highest determined content of mercury, 16.7 $\text{ng}\cdot\text{g}^{-1}$, taken for vitamin C and rutin deficiency, within a week we consume 207.2 $\text{Hg}\cdot 60\text{kg}^{-1}$ b.w.. This means 0.09% PTWI.

The study of mercury content in medicines and dietary supplements available on the Polish market proved that they are safe and do not pose a threat for the health of people taking the medicines. The mercury contained in the analyzed samples was only a little fraction of the weekly mercury consumption acceptable by the WHO. However, we need to remember that mercury also occurs in the air, drinking water and food, and in combination with medications, this can be dangerous for human health.

Quality assurance

In order to verify and control the applied method, certified reference materials were used for soil: SRM 2709 (National Institute of Standards and Technology, USA) and 2711 (National Institute of Standards and Technology, USA) for estuarine sediment LGC 6137 (European Reference Material, Belgium) and leaves SRM 1515 (National Institute of Standards and Technology, USA). The results of mercury concentration in the certified reference materials are presented in Table 1.

Table.1. Determination results of the certified reference materials.

		SRM 2711	SRM 2709	LGC 6137	SRM 1515
Certified value	[ng·g ⁻¹]	6250 ± 190	1400 ± 80	340 ± 50	44 ± 4,0
Determined value	[ng·g ⁻¹]	6060 ± 70	1440 ± 30	373 ± 10	43 ± 2,4
Accuracy	[%]	3,0	2,9	9,7	2,3

Summary of the scientific achievement

The results of the research obtained in a series of eleven works devoted to the same subject provide the basis for the summary of my scientific achievement:

1. I developed a single-step and a multi-step extraction procedures for mercury determination, which I applied for the determination of mercury fractions in samples with complex matrix.
2. I implemented the single-step extraction with the use of 3M HCl as solvent in geochemical survey for mobile forms of mercury in sedimentary rocks investigation.
3. The sequential extraction procedure for mercury determination provided by author, was applied for various mercury fraction determination differing with bioavailability and mobility in the samples of soils, river sediments and sediments plotted after tsunami passing.
4. The proposed scheme of sequential extraction made it possible to obtain results that provide more comprehensive information on the environment condition from the perspective of environmental protection. The proposed scheme of sequential extraction can be used to describe changes occurring in the environment in samples characterized with different degrees of contamination.

5. I was the first researcher in the world who determine of mercury content in chemical fractions in samples of sediment deposited by a tsunami wave in Thailand.
6. I performed comprehensive determination of mercury in different components of the environment (soils, sediments, leaves, surface and ground waters). This was the first study involving determination of mercury in ground and surface water samples for the city of Poznań.
7. I was the first researcher in Poland, I determined mercury in the floodplain soils of the Warta river. The study is very important from the perspective of environmental protection.
8. On the basis mercury analyses in samples of soils and leaves of two species, *Tilia platyphyllos* and *Acer platanoides*, I demonstrated that the main source of mercury in the studied leaves is mercury from dust adsorbed on the leaf surface and mercury connected with gas exchange through leaf stomas. I did not observe a correlation between the mercury contents in soils and maple or linden leaves. I did not find any differences in the content or mercury in the leaves of the two tree species.
9. I carried out the first determination of mercury content in soils and plant material in the whole vegetation period in perennial trees. I demonstrated that the highest content of mercury both in soils and in tree leaves occurs in the spring.
10. The analysis of mercury content in medicines and dietary supplements available on the Polish market showed that they are safe and do not pose a threat for the health of people taking them. I proved that the mercury contained in the analyzed samples was only a little fraction of the weekly mercury consumption acceptable by the WHO.
11. The determination of mercury content in medicines available from pharmacies was the first in Poland and in Europe. The fact that higher mercury content was observed in medicines than in dietary supplements may be the basis for regulating the acceptable mercury content in medicines, just like it is done for dietary supplements.

References

- [1] Amorós Jose-Angel, Esbrí J. M., García-Navarro Francisco-Jesús, Pérez-de-los-Reyes C., Bravo S., Villaseñor B., Higuera P. (2014) Variations in mercury and other trace elements contents in soil and in vine leaves from the Almadén Hg-mining district. *J Soils Sediments*. 14: 773–777
- [2] Ang H. H., Lee K. L. (2006) Contamination of mercury in Tongkat Ali hitam herbal preparations. *Food and Chem Toxicol*. 44:1245–1250

- [3] Beldowski J., Pempkowiak J., (2003) Horizontal and vertical variabilites of mercury concentration and speciation in sediments of the Gdańsk Basin, Southern Baltic Sea. *Chemosphere* 52:645-654
- [4] Biester H, Muller G, Schöler H.F. (2002) Binding and mobility of mercury in soils contaminated by emissions from chloralkali plants. *Sci Total Environ.* 284:191–203
- [5] Bloom N., Preus E., Katon J., Hiltner M. (2003) Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils. *Analytica Chimica Acta* 479: 233-248
- [6] Boszke L., Kowalski A. (2006) Spatial distribution of mercury in bottom sediments and soils from Poznań, Poland. *Polish J. Environ. Stud.* 2: 211-218
- [7] Boszke L., Kowalski A., Głosińska G., Szarek R., Siepak J. (2003) Environmental factors affecting speciation of mercury in the bottom sediments; an overview. *Polish Journal of Environmental Studies* 12 (1): 5-13
- [8] Ching I.L., Hongxiao T. (1985) Chemical studies of aquatic pollution by heavy metals in China. In: Irgolic K.J., Martel A.E. (eds) *Environmental inorganic chemistry*. VCH Publishers, 359–371
- [9] Dąbkowska-Naskręt H., Bartkowiak A., Różański S. (2008) Zawartość rtęci w glebach intensywnie użytkowanych rolniczo obszaru Pomorza i Kujaw. *Ochrona Środowiska i Zasobów Naturalnych* 35/36, IOŚ, Warszawa: 153–156
- [10] Dolan S. P. , Nortrup D. A., Bolger P. M., Capar S. G. (2003) Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry. *J. Agric. Food Chem.* 51, 1307–1312
- [11] Eguchi T., Tomiyasu T., (2002) The speciation of mercury in sediments from Kagoshima bay and Minamata Bay, Souyhern Kyusyu, Japan by fractional extraction/cold-vapor AAS (in Japan), *Bunseki Kagaku* 51: 859-864
- [12] Ericksen J.A., Gustin M.S. (2004) Foliar exchange of mercury as a function of soil and air mercury concentrations. *Science of the Total Environment.* 324: 271–279
- [13] Ericksen J.A., Gustin M.S., Schorran D.E., Johnson D.W., Lindberg S.E., Coleman J.S. (2003) Accumulation of atmospheric mercury in forest foliage. *Atmospheric Environment.* 37: 1613–1622
- [14] Fay L., Gustin M. (2007) Assessing the influence of different atmospheric and soil mercury concentrations on foliar mercury concentrations in a controlled environment. *Water Air Soil Pollut.* 181: 373–384
- [15] Feng X., Hong Y., (1999) Modes of occurrence of mercury in coals from Guizhou, People’s Republic of China *Fuel.* 78:1181-1188
- [16] Frankowski M., Kowalski A., Ziola A., Siepak J. (2007) Determination of mercury in leaves of *Acer plantanoides* and *Tilia platyphyllos* in the area of the city of Poznań and its vicinity. *Oceanological and Hydrobiological Studies*, Vol. XXXVI, No. 3: 1-12
- [17] Frankowski M., Ziola A., Siepak M., Siepak J. (2008) Analysis of Heavy Metals in Particular Granulometric Fractions of Bottom Sediments in the Mała Welná River (Poland). *Polish J. of Environ. Stud.* Vol. 17: 343-350
- [18] Genuis S. J., Schwalfenberg G., Siy A. K. J., Rodushkin I. (2012) Toxic element contamination of natural health products and pharmaceutical preparations. *Plos One.* 11:1-12
- [19] GUS. (2011) Stan zdrowia ludności Polski w 2009 roku. Główny Urząd Statystyczny. Warszawa.
- [20] Han Y., Holsen T., Lai S., Hopke P., Yi S., Liu W., Pagano J., Falanga L., Milligan M., Andolina C. (2004) Atmospheric gaseous mercury concentrations in New York State: relationships with meteorological data and other pollutants, *Atmospheric Environment* 38: 6431-6446
- [21] Harris E. S., Cao S., Littlefield B. A., Craycroft J. A., Scholten R., Kaptchuk T., Fu Y., Wang W., Liu Y., Chen H., Zhao Z., Clardy J., Woolf A. D., Eisenberg D. M. (2011) Heavy metal and pesticide content in commonly prescribed individual raw Chinese Herbal Medicines. *Sci Total Environ.* 409: 4297-4305
- [22] Harvey J.W., Krupa S.L., Gefvert C., Mooney R.M., Choi J., King S.A., Giddings J.B. (2002) Ground-water component of the mercury budget at ENR. Interactions between surface water and ground water and effects on mercury transport in the north-central everglades. U.S. Department of the Interior, U.S. Geological Survey, Center for Coastal Geolog.
- [23] Hurley J., Rolffhus K., Cowell S., Shafer M., Hughes P. (2000) Inlues of watershed characteristic on total

and methyl mercury levels in Lake Michigan tributaries, In: 11th Annual International Conference on Heavy Metals in the Environment. J. Nriagu (red.). University of Michigan School of Public Health, Ann Arbor, Michigan

[24] Jackowska I., Bojanowska M. (2000) Badania nad formami i rozpuszczalnością metali ciężkich w glebie lessowej. Roczniki Gleboznawcze, Tom LI nr ½ Warszawa 2000: 65-72

[25] JECFA (2010) Food and Agriculture Organization of the United Nations World Health Organization Joint FAO/WHO Expert Committee On Food Additives. Seventysecond meeting Rome

[26] Kloke A., Sauerbeck D. R., Vetter J. (1984) The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains. Changing Metal Cycles and Human Health, ed. Nriagu, J. O. 113–141

[27] Kot F.S., Matyushkina L.A. (2002) Distribution of mercury in chemical fraction of contaminated urban soils of Middle Amur, Russia. J Environ Monit 4:803–908

[28] Kowalski A., Kurzyca I., Boszke L. (2005) One Year Observation of Mercury in Wet Precipitation of Poznań City (Poland). Polish Journal Environmental Studies Vol. 14, suppl. V: 37-42

[29] Kumar G., Srivastava A., Sharma S. K., Gupta Y. K. (2012) Safety evaluation of an Ayurvedic medicine, Arogyavardhini vati on brain, liver and kidney in rats. Journal of Ethnopharmacology. 140: 151– 160

[30] Lechler P.J., Miller J.R., Hsu L.C., Desilets M.O. (1997) Mercury mobility at Carson River superfund site, west-central Nevada, USA: interpretation of mercury speciation data in mill tailing, soils, and sediments. Journal of Geochemical Exploration 58: 259-267

[31] Levine K. E., Levine M. A., Weber F. X., Hu Y., Perlmutter J., Grohse P. M. (2005) Determination of mercury in an assortment of dietary supplements using an inexpensive combustion atomic absorption spectrometry technique. Journal of Automated Methods & Management in Chemistry. 4: 211–216

[32] Lis J., Piaseczna A., (1995) Geochemiczny Atlas Polski. Państwowy Instytut Geologiczny, Warszawa

[33] Liu R. H., Wang Q. C., Wang Y., Zhang L., Shao Z. G. (2003) Distribution of mercury in typical wetland plants in the Sanjiang Plain. Chin. Geogr. Sci. 3: 242–246

[34] Lodenius M. (2013) Use of plants for biomonitoring of air borne mercury in contaminated areas. Environmental Research. 125: 113–123

[35] Martin-Doimeadios R.C.R., Tessier E., Amouroux D., Guyoneaud R., Duran R, Caumette P., Donard O.F.X. (2004) Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species-specific enriched stable isotopes. Marine Chemistry 90:107-123

[36] Miller E.L., Dobb D.E., Heithmar E.M. (1995) Speciation of mercury in soils by sequential Extraction. Presented at the USEPA Metal Speciation and Contamination of Surface Water Workshop. Jekyll Island

[37] NFESC - Naval Facilities Engineering Command. (2000) Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Navy and Marine Corps Facilities. Part 2: Technical Background Document for Assessing Metals Bioavailability. Washington, DC 20374-5065

[38] Niu Z., Zhang X., Wang S., Ci Z., Kong X., Wang Z. (2013) The linear accumulation of atmospheric mercury by vegetable and grass leaves: Potential biomonitors for atmospheric mercury pollution. Environ Sci Pollut Res. 20: 6337–6343

[39] Pérez-Sanz A., Millán R., Sierra M. J., Alarcón R., García P., Gil-Díaz M., Vazquez S., Lobo M. C. (2012) Mercury uptake by *Silene vulgaris* grown on contaminated spiked soils. Journal of Environmental Management. 95: 233-237

[40] Poissant L., Pilote M., Yumvihoze E., Lean D. (2008) Mercury concentrations and foliage/atmosphere fluxes in a maple forest ecosystem in Québec, Canada. 2008. Journal of Geophysical Research. 113: 1-12

[41] Poissant L., Pilote M., Yumvihoze E., Lean D. (2008) Mercury concentrations and foliage/atmosphere fluxes in a maple forest ecosystem in Québec, Canada. Journal of Geophysical Research. 113, D10307

[42] Prochackova T., Góra R., Kadrač J., Hutta M. (1998) Distribution of mercury in soil organic matter fractions obtained by dissolution/precipitation method, Journal of Radioanalytical and Nuclear Chemistry 229:61-65

- [43] Reimann, A., Arnoldussen, A., Boyd, R., Finne, T. E., Koller, F., Nordguen, O., Englmaier, P. (2007) Element contents in leaves plant species (brich, mountains ash, fern and spruce) along antropogenic and geogenic concentration gradients. *Sci. Tot. Environ.* 2–3: 416–433
- [44] Renneberg A.J., Dudas M.J. (2001) Transformations of elemental mercury to inorganic and organic forms in mercury and hydrocarbon co-contaminated soils. *Chemosphere* 45:1103–1109
- [45] Rozporządzenie komisji (WE) nr 629/2008 z dnia 2 lipca 2008 r. zmieniające rozporządzenie (WE) nr 1881/2006 ustalające najwyższe dopuszczalne poziomy niektórych zanieczyszczeń w środkach spożywczych
- [46] Rutter A. P., Schauer J. J., Shafer M. M., Creswell J. E., Olson M. R., Robinson M., Collins R. M., Parman A. M., Katzman T. L., Mallek J. L. (2011) Dry deposition of gaseous elemental mercury to plants and soils using mercury stable isotopes in a controlled environment. *Atmospheric Environment.* 45: 848-855
- [47] Sakamoto H., Tomiyasu T., Yonehara N. (1995) The content and chemical forms of mercury in sediments from Kagoshima Bay, in comparison with Minamata Bay and Yatsushiro Sea, Southwestern Japan. *Geochem Journal.* 29: 97-105
- [48] Saniewska, D., Beldowska, M., Beldowski, J., Falkowska, L. (2014). Mercury in Precipitation at an Urbanized Coastal Zone of the Baltic Sea (Poland). *AMBIO* 43 (7): 871-877
- [49] Saper R. B., Kales S. N., Paquin J., Burns M. J., Eisenberg D. M., Davis R. B., Phillips R. S. (2004). Heavy metal content of ayurvedic herbal medicine products. *JAMA.* 292 (23): 2868-73
- [50] Saper R. B., Phillips R. S., Sehgal A., Khouri N., Davis R. B., Paquin J., Thuppil V., Kales S. N. (2008). Lead, mercury, and arsenic in US- and Indian-manufactured ayurvedic medicines sold via the internet. *JAMA.* 300(8): 915–923
- [51] Sladek C., Gustin M.S. (2003) Evaluation of selective extraction methods for determination of mercury speciation and mobility in mine waste. *Applied Geochemistry* 18:567-576
- [52] Socha K., Michalska-Mosiej M., Lipka-Chudzik K., Borawska M. H. (2013) Zawartość rtęci w suplementach. *Probl Hig Epidemiol* 94(3): 645-647
- [53] Stein E.D., Cohen Y., Winer A.M. (1996) Environmental distribution and transformation of mercury compounds, *Critical Review in Environ. Sci. Technol.* 26:1-43
- [54] Stoichev T., Martin-Doimeadios R., Tessier E., Amouroux D., Donard O. (2004) Improvement of analytical performances for mercury speciation by on-line derivatization, cryofocussing and atomicfluorescence spectrometry, *Talanta* 62: 433–438
- [55] Sutherland R.A., Tack F.M.G., Tolosa C.A., Verloo M.G. (2001) Metal Extraction from Road Sediment using Different Strength Reagents: Impact on Anthropogenic Contaminant Signals. *Environmental Monitoring and Assessment.* 71:221-242
- [56] Szczuciński W., Niedzielski P., Kozak L., Frankowski M., Ziola A., Lorenc, S. (2007) Effects of rainy season on mobilization of contaminants from tsunami deposits left in a coastal zone of Thailand by the 26 December 2004 tsunami. *Environ. Geol.* 53:253-264
- [57] Tabatchnick M. D., Nogaro G., Hammerschmidt C. R. (2012) Potential sources of methylmercury in tree foliage. *Environmental Pollution.* 160: 82-87
- [58] Tessier A., Campbell P. G. C., Bisson M. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51 (7): 844–851
- [59] Tumir H., Bošnjir J., Vedrinar-Dragojević I., Dragun Z., Tomić S., Puntarić D., Jurak G. (2010) Monitoring of metal and metalloid content in dietary supplements on the Croatian market. *Food Control.* 21: 885–889
- [60] Ullrich S.M., Tanton T.W., Abdrashitowa S.A. (2001) Mercury in the aquatic environment: a review of factors affecting methylation. *Crit Rev Environ Sci. Technol* 31:241–293
- [70] Wallschläger D., Desai M., Spengler M., Wilken R., (1998) Mercury speciation in floodplai soils and sediments along a contaminated river transect. *Journal of Environmental Quality,* 27: 1034–1044
- [71] Wang J, Feng X, Anderson CWN, Xing Y, Shang L (2012) Remediation of mercury contaminated sites—a review. *Environ Res* 125:113–123
- [72] Wasay S.A., Barrington S., Tokunaga S., (1998) Retention form of heavy metals in three polluted soils, *Journal of Soil Contamination* 7: 103-119

[73] Wolle M. M., Mizanur G. M. Rahman, Kingston H. M., Pamuku M. (2014) Speciation analysis of arsenic in prenatal and children's dietary supplements using microwave-enhanced extraction and ion chromatography-inductively coupled plasma mass spectrometry. *Analytica Chimica Acta*. 818: 23–31

Artur Kowalski